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ORIGINAL PAPER

QTL mapping of resistance to gray leaf spot in maize

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Abstract Gray leaf spot (GLS), caused by the causal fungal pathogen *Cercospora zeae-maydis*, is one of the most serious foliar diseases of maize worldwide. In the current study, a highly resistant inbred line Y32 and a susceptible line Q11 were used to produce segregating populations for both genetic analysis and QTL mapping. The broad-sense heritability (H^2) for GLS resistance was estimated to be as high as 0.85, indicating that genetic factors played key roles in phenotypic variation. In initial QTL analysis, four QTL, located on chromosomes 1, 2, 5, and 8, were detected to confer GLS resistance. Each QTL could explain 2.53–23.90 % of the total phenotypic variation, predominantly due to additive genetic effects. Two major QTL, *qRgls1* and *qRgls2* on chromosomes 8 and 5, were consistently detected across different locations and replicates. Compared to the previous results, *qRgls2* is located in a 'hotspot' for GLS resistance; while, *qRgls1* does not overlap with any other known resistance QTL. Furthermore, the major QTL-*qRgls1* was fine-mapped into an interval of 1.4 Mb, flanked by the markers GZ204 and IDP5. The QTL-*qRgls1* could enhance the resistance percentages by 19.70–61.28 %, suggesting its usefulness to improve maize resistance to GLS.

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Introduction

Gray leaf spot (GLS), caused by the causal fungal pathogen *Cercospora zeae-maydis*, poses a grave threat to maize production worldwide (Wang et al. 1998). The yield loss due to GLS ranges from 20 to 60 % (Donahue et al. 1991; Elwinger et al. 1990; Huff et al. 1988; Ward et al. 1997a), to as high as 100 % under severe epidemics (Donahue and Stromberg 1989; Latterell and Rossi 1983; McGee 1988). The fungus predominantly overwinters in diseased plant debris that remains in the soil (Payne and Adkins 1987), and conidia produced by the fungus are disseminated to corn plants by wind and rain splash (Denazareno et al. 1993; Donahue et al. 1991). Lesions caused by GLS first appear as small tan spots, which are not easily distinguished from lesions caused by other foliar pathogens of maize (Stormberg and Donahue 1986). Mature GLS lesions, typically run parallel to leaf veins (Ayers et al. 1984; Stormberg and Donahue 1986), are distinctly rectangular in shape with gray to tan color, which are readily distinguished from other foliar diseases of maize (Ward et al. 1999). Further expansion of GLS is favored by extended periods of high humidity and moderate

temperatures, resulting in severe leaf senescence and predisposing maize to stalk decay, which can lead to increased stalk lodging (Rupe et al. 1982). Reduced conservation tillage and application of chemical fungicides may diminish incidence of GLS, but both methods are neither economical nor environmentally friendly for grain production (Ward et al. 1997b). Thus, deployment of resistant hybrids would provide a cost-effective mean of controlling GLS.

