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ORIGINAL ARTICLE

Fine mapping of a quantitative resistance gene for gray leaf spot of maize (*Zea mays* L.) derived from teosinte (*Z. mays* ssp. *parviglumis*)

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Abstract

Key message **In this study we mapped the QTL Qgls8 for gray leaf spot (GLS) resistance in maize to a ~130 kb region on chromosome 8 including five predicted genes.**

Abstract In previous work, using near isogenic line (NIL) populations in which segments of the teosinte (*Zea mays* ssp. *parviglumis*) genome had been introgressed into the background of the maize line B73, we had identified a QTL on chromosome 8, here called *Qgls8*, for gray leaf spot (GLS) resistance. We identified alternate teosinte alleles at this QTL, one conferring increased GLS resistance and one increased susceptibility relative to the B73 allele. Using segregating populations derived from NIL parents carrying these contrasting alleles, we were able to delimit the QTL region to a ~130 kb (based on the B73 genome) which encompassed five predicted genes.

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Xinye Zhang and Qin Yang have contributed equally to the work.

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Introduction

Gray leaf spot (GLS) is a major foliar disease of maize, caused by the fungal pathogens *Cercospora zeae-maydis* and *Cercospora zeina* (Crous et al. 2006; Meisel et al. 2009; Korsman et al. 2012). The disease was first reported in Illinois (USA) (Tehon and Daniels 1925) and occurs in many maize-growing countries (Ward et al. 1999; Okori et al. 2004; Juliatti et al. 2009; Meisel et al. 2009; Kinyua et al. 2010; Manandhar et al. 2011; Liu and Xu 2013; Nega et al. 2016). The fungus can overwinter in the debris of diseased corn plants left on the soil surface and conidia produced in the following spring can be spread by wind or rain splashing. High relative humidity and prolonged periods of leaf wetness are ideal conditions for GLS epidemics (Rupe et al. 1982; Lipps 1998; Clements et al. 2000; Zhang et al. 2012; Dhami et al. 2015). GLS lesions are usually first observed on the lower leaves and then spread up the plant during the season. Yield losses are generally due to reduction in photosynthetic leaf area (Lipps 1998; Ward et al. 1999; Zhang et al. 2012). Compared with other methods, such as reduced conservation tillage, crop rotation, and the application of fungicides, improvement of host resistance is accepted as the most economical and environmentally friendly method for controlling GLS (Lehmensiek et al. 2001).

Resistance to GLS is quantitatively inherited, and quantitative trait loci (QTL) for GLS resistance have been identified on all 10 chromosomes of maize across different mapping populations (Bubeck et al. 1993;

Saghai Maroof et al. 1996; Clements et al. 2000; Lehmensiek et al. 2001; Gordon et al. 2004; Danson et al. 2008; Balint-Kurti et al. 2008; Pozar et al. 2009; Zwonitzer et al. 2010; Asea et al. 2012; Chung et al. 2011; Veiga et al. 2012; Zhang et al. 2012; Berger et al. 2014; Benson et al. 2015). In meta-analyses, Shi et al. (2007) identified seven consensus GLS resistance QTLs in bins 1.06, 2.06, 3.04, 4.06, 4.08, 5.03, and 8.06 (Davis et al. 1999), and Yan et al. (2016) confirmed 13 consensus QTLs including major QTL on chromosomes 1 and 4.

The narrow genetic basis of cultivated maize is an important factor limiting the breeding of new maize varieties for high-yield and disease resistance (Wallace et al. 2014). The genus *Zea* consists of five species divided into two sections; Section *Luxuriantes* including the species Z. *diploperennis, Z. perennis, Z. luxurians*, and *Z. nicaraguensis* and Section *Zea*, which includes only one species, *Zea mays. Zea mays* is further categorized into 4 subspecies one of which is modern maize (*Zea mays* ssp. *mays*). Another subspecies, *Z. mays* ssp. *parviglumis* is thought to be the immediate ancestor of cultivated maize (Matsuoka et al. 2002). The term teosinte is often used to describe all the species and subspecies within genus *Zea* that are not cultivated maize (Doebley and Iltis 1980; Iltis and Doebley 1980).

It has been proposed that teosintes can be used as genetic resources for the improvement of modern maize through the introduction of pre-domestication alleles (Liu et al. 2016); however, the substantial anatomical differences between teosintes and cultivated maize limit their use in breeding programs. Liu et al. (2016) developed a set of 928 near isogenic lines in which genomic fragments from 10 different *Z. mays* ssp. *parviglumis* accessions were introgressed in the background of the maize inbred B73. Lennon et al. (2016) screened this population for GLS resistance and identified and validated several alleles derived from teosinte which conferred GLS resistance. In one case, alternate alleles were identified from different teosinte accessions at a QTL in bin 8.06, one of which conferred GLS resistance and the other susceptibility with respect to the corresponding B73 allele. We reasoned that if these alleles were segregating against each other in a mapping population, the contrast between resistant and susceptible individuals would be enhanced and the locus would be easier to fine-map than the other QTL we identified. In this study, we constructed such populations in a B73 NIL background and used them to map the QTL, which we refer to here as *Qgls8*, to an interval of ~130 kb.

Materials and methods

Plant materials

In our previous study (Lennon et al. 2016), a previously developed population of teosinte/B73 NILs (Liu et al. 2016) was screened for resistance to GLS, and a pair of maize-teosinte (*Zea mays* ssp. *parviglumis*) B73 NILs—Z032E0081 and Z033E0056 were identified, which both harbored teosinte introgressions at the QTL region in Bin 8.06 (Fig. 1). The alleles from Z032E0081 and Z033E0056, respectively, increased and decreased GLS resistance compared with the allele from the recurrent parent, the maize inbred line B73. In this study, $F_{2:3}$ and F_4 populations developed from crosses between these two lines were used in the validation and fine mapping of this QTL which was named *Qgls8* (Fig. 2).