Introgression of resistance gene/QTL from donors into elite maize germplasm is expected to be a feasible approach to develop resistant germplasm (Gordon et al. 2004). It was reported that most sources of GLS resistance inherited in quantitative manner (Gevers et al. 1994; Huff et al. 1988; Thompson et al. 1987; Ulrich et al. 1990). Numerous studies focused on heritability and QTL analysis of maize resistance to *Cercospora zeae-maydis* (Gordon et al. 2006; Ininda et al. 2007; Menkir and Ayodele 2005). Moderate to high heritability, controlled predominantly by additive gene action, was reported in maize resistance to GLS (Clements et al. 2000; Gordon et al. 2006). A large number of QTL for GLS resistance have been detected in different populations under various environmental conditions. Three major QTL located on chromosomes 1, 4, and 8 have been consistently identified in the F_2 population derived from the cross between B73 (susceptible) and Va14 (resistant) across three disease evaluations over 2 years and two generations (Saghai Maroof et al. 1996). Five QTL for GLS resistance, together with six QTL that were identified with maturity-dependent association to GLS severity, have been detected in populations derived from the susceptible parent FR1141 and the resistant parent 061 (Clements et al. 2000). Bulked segregant analysis in combination with amplified fragment length polymorphism (AFLP) technique identified three QTL loci on chromosomes 1, 3, and 5, which explained about 37, 10, and 11 % of the phenotypic variance, respectively (Lehmensiek et al. 2001). QTL located on chromosome arms 2L and 4L together explained 40–47 % of the total phenotypic variation in the $F_{2:4}$ population derived from the resistant inbred VO613Y and susceptible inbred Pa405 (Gordon et al. 2004). Five significant QTL were detected in bins 1.05, 2.04, 4.05, 9.03, and 9.05 in an advanced IBM population, and each of them was located to a region <3 centiMorgans (cM) (Balint-Kurti et al. 2008). Two potential GLS QTL 'hotspots' were identified in bins 1.05–1.06 and 2.03–2.05, compared with results from precious QTL analysis of GLS (Balint-Kurti et al. 2008). Three QTL located in bins 1.05, 1.07, and 3.07 that significantly reduced the GLS severity have been mapped and confirmed using near-isogenic lines (NILs) (Pozar et al. 2009). Additional QTL for GLS resistance have also been reported by other researchers (Danson et al. 2008; Juliatti et al. 2009). In summary, an integrated QTL linkage map for GLS resistance was constructed by compiling totally 57 QTL, in which 26 'real' and 7 'consensus' QTL were located in bins 1.06, 2.06, 3.04, 4.06, 4.08, 5.03 and 8.06 (Shi et al. 2007).

Although lots of putative QTL for GLS resistance have been identified and validated, no any QTL has ever been fine mapped or even cloned. In this study, a QTL analysis of maize resistance to GLS was performed, followed by fine mapping and genetic analysis of a major resistance QTL. In addition, a high-resolution linkage map was constructed for the major resistance QTL, which could be used to identify markers to clone the QTL or to introduce the QTL via MAS to improve maize resistance to GLS.

Materials and methods

Plant materials

A tropical inbred line Y32, developed from Suwan 1 (a tropical population in Thailand), was highly resistant to GLS and thus used as the donor parent; while a susceptible line Q11, derived from HL1999 (a temperate hybrid in America), was selected as the recurrent parent. All mapping populations were developed from the same cross of Y32 × Q11 (Fig. 1), including self-pollinated populations (F_2, F_3, and F_4) and a backcross population (BC_1F_4). In 2009, the F_2 population was grown at the experimental station in Kunming, Yunnan province, and the selective 161 F_2 individuals were self-pollinated to generate respective 161 $F_{2:3}$ families. In 2010, these 161 $F_{2:3}$ families were planted in two replicates at each of two locations, Dehong and Baoshan, Yunnan province, where the plants could be naturally infected by *Cercospora zeae-maydis*. Every F_3 individual was investigated for its phenotype in the field. Meanwhile, the $F_{2:3}$ families grown in Dehong were self-pollinated to produce the F_4 population. The molecular markers within a major QTL region were used to identify recombinants from the F_2 population, which resulted in 15 F_2 recombinants. During 2010/2011 winter nursery, a total of 1,149 F_4 individuals from the 63 F_3 plants which corresponded to the 15 F_2 recombinants were grown in Jinghong, Yunnan province. The F_4 plants were further backcrossed to the susceptible parent Q11 to generate BC_1F_4 progeny. In 2011, BC_1F_4 progeny derived from 27 F_4 recombinant individuals were planted in Baoshan, and investigation of both genotype and phenotype for every BC_1F_4 progeny was conducted to narrow down the QTL region.

GLS disease evaluation

Two parents and the 161 $F_{2:3}$ families were naturally evaluated for their resistance to GLS in two replicates at each of the two locations, Baoshan and Dehong, Yunnan province. These

four replications are thus represented as B1, B2, D1, and D2, respectively. The BC_1F_4 population was evaluated for GLS resistance in Baoshan. A scoring system with 5 scales (1, 3, 5, 7, and 9) was adopted to evaluate GLS resistance, and infected leaves corresponding to different scales were depicted in Fig. 2. The individuals with disease scales 1 and 3 were considered as resistant; whereas, those with scales 5, 7, and 9 were considered as susceptible. In an initial QTL mapping in 2010, each F_3 individual was independently scored in 15 days post pollination; while, in QTL fine mapping in 2011, each BC_1F_4 progeny was scored three times in 7, 15, and 30 days post pollination. In addition, to explore a more accurate phenotyping method, we picked up the leaf at ear node from every F_3 individual for scanning by the scanner. Thereafter, we analyzed the pictures by the software namely 'Compu Eye, Leaf & Symptom Area' (Bakr 2005), by which the total leaf area and the area of any visible symptom on the leaf could be measured. Finally, we assessed the percentage of lesions caused by GLS on the whole leaf for all picked leaves, which were used to represent the disease severity and subjected to QTL analysis.

Analysis of phenotypic data

Variance of resistance was estimated by PROC GLM in SAS 8.02 software (SAS Institute 1999). The model for variance analysis was $Y = \mu + \alpha_G + \beta_L + (\alpha\beta)_{GL} + \varepsilon_{GLR}$, in which α_G represented the effect of Gth line, β_L was the effect of the Lth location, $(\alpha\beta)_{GL}$ was the effect of the line by location interaction, and ε_{GLR} was the residual effect. All effects were considered to be random. Broad-sense heritability (H^2) was estimated as $H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_{GL}^2/R + \sigma_e^2/LR)$ (Knapp et al. 1985), where σ_G^2 was the genetic variance, σ_{GL}^2 was an interaction variance of genotype by location, σ_e^2 was the residual error, R was the number of replications, and L was the number of locations.

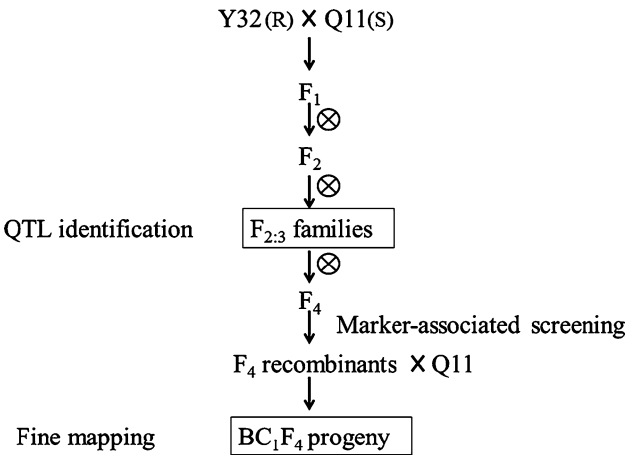


Fig. 1 Experimental flow chart of QTL identification and fine mapping. Both $F_{2:3}$ families and BC_1F_4 progeny in the *boxes* were individually evaluated for resistance to GLS, The $F_{2:3}$ families were used for QTL identification; while, the BC_1F_4 progeny were used for fine mapping of the major resistance QTL-*qRgls1*

Genotyping

DNA extraction was performed according to the method described by Murray and Thompson (1980), using immature leaf tissue collected from each plant from all mapping populations. The SSR and STS markers retrieved from the Maize Genetics and Genomics Database (http://www.maizegdb.org/) or newly designed in the current study were synthesized by Invitrogen (Beijing, China). SSR markers polymorphic for two parents were used to construct a linkage map. PCR products were separated by electrophoresis on either 2 % agarose gel or 6 % polyacrylamide gel.

Construction of a linkage map and detection of QTL for resistance to GLS

Linkage map was constructed by MAPMAKER3.0 with polymorphic SSR markers between two parents (Lincoln et al. 1992). Linkage groups were identified using the 'Group' command that was used to identify linkage groups with a logarithm of odds (LOD) score of 3.0, and recombination frequency was converted into cM using the Kosambi mapping function (Kosambi 1944). QTL detection was performed using the composite interval mapping method (CIM) (Zeng 1994) as in the QTL cartographer (version 2.5) (Basten et al. 1997). A significant threshold for confirming a putative QTL was obtained from 1,000 permutations at $P < 0.05$ for each data set.