Field trials and disease scoring

Seventy-four recombinant $F_{2:3}$ families were planted in Andrews (North Carolina) and at Kentland Research Farm, Blacksburg (Virginia) as one replication in each location in the summer of 2015. In summer of 2016, 142 homozygous recombinant F_4 lines were planted in Andrews and Blacksburg using a randomized complete block design with two replicates in each environment. In all trials, 15 seeds of each $F_{2:3}$ family or F_4 line were planted in each row. The length of each row was 4 m, and the width between rows was 1 m.

Andrews is an ideal environment for GLS resistance identification because of the favorable epidemic conditions for GLS (the morning mists and dews and moderate temperatures). In addition, there are sufficient sources

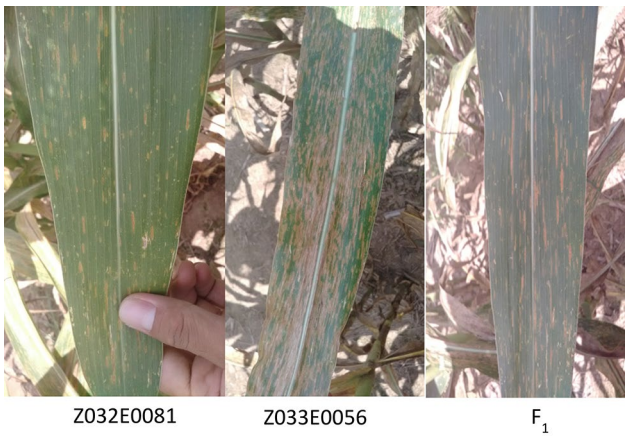

Fig. 1 The field performance of Z032E0081 and Z033E0056 and their F_1. Pictures were taken of the ear leaves of plants in neighboring rows on the same day in Andrews, NC

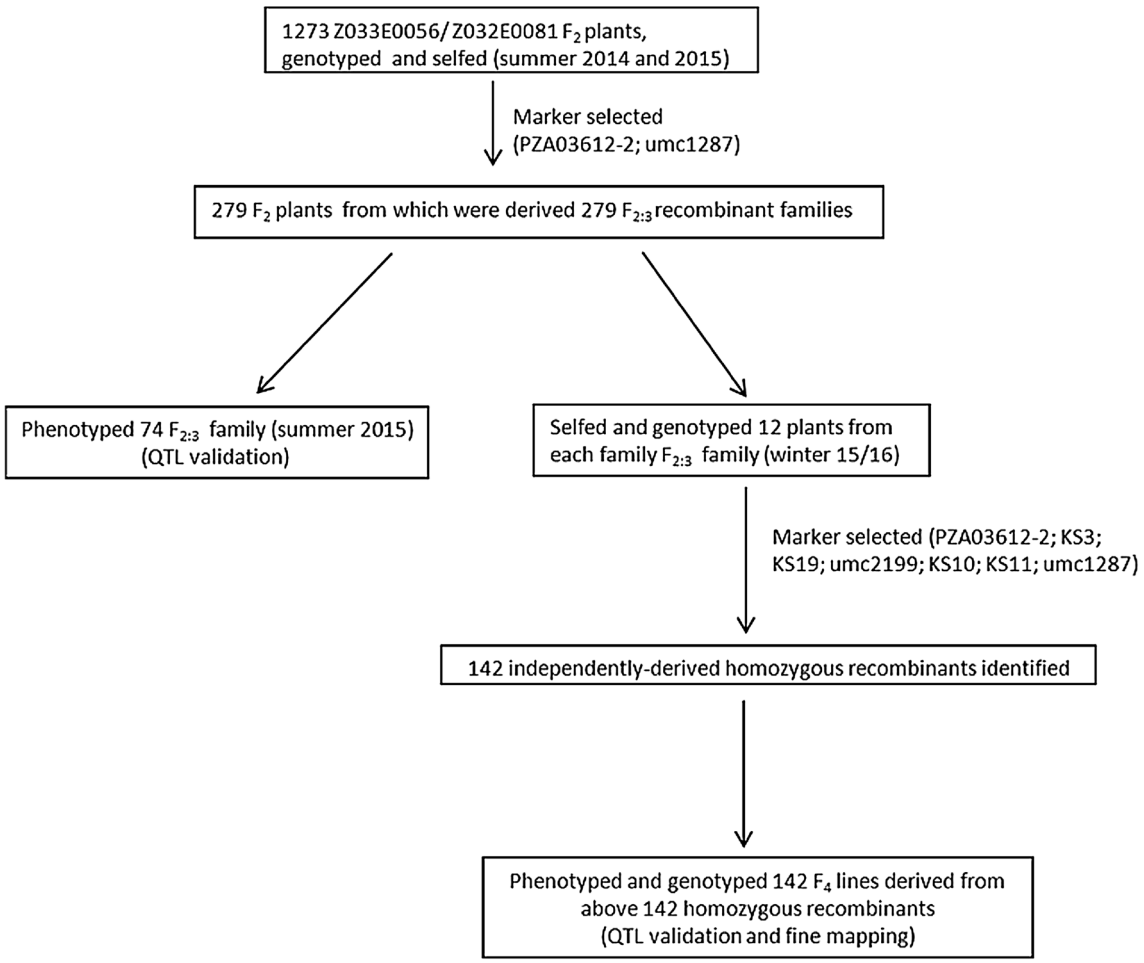

Fig. 2 The scheme used for fine mapping *Qgls8*

of inoculum left on the field surface from previous year (Balint-Kurti et al. 2008; Lennon et al. 2016), so GLS can occur naturally with inoculation. In Blacksburg, all the materials were inoculated artificially 30–45 days after planting with sorghum seeds infested by several isolates of *Cercospora zeae-maydis*. In each environment, disease scoring was performed two times with 10 days interval, starting ~2 weeks after anthesis. The scales used for GLS rating were from 1 to 9, where 1 means a completely dead plant and 9 represents no evidence of disease (Lennon et al. 2016). The average GLS score of each row was calculated.