Development of PCR-based markers

As those SSR markers retrieved from the Maize Genetics and Genomics Database were not sufficient to construct a relative high-resolution linkage map, new SSR markers were designed to saturate the linkage map. In addition, the QTL-*qRgls1* detected in the initial QTL mapping covered the confidence interval between SSR markers phi420701 and GZ34 with the physical distance of 23 Mb according to the B73 whole-genome physical map. So, high-density markers are needed to narrow down the QTL locus. Firstly, BAC sequences which were used to develop new markers in the QTL region were downloaded from the website (http://www.maizesequence.org/index.html). The sequences retrieved were first scanned by the software SSRHunter1.3 (Zeng 1994) to mine simple-sequence-repeats (SSRs). These SSR sequences were then compared with the MAGI database (http://magi.plantgenomics.iastate.edu/) to find

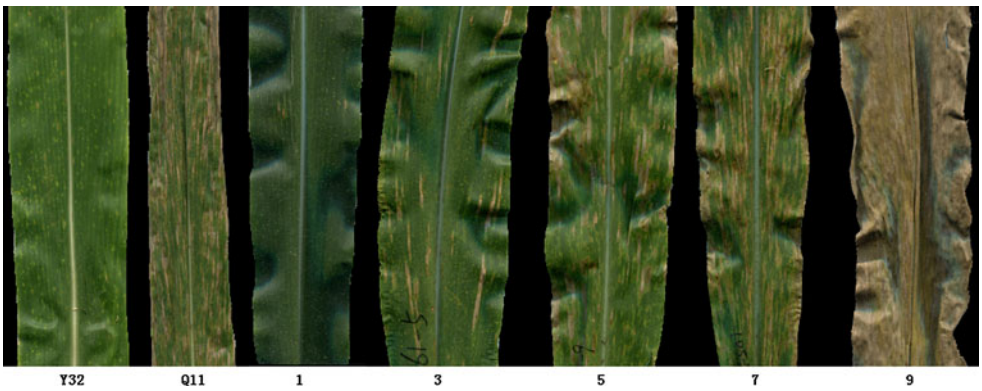


Fig. 2 Symptoms of GLS for parental lines and leaves with disease scales 1–9. The criteria of scales for GLS disease rating in the field is based on percentages of lesion area: scale *1* 0–5 %, scale *3* 6–10 %, scale *5* 11–30 %, scale *7* 31–70 %, and scale *9* 71–100 %

those single-/low-copy sequences to design primers by the software Primer5.0 with the following criterion: ~20 nucleotides with 40–60 % GC content, no consecutive tracts of a single nucleotide, and no secondary structure. Once the PCR products showed polymorphism between the two parent lines Y32 and Q11, the development of a SSR marker was deemed successful.

In addition, sequence-tagged sites (STS) and cleaved amplified polymorphic sequence (CAPS) markers have also been exploited to develop new polymorphic markers. Firstly we masked the repetitive sequences for downloaded sequences using the software RepeatMasker (http://www.repeatmasker.org/). Then primers were designed on those single-/low-copy sequences to amplify both parental lines. The PCR products of two parents were subjected to sequencing. The sequenced amplicons were compared with the B73 genome sequence using CLUSTALX to ensure that the correct sequence was obtained. Thereafter, pairwise multiple alignments between two parents were performed by DNAMAN software to find out all polymorphic sites. Single nucleotide polymorphism (SNP) that was related to a particular common restriction site could be used to design CAPS, and insertion/deletions (InDels) were used to develop STS markers. In an attempt to find out more InDels, we predicted putative genes in the single-/low-copy sequence using tools in software SoftBerry (http://linux1.softberry.com/berry.phtml), and designed primers in either 5′- or 3′- ends of the predicted genes as high-level polymorphism is usually present in these regions of a gene.