The infested sorghum seeds were prepared in the following way: The seeds were soaked in water over 2–3 days and autoclaved in 1 L Erlenmeyer flasks. *Cercospora zeae-maydis* was grown on V8 agar plates for about 2 weeks at room temperature. Mycelia were macerated in water under sterile conditions and 5 ml of this mixture was added to each flask. The fungus was grown in the flask for about 4 weeks, until the seeds were visibly heavily infested. The infested seeds were then dried using simple fans in an open (non-sterile) space. The dried infested seed were used as inoculum.

DNA extraction

All DNA extraction was performed using 96-well plate (Stripes of 8 Attached 1.1 ml Micro Tubes™ in Convenient BulkPacks™, National Scientific Supply Co, Inc. Reorder#: TN0946-08B). For each sample, about 100 mg leaf tissue was frozen and ground with a stainless steel ball using a shaker (Retsch, Inc.). 500 µl of SDS buffer was added into each sample (500 ml SDS buffer contains 14.6 g NaCl, 9.3 g EDTA-2Na, 50 ml 1 M pH 8.0 Tris–HCl, 7.5 g SDS). The 96-well plate carrying mixtures of leaf tissues and SDS buffer was incubated in 65 °C water bath about 30 min. 450 µl Tris-Phenol/$CHCl_3$ (1:1) was added into each sample, mixed and the 96-well plate was spun at 3800 rpm for 10 min. The upper aqueous was transferred into another new 96-well plate. 0.7 volume of isopropanol was added, mixed, and the plate was spun at 3800 rpm for

10 min. The supernatant was removed and the DNA pellet was washed with 100 µl 70% ethanol. The plate was centrifuged at 3800 rpm for 5 min. The supernatant was discarded and the DNA allowed to dry for ~30 min. The dried DNA was suspended in 50 µl sterile TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.0).

Development and scoring of molecular markers

We developed 14 novel SNP/InDel assays for this work (Table 1). The B73 reference genomic sequence in the QTL region (from PZA03612-2 to umc1287) was used to design PCR primers to amplify ~500–1000 bp regions at ~1 cM intervals from the two parents. In some cases, the PCR primers used were derived from a previous paper (Chung et al. 2010). PCR products were cleaned using QIAquick® Gel Extraction Kit (Cat# 28704). All these products were sequenced at the Genomic Sciences Laboratory (North Carolina State University), and the sequences of Z033E0056 and Z032E0081 were aligned to identify single-nucleotide polymorphisms (SNPs) or insertion–deletion (InDel) polymorphisms. If a SNP or InDel polymorphism was identified, the context sequence was provided to LGC Genomics LLC (Beverly, MA, USA) in order to design a "Kompetitive Allele Specific PCR" (KASP) assay (He et al. 2014) that could be used to genotype plants at the corresponding locus. Information on the SNPs and the primers used for the KASP assays is listed in Table 1. KASP primers designed using sequences of PCR products amplified with primers from Chung et al. (2010) have a "ctg" prefix, while others have a "KS" prefix. Markers PZA03612-2 and PZA00429-1 were derived from our previous study (Lennon et al. 2016).

In addition to the markers that were scored by KASP assays, three other classes of markers were used. Three previously characterized SSR markers (http://www.maizegdb.org/) were used (umc2199, umc2210, umc1287—Table 2). These SSR markers were genotyped

Table 1 SNP/InDel marker information

Primer name	Position (v3) (bp)[a]	Allele FAM[b] primer	Allele HEX[c] primer	Common primer	Polymorphism[d]
KS11	153,797,365	GCAGAGCCTCAGGTCCTCAGA	CAGAGCCTCAGGTCCTCAGC	CTGCGGAATGGACAGGTGAGCTT	A/C
KS4	136,581,449	GTTCTCCCAGCACTGAGCAAGT	CTCCCAGCACTGAGCAAGC	CTTTCGGATCAACAGAATTTGCAGCTTAT	A/G
KS3	134,269,830	ACGTAATAATGATCCAAAAAATCAACAACG	ATAACGTAATAATGATCCAAAAAATCAACAACT	GATGGCTGCATCTCCCATAGGAATA	G/T
KS10	150,740,579	GCCTTGATATGTACTTGTCTACA	GCCTTGATATGTACTTGTCTACC	ACAATCACTGGTGGTATGGGCCAT	A/C
KS20	146,007,753	GGAGCTGGCTTTGCTGCTGTT	GGAGCTGGCTTTGCTGCTGTG	GACGAGGACGACCACCGGTA	TGT/-
KS27	152,677,635	GCCCACAGGCGTCGGGC	GCCCACAGGCGTCGGGG	AGTTGGCGCCGATGAGGAGGTT	C/G
KS28	151,638,826	GAATTCACTTGTGATGTTTTTTTGTGG	CTGAATTCACTTGTGATGTTTTTTTTGTGA	ACTAGACACAGGAATGCAAYGGCAA	C/T
KS19	146,364,681	GAGCATATTTGAATTGTAAGGTGATACC	CGAGCATATTTGAATTGTAAGGTGATACT	CCGCCACAAGCTWATGTTAATAGACAATA	G/A
KS30	141,338,119	AACAGCCAAAGCAACTAAAGCAAACTA	CAGCCAAAGCAACTAAAGCAAACTG	GTTGTTTGTACTAGKCGGGCATCAATT	T/C
ctg358-01	152,257,137	CGGGAAAGCTTATACAGCTCTCA	CGGGAAAGCTTATACAGCTCTCG	GCCAGCCTCTTGTGAACGCTATTAT	T/C
ctg358-37	151,680,633	CGACTTCCCCATTAACCATCCGA	GACTTCCCCATTAACCATCCGG	CGGTCCCGAATTTACAGTCGAACAA	TTT/-
ctg358-05	151,668,694	ATCTAATAGAGTTCTTGGAAATCTACGATA	CTAATAGAGTTCTTGGAAATCTACGATG	CAGAAACAGAACATTGTTAGTGCAAGACTA	A/G
ctg358-14	151,446,446	GCCGCACGAGGACGGGTC	GCCGCACGAGGACGGGTT	GAGCTTAAGCGCGTCAGTCCAAATT	G/A
ctg359-01	153,687,938	TCCCAGGCCACCAAAATGGTC	AATTTCCCAGGCCACCAAAATGGTT	CCCAAGGATTTGAGAAAGGGTTTTGAAAA	C/T