Fine mapping of the major QTL for GLS resistance

Recombinants screened from the F_4 population were backcrossed to Q11 to generate backcross progeny, which were planted in the field to score their disease severities. The backcross progeny from a single recombinant was divided into two subgroups based on their genotypes, homozygous (Q11/Q11) and heterozygous (Q11/Y32) genotypes, at the QTL region. Significant difference in disease severity between two subgroups was tested using a two-way ANOVA, followed by a *t* test. The significant ($P < 0.05$) or no significant ($P > 0.05$) difference in GLS resistance between two subgroups indicated the presence or absence of the resistance QTL in the donor region. The phenotype of a recombinant was deduced as resistant if it harbored the resistance QTL or susceptible if it did not harbor the resistance QTL. The availability of both donor size and deduced phenotype for each recombinant allowed fine mapping of the resistance QTL (Yang et al. 2012).

Results

Phenotypic analysis

Maize resistance to GLS was evaluated for each individual in $F_{2:3}$ families as well as two parental lines in Baoshan and Dehong in 2010. The resistant parent Y32 showed highly resistant to GLS in Baoshan and Dehong, rated as scales 1 and 1.5, respectively. The susceptible parent Q11 was highly susceptible to GLS, rated as scale 7 in both Baoshan and Dehong. The average scale of each $F_{2:3}$ family was used to represent the disease scale of its corresponding F_2 individual (Fig. 3). In the F_2 population, no bimodal distribution in disease severity was observed, indicating that GLS resistance did not fit the genetic model of a single dominant gene. In contrast, the continuous distribution from highly resistant to complete susceptible implied that maize resistance to GLS was quantitatively inherited in nature.

In the backcross progeny developed from a F_4 recombinant, there are two genotypes at the QTL-*qRgls1* region, homozygote (without Y32 donor segment) and heterozygote (with Y32 donor segment). For those F_4 recombinants with the resistance QTL-*qRgls1* in their donor segments,