[a]Position from reference genome sequence of B73 (v3); SNP markers developed for KASP genotyping comprised a common primer and two allele-specific primers which were labeled with two different fluorophores: FAM[b] and HEX[c], respectively; [d]polymorphism between the Z033E0056 and Z032E0081 alleles which can be distinguished by the corresponding KASP marker

Table 2 Information of SSR and other markers

Primer name	Position (v3)[a]	Marker type[b]	Forward	Reverse
umc2199[c]	149,146,480	SSR	ACCGAGTTAAGATTACATCACGCC	TGTTTCCCCTAATAAAGCAAATGAA
umc2210[c]	152,001,123	SSR	GATGCTACCATTTCAGTGAGCGAT	AGCGGGTCGATCTTTCTCTTAGTT
umc1287[c]	156,640,498	SSR	ATGGGATGATCAGTCGTTTCAGTC	AGAAGGAGGCCCACTACGAGAG
ctg358-18[d]	151,442,614	Size	AACATCCAGGGCGAGTGTCT	AGCATCGGTTAGGGTTTCCA
ctg358-20[d]	151,528,279	Size	CAGCGTCCAACAACACATCC	ACGACCTCCGACTCCTACCC
ctg358-33[d]	152,129,338	Size	GCTTGCCTGTTCATCCTGGT	TCGCTCTTCCTTGACGCTTT
ctg358-32[d]	152,128,452	Sequence	CAACCTCTCCTCCCTCCAGA	CAGCGTTCCCTTCCAGTGA
P1[e]	152,243,515	Sequence	AAGCACTGTATGTCGGACGG	CCAGAGAGACGGTTCCCATC
P6[e]	152,240,171	Sequence	GACGGAGATGCAGATCGTTA	ATCCTCAAAACAAGTGCCCG

[a]Position from reference genome sequence of B73 (v3) of the 5′end of the forward primer

[b]SSR- simple sequence repeat; Size- alleles were distinguished based on their sizes assessed when run on a 1% agarose gel; sequence-alleles were distinguished based on direct sequencing of PCR products

[c]These primers are from MaizeGDB (http://www.maizegdb.org/)

[d]These primers are from Chung et al. (2010)

[e]The annealing temperatures of P1 and P6 are 68°C and 65 °C, respectively

by running the products on a conventional 4% agarose gel. The Z033E0056 and Z032E0081 products of three markers, ctg358-18, ctg358-20, and ctg358-33, that had previously been described as CAPS (cleaved amplified polymorphic site) markers in Chung et al. (2010) could be distinguished based on size alone when run 1% agarose gels. Finally, PCR products from two primers pairs we designed, P1 and P6, and ctg358-32 in Chung et al. (2010) were directly sequenced to distinguish the Z033E0056 and Z032E0081 alleles. Positions of all markers used in this paper are based on the reference genome sequence of B73 (v3).

Genotyping

PCR reactions for SSR genotyping (umc2199, umc2210, umc1287) were performed in total volumes of 15 µl, including 1.5 µl 10× buffer (DreamTaq Green buffer, Thermo Scientific, Cat# EP0713), 0.3 µl 10 mM dNTPs (Promega Corporation, Cat# U151B), 0.2 µl forward and reverse primers, 0.1 µl Taq DNA polymerase (DreamTaq Green DNA Polymerase, Thermo Scientific, Cat# EP0713), 1 µl 50 ng/µl DNA, and 11.7 µl distilled H_2O.

Touchdown PCR was employed for the detection of SSR markers. The PCR procedure was as follows: 95 °C for 5 min, 10 cycles of 95 °C for 30 s, 67–57 °C for 30 s (1 °C decrease per cycle) and 72 °C for 40 s, 35 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 40 s, with the final extension at 72 °C for 10 min. The PCR products of SSR markers were distinguished on 4% agarose gels.

PCR reaction conditions for ctg358-18, ctg358-20, ctg358-33, and ctg358-32 were the same as that of SSR markers while the PCR program used was the same as reported in Chung et al. (2010). The PCR products of ctg358-18, ctg358-20, and ctg358-33 were distinguished on 1% agarose gels, while PCR product of ctg358-32 was cleaned and sequenced to get polymorphic information between Z033E0056 and Z032E0081.

The KASP assays were run on 384-well plates and were set up in a total volume of 5.07 µl, consisting of 2.5 µl 20 ng/µl genomic DNA, 2.5 µl 2× KASP Master mix (Cat# KBS-1016-017), and 0.07 µl KASP Assay mix (KASP primers). Touchdown PCR was used for as follows: 94 °C for 15 min, 10 cycles of 94 °C for 20 s, 61–55 °C or 1 min (0.6 °C decrease per cycle), 25 cycles of 94 °C for 20 s, and 55 °C for 1 min. After PCR, end-point genotyping on a LightCycler 480 (Roche Molecular Systems, Inc.) was used to analyze the fluorescence of different genotypes of each KASP marker. Fluorescence can be clustered into three types: allele Z033E0056 type, heterozygous, or allele Z032E0081 type.

Markers P1 and P6 were amplified in 25 µl carrying 5 µl 5× Q5 reaction buffer (New England BioLabs Inc., Cat# M0491S), 0.5 µl 10 mM dNTPs (Promega Corporation, Cat# U151B), 1.25 µl forward and reverse primers, 0.25 µl Q5 high-fidelity DNA polymerase (New England BioLabs Inc., Cat# M0491S), 1 µl 50 ng/µl DNA, and 15.75 µl distilled H_2O. PCR program was applied as follows: 98 °C for 30 s, 30 cycles of 98 °C for 10 s, 68/65 °C for 30 s, and 72 °C for 30 s, with the final extension at 72 °C for 2 min. PCR products were cleaned using QIAquick® Gel Extraction Kit (Cat# 28704) and sequenced in Genomic Sciences Laboratory (North Carolina State University). Sequences of Z033E0056 and Z032E0081 were aligned to get polymorphism information.

Data analysis and QTL mapping

Statistical analysis was performed using SPSS 13.0 (SPSS, Chicago, USA). The genetic positions of the markers were assigned using the NAM genetic map (McMullen et al. 2009; Olukolu et al. 2014), and the physical positions were based on the B73 version 3 genome assembly (http://www.maizegdb.org/). QTL mapping was performed using Windows QTL Cartographer software v2.5 based on composite interval mapping (CIM, Silva et al. 2012). The walk speed was 1.0 cM. 1000 permutation tests at a significant level of 0.05 were performed to determine likelihood of odds ratio (LOD) threshold value for each trait.

Results

Dominance of the resistance allele at *Qgls8*

From our previous study (Lennon et al. 2016), we demonstrated that the two B73 NILs Z032E0081 and Z033E0056 carry introgressions from different teosinte accessions with different effects at *Qgls8* (Fig. 1). The allele from Z032E0081 conferred GLS resistance, while that from Z033E0056 conferred GLS susceptibility relative to the B73 allele. The effects of the Z032E0081 and Z033E0056 alleles measured in the Lennon et al. (2016) study were, respectively, 0.51 and −0.61 on the 1–9 scale employed. We produced F_1 plants from reciprocal crosses of Z032E0081 and Z033E0056 and assessed them for GLS resistance relative to each of its parents in replicated field trials in Andrews NC in the summer of 2016. The Z032E0081 and Z033E0056 parents had average scores of 6.25 and 4.13, respectively, while the F_1 had an average score of 5.88, indicating that the resistance allele is largely dominant at *Qgls8*.

Validation of *Qgls8*

Before fine mapping of *Qgls8*, we needed to verify that this QTL segregated and was detectable in an F_2 population derived from Z032E0081 (resistant) and Z033E0056 (susceptible). Using two markers PZA03612-2 and umc1287 that were predicted to flank *Qgls8* based on our previous work (Lennon et al. 2016), we screened 1273 F_2 plants and identified 279 individuals recombinant between these markers. These plants were selfed to produce 279 $F_{2:3}$ families (see Fig. 2). 74 of these 279 $F_{2:3}$ families were scored for GLS resistance in Andrews and Blacksburg, respectively, in the summer of 2015. The families were genotyped with 13 markers (PZA03612-2, KS3, KS4, KS30, PZA00429-1, KS20, KS19, umc2199, KS10, KS28, KS27, KS11, umc1287) dispersed evenly in the ~15 cM region. After analysis of the data from Andrews, a QTL associated with GLS resistance was identified in the *Qgls8* region with a peak at marker KS28 (see Ad2015 result in Fig. 3; Table S1). But there was no obvious QTL peak identified after analysis using phenotypic data from Blacksburg (VA2015 result in Fig. 3; Table S1). We concluded that the chromosome region defined by PZA03612-2 and umc1287 did harbor *Qgls8* but that the QTL appeared to be environment-specific.

Fine mapping of the GLS resistance QTL

142 independently derived homozygous recombinant plants were identified from the 279 recombinant $F_{2:3}$ families and genotyped with the markers PZA03612-2, KS3, KS4, KS30, PZA00429-1, KS20, KS19, umc2199, KS10, KS28, KS27, KS11, umc1287, in order to saturate the ~15 cM *Qgls8* region (Fig. 4). 142 independent homozygous recombinant F_4 lines were derived from these plants and were assessed for GLS resistance in randomized replicated field trials in Andrews NC and Blacksburg VA during the summer of 2016.

Similar to our observations from the analysis of the $F_{2:3}$ populations, the effect of *Qgls8* was not apparent in the data from Blacksburg, VA (compare Ad2016 and VA2016 results in Fig. 3), in fact even the phenotypes of the parental lines were not consistently distinct in Blacksburg VA (scores of Z032E0081 and Z033E0056 were 6.4 and 6, respectively, in Blacksburg compared with 6.3 and 4.1 in Andrews NC). This confirmed our conclusion that *Qgls8* was environment-specific, and therefore only phenotypic data from Andrews NC were used in the subsequent analyses. Using these data we were able to 'Mendelize' the trait; in other words, we were able to designate each line as either resistant or susceptible based on whether the phenotype was significantly different to the susceptible parent Z033E0056 (Fig. 4a). The 142 lines were divided into 23 classes, A through W, based on their recombinant types. Classes C and N were further sub-divided based on the fact that in each of these classes resistant and susceptible lines were found (Fig. 4a).