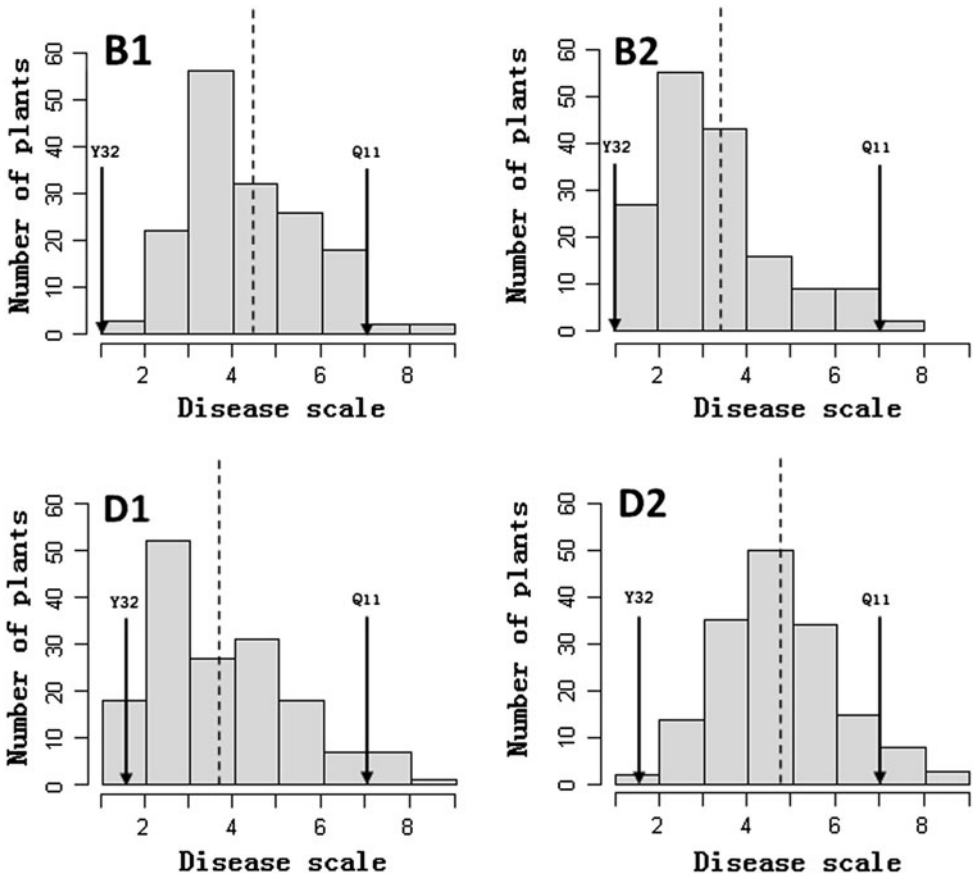


Fig. 3 Distribution of GLS disease scales in the F_2 population. The *vertical dash line* indicates an average disease scale of the F_2 population. Two *vertical solid lines* represent the disease scales of two parental lines. *B1* replicate #1 in Baoshan, *B2* replicate #2 in Baoshan, *D1* replicate #1 in Dehong, *D2* replicate #2 in Dehong

the frequencies of resistant plants ranged from 39.27 to 92.11 % for heterozygotes and from 9.3 to 72.41 % for homozygotes. However, for those F_4 recombinants without the resistance QTL-*qRgls1* in their donor segments, homozygote and heterozygote showed no significant difference in resistance to GLS.

Genetic heritability of maize resistance to GLS

Statistical analysis on the average scales of GLS resistance for 161 $F_{2:3}$ families in four replications indicated the presence of significant difference among genotypes and between locations (Table 1). The broad-sense heritability for GLS resistance was estimated to be as high as 0.85, indicating that the phenotypic variance was predominantly controlled by genetic factors in the F_2 population.

Construction of the linkage map

The F_2 population, derived from Y32 and Q11, was selected to construct the linkage map. A total of 816 SSRs were screened for polymorphisms between the two parents, and 183 of them were polymorphic markers and thus used to genotype the F_2 population. A linkage map was constructed using MAPMAKER3.0, which included 10 linkage groups and spanned a total of 1,667.9 cM in genetic distance with an average 9 cM every two adjacent markers. Since these SSR markers were evenly distributed on 10 chromosomes, the linkage map was suitable for QTL detection.

Initial QTL mapping for GLS resistance

Four QTL for resistance to GLS were detected in the F_2 mapping population, which could totally explain 44.19–66.83 % of the total phenotypic variation (Figs. 4a, 5; Table 2). The resistance alleles at *qRgls1*, *qRgls2*, and *qRgls4* were derived from the resistant parent Y32; while, the resistance allele at *qRgls3* originated from the susceptible parent Q11. The QTL-*qRgls1* was consistently present in four replications and could account for 10.87–20.34 % of the total phenotypic variation. The confidence interval was the exactly same among B1, B2, and D1 replication (bins 8.01–8.03) and overlapped with that detected in D2 replication (bins 8.02–8.05) (Fig. 4b). The second QTL-*qRgls2* located in bins 5.03–5.04 was detected in three replications (B1, B2, and D2) and explained 18.93–23.90 % of the total phenotypic variation. The QTL-*qRgls4*, located in bins 2.05–2.09, was only detected in the B2 replication, which could account for 11.86 % of the

Table 1 Analysis of variances for GLS resistance

Sources	*df*	SS	MS	*F* value	Pr ($>F_{0.001}$)	Significance
Genotype (G)	160	1,025.2	6.41	5.606	<2.2e−16	***
Location (L)	1	35.58	35.58	31.1285	5.14e−08	***
Replication	1	0.02	0.02	0.0173	0.8954	
G × L	160	150.07	0.94	0.8206	0.9209	
Residual error (σ)	321	366.89	1.14			

*** Significance at $P < 0.001$

df degree of freedom, *SS* sum of squares, *MS* mean squares, *Pr* probability

total phenotypic variation. The QTL-*qRgls3*, located in bins 1.09–1.11, was detected in D1 and D2 replications and could only explain 2.53–10.73 % of the phenotypic variation. In addition, resistance to GLS conferred by *qRgls1*, *qRgls2*, and *qRgls4* was predominantly controlled by additive gene action, and the resistance controlled by *qRgls3* showed both additive and dominant gene action.