Referring to Fig. 4a, recombinant classes A and B are both resistant, class C is split into class C-1 (resistant) and class C-2 (susceptible), and classes D through M are susceptible. This implies that the causal gene underlying *Qgls8* is located in the region flanked by markers KS28 and KS27, where the C class recombination events were determined to have occurred. This conclusion was confirmed by the inspection of classes N through W. The recombination events defining class N also occur in the KS28–KS27 interval and again this class can be sub-divided into both resistant (class N-2) and susceptible (N-1) lines (Fig. 4a).

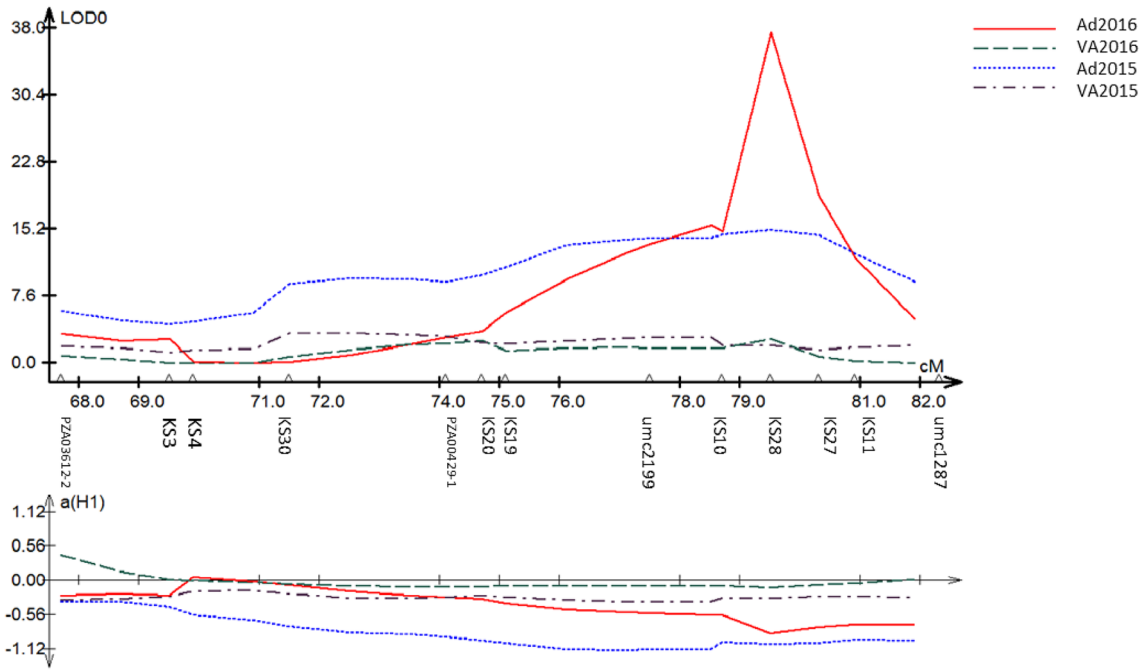

Fig. 3 Validation of *Qgls8* using 74 recombinant $F_{2:3}$ families in 2015 with one replicate in each location and 142 homozygous recombinant F_4 lines in 2016 with two replicates in each location. Each year the two locations used were Andrews, NC (noted above as Ad) and Blacksburg ,VA (VA)

Classes O through W are all resistant, again consistent with the positioning of *Qgls8* in the KS28-KS27 interval.

We developed or identified eight markers (ctg358-05, ctg358-37, umc2210, ctg358-32, ctg358-33, P6, P1, ctg358-01) located between KS28 and KS27 and used them to screen all the C-1, C-2, N-1, and N-2 class lines. With the addition of new markers, the C-2 and N-1 classes could be further sub-divided by recombination location (Fig. 4b). Inspection of these data (Fig. 4b) unequivocally places *Qgls8* in the ~130 kb region defined by ctg358-32 and ctg358-01. According to B73 reference genome sequence (v3), the ~130 kb region harbored five genes: GRMZM2G360219, GRMZM2G060886, GRMZM2G316907, GRMZM2G316904, and GRMZM2G014089 (Fig. 4c). Table 3 shows the information of five candidate genes. Both GRMZM2G360219 and GRMZM2G316907 were annotated as receptor-like protein kinase. GRMZM2G014089 was predicted as ABC transporter ATP-binding protein.

Discussion

GLS is a very serious foliar disease of maize. Yield losses could be up to 80–100% because of lodging caused by stalk deterioration during severe epidemics which impeded harvesting mechanically (Latterell and Rossi 1983; Danson et al. 2008). Cultivation of varieties resistant to GLS has been considered as an effective method to control GLS (Danson et al. 2008; Zhang et al. 2012). Numerous studies investigating the genetic basis of GLS resistance have concluded that resistance is based on numerous loci each with a small effect; in other words, GLS resistance in corn is inherited as a typical quantitative trait.

Here we report the fine mapping of a GLS QTL, *Qgls8* located in Bin 8.06 to a ~130 kb region defined by the markers ctg358-32 and ctg358-01 (152,128,452-152,257,137 bp on the B73 v3 genome). We set up the mapping population used in this study so that previously identified resistance and susceptibility alleles (both relative to the B73 allele) were segregating against each other. This had the intended effect of increasing the perceived effect at *Qgls8* and allowed us to categorize lines as either resistant or susceptible. The effect observed at *Qgls8* in the F_4 populations assessed at Andrews was −0.85 on our 1–9 scale (Table S1), compared to effects of 0.51 and −0.61 observed previously (Lennon et al. 2016) for the resistance and susceptibility alleles segregating against the B73 allele, respectively. While comparison of effects across environments and years is not entirely legitimate due to differences in disease pressure, scoring date etc., this does suggest that by producing a population in which these alleles segregated against each other, we were able to somewhat increase the contrast at *Qgls8*.

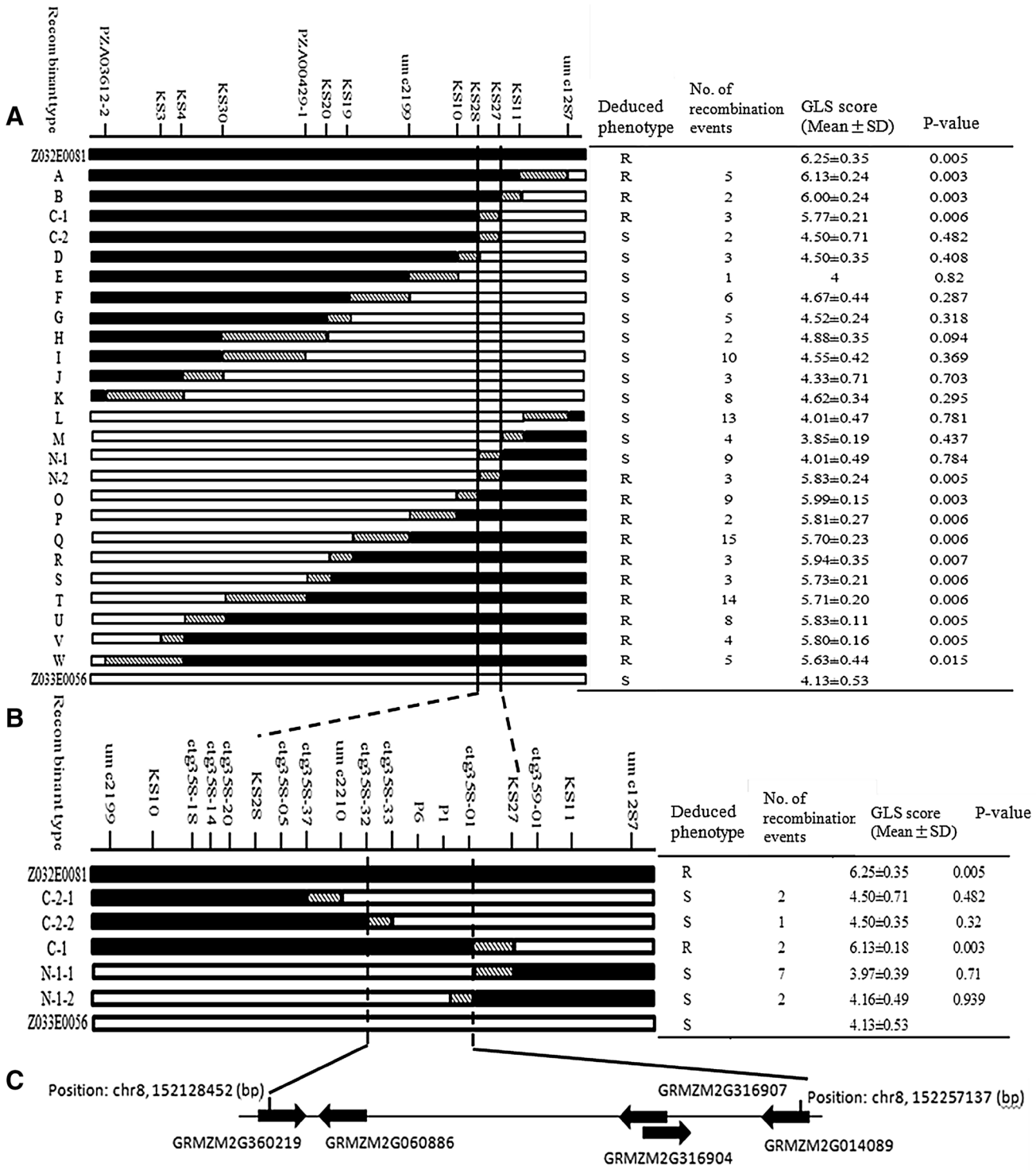

Fig. 4 Fine mapping of *Qgls8* using selected homozygous recombinant F_4 lines. *Black rectangle* represents homozygous genotypes of Z032E0081 (resistant); *White rectangle* represents homozygous genotypes of Z033E0056 (susceptible); *Gray rectangle* represents the region in which recombination occurred; Recombinant types C-2-1 (**b**) and C-2-2 (**b**) are both from the recombinant C-2 (**a**); Recombinant types N-1-1 (**b**) and N-1-2 (**b**) are both from the recombinant N-1 (**a**); The *P* value is the likelihood that the resistance of each line is not different to that of the susceptible parent Z033E0056; The position showed here is from reference genome sequence of B73 (v3) (**c**); *black arrows* in different directions represent different genes (**c**); the *left* and *right vertical lines* represent the positions of the markers ctg358-32 and ctg358-01, respectively

Table 3 Information of candidate genes underlying the ~130 kb region of *Qgls8*

Gene ID	Start–end (v3)[a]	Function annotation
GRMZM2G360219	152127054.0.152130466	Receptor-like protein kinase 2 precursor
GRMZM2G060886	152130931.0.152135975	CPuORF25—conserved peptide uORF-containing transcript expressed
GRMZM2G316907	152240805.0.152244210	Receptor-like protein kinase 2 precursor
GRMZM2G316904	152241660.0.152244983	Uncharacterized protein
GRMZM2G014089	152253211.0.152257798	ABC (ATP-binding cassette) transporter

[a]Position of the reference genome sequence of B73 (v3)

Several studies have identified GLS resistance QTL in the same region. A QTL for GLS resistance in the same region on chromosome 8 identified by Saghai Maroof et al. (1996) explained 7.7–11.0% of phenotypic variance using F_2 and $F_{2:3}$ populations derived from the cross between B73 and Val4. A consensus GLS resistance QTL was detected in bin 8.06 using meta-analysis based on data from 5 publications (Shi et al. 2007). Benson et al. (2015), using the extremely powerful 5000-line NAM population (McMullen et al. 2009), found one SNP (PZA03651.1) in bin 8.06 associated GLS resistance, about 5 Mb from the *Qgls8* region defined here.

It should be noted that flowering time has been reported to have an effect on perceived resistance levels to GLS (e.g., Zwonitzer et al. 2010). While we did not score flowering time in this study, previous studies using the original population in which *Qgls8* was identified have reported flowering time QTL at around 67 cM on chromosome 8 (NAM map coordinates) and have identified *ZCN8*, located at 123,030,387-123,032,175 bp (B73v3 assembly) as a possible causative gene (Lennon et al. 2016; Liu et al. 2016). This would place the flowering time locus as ~9 cM and ~30 Mbp from *Qgls8*, making it unlikely that *Qgls8* was influencing flowering time.

Resistance genes for several other diseases map near *Qgls8*. Chung et al. (2010) reported that $qNLB8.06_{DK888}$, a QTL for NLB resistance was found near *Qgls8*. The *Ht2* and *Htn1* genes for resistance to NLB are also found in this region (Zaitlin et al. 1992; Simcox and Bennetzen 1993). *Htn1* was recently shown to encode a wall-associated receptor-like kinase (Hurni et al. 2015). According to our data, *Htn1* is ~600 kb upstream of *Qgls8*, which partially overlaps $qNLB8.06_{DK888}$.

No other GLS resistance QTL have been as precisely defined as *Qgls8*. Zhang et al. (2012) identified two major QTL, *qRgls1* and *qRgls2* for GLS resistance on chromosomes 8 and 5, respectively, and finely mapped *qRgls1* to an interval of 1.4 Mb. In the subsequent study, Xu et al. (2014) narrowed the region of the second major QTL—*qRgls2* from an initial ~110 Mb to an interval of ~1 Mb which harbored 15 predicted genes. The NAM study performed by Benson et al. (2015) mapped two GLS resistance QTL on chromosome 1 to regions of 6.5 and 5.2 Mb. We have defined a relatively small ~130 kb region encompassing five predicted genes (Table 3). Of these five genes, three are similar to genes with defined roles in disease resistance: Both GRMZM2G360219 and GRMZM2G316907 encode receptor-like protein kinases. Proteins of this type function as pattern-recognition receptors (PRRs) involved in sensing microbe-associated molecular patterns (MAMP) and inducing MAMP-triggered immunity (MTI) (Newman et al. 2013). GRMZM2G014089 is predicted to be an ABC transporter ATP-binding protein. A protein of this type, LR34, regulates resistance to several fungal diseases in wheat (Krattinger et al. 2009). We continue to screen new recombinants and insertional mutants to definitively identify the causal gene at *Qgls8*. It should be noted that the ~130 kb region and the genes within it are all defined based on the sequenced B73 genome. However, in this study, both the resistance and the susceptibility alleles derive from teosinte, *Z. mays* ssp *parviglumis*. Gene complements are known to vary extensively between maize genotypes. For instance, comparisons of the B73 and PH207 maize genomes suggest that more than 2500 genes (about 8% of the total number) occur in only one or other of the genomes (Hirsch et al. 2016). It is therefore quite possible that the actual causal gene for *Qgls8* may not be any of the genes listed in Table 3. If that is the case, the *Qgls8* regions from the lines Z032E0081 and Z033E0056 will need to be sequenced to determine the identity of the causal gene.

In this study, we assessed GLS resistance in two different environments: Andrews, NC and Blacksburg, VA. But the effect of *Qgls8* was not apparent in Blacksburg, VA in the original lines and in $F_{2:3}$ families and F_4 lines (Fig. 3). In our previous study in which *Qgls8* was identified, only the Andrews, NC environment was used (Lennon et al. 2016). Quantitative traits in general are well known for often having environment-specific effects (e.g., Snape et al. 2007). Environment-specific QTL for GLS have been noted previously (Bubeck et al. 1993; Gordon et al. 2004; Balint-Kurti et al. 2008). The disease pressure in Andrews was much higher, which meant the disease appeared earlier and progressed more quickly. We only used artificial inoculation at the Blacksburg site. The Andrews site was also

more prone to morning mists, which are conducive to GLS development (Beckman and Payne 1982). The environment-specificity may have been due to any of these factors, a combination of them or to factors we have not considered. As we identify the causal gene, it will be extremely interesting to determine the reason for its conditional effects.

Author contribution statement XZ, QY, and PBK planned the research; XZ, ER, WT, QY, and PBK executed the field trials; XZ and QY performed the genotyping and developed the molecular markers; XZ and QY performed the analysis; XZ and PBK wrote the manuscript; and QY, ER, and WT edited the manuscript.

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Compliance with ethical standards

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

Research involving animal and human rights This article does not contain any studies with human participants or animals performed by any of the authors.

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