To confirm the genetic effect of QTL-*qRgls1*, we selected the closest marker, um1913, to identify three genotypes: homozygous Q11/Q11, heterozygous Q11/Y32, and homozygous Y32/Y32, in the F_2 population, and calculated resistance scales at these three genotypes (Fig. 6). In all four replications, the homozygous genotype Y32/Y32 showed the highest resistance to GLS, followed by the heterozygous genotype Q11/Y32, and the homozygous genotype Q11/Q11 displayed the lowest GLS resistance. The evidence strongly indicated the presence of a valid resistance QTL in bins 8.01–8.03.

An attempt was also made to detect the QTL using the phenotypic data gained from scanned leaves and unfortunately this resulted in obscure and controversial QTL loci. It seems that the scanned data could not be readily used in QTL analysis for GLS resistance.

Saturation of the *qRgls1* region with newly developed markers

To resolve all recombinants for fine mapping of the *qRgls1* locus, high-density markers need to be developed in the *qRgls1* region. In the current work, as many as 1,054 simple-sequence repeats (SSRs) were obtained by mining the B73 genome sequence within the confidence interval of *qRgls1*. Compared with the MAGI database, 583 of the 1,054 SSRs were low-copy sequences. Of the 583 low-copy SSR sequences, 337 were suitable to design SSR primers and 38 of them showed polymorphic for two parents. Finally, only 14 SSR markers were used to saturate the linkage map and to resolve recombination breakpoints (Table 3). The remaining 24 SSR markers were either redundancy or tightly linked to the above 14 SSR markers, and thus they were not used in the current study. In addition, we sequenced 84 single-/low-copy sequences to develop STS or CAPS markers to saturate those segments in the *qRgls1* region lacking of sufficient markers. This resulted in two STS markers based on two InDels between two parental lines (Table 3).

Fine mapping of *Rgls1*

In the initial QTL mapping, QTL-*qRgls1* was detected within a 23 Mb confidence interval in bins 8.01–8.03. Thereby, two flanking markers, phi420701 and GZ34, were used to screen the recombinants in the *qRgls1* region from the F_4 population. The resultant 188 recombinants were backcrossed to Q11 to develop respective BC_1F_4 progeny. Moreover, another 16 markers (GZ199, GZ204, IDP2, IDP5, GZ92, GZ9, GZ175, bnlg2235, phi119, GZ102, GZ193, GZ123, umc1913, GZ126, GZ30, and GZ164) equally distributed (1–2 Mb between two adjacent markers based on the B73 genome) within the *qRgls1* region were further used to genotype the 188 recombinants, resulting in 15 genotypes based on their donor sizes. A total of 2,004 BC_1F_4 progeny derived from the 27 recombinants which covered all 15 genotypes were planted in Baoshan in 2011 (Fig. 7). The backcross progeny of each recombinant was divided into two genotypes, homozygous Q11/Q11 and heterozygous Q11/Y32, and the resistance percentage was calculated for each genotype. Of these 15 types of recombinants, types II, III, IV, V, VI, VII, VIII, and IX showed no significant difference ($p > 0.05$) between their homozygous (Q11/Q11) and heterozygous (Q11/Y32) BC_1F_4 progeny, indicating absence of *qRgls1*. In contrast, types I, X, XI, XII, XIII, XIV, and XV recombinants exhibited significant difference ($p < 0.05$) between homozygous and heterozygous genotypes, suggesting the presence of *qRgls1* in the donor regions. The types I, II and IX had the closest recombination breakpoints to the *qRgls1* locus. The resistant type I recombinants had recombination breakpoints downstream of GZ204, and the susceptible type IX recombinants had recombination breakpoints upstream of IDP2. These findings clearly restricted the *qRgls1* locus downstream of GZ204. The type II recombinant was susceptible to GLS and had a breakpoint between IDP2 and IDP5, indicating the *qRgls1* locus was located upstream of IDP5. The other recombinant types showed perfect match between the donor regions and deduced phenotypic performance. In other words, the presence/absence of the donor region between GZ204 and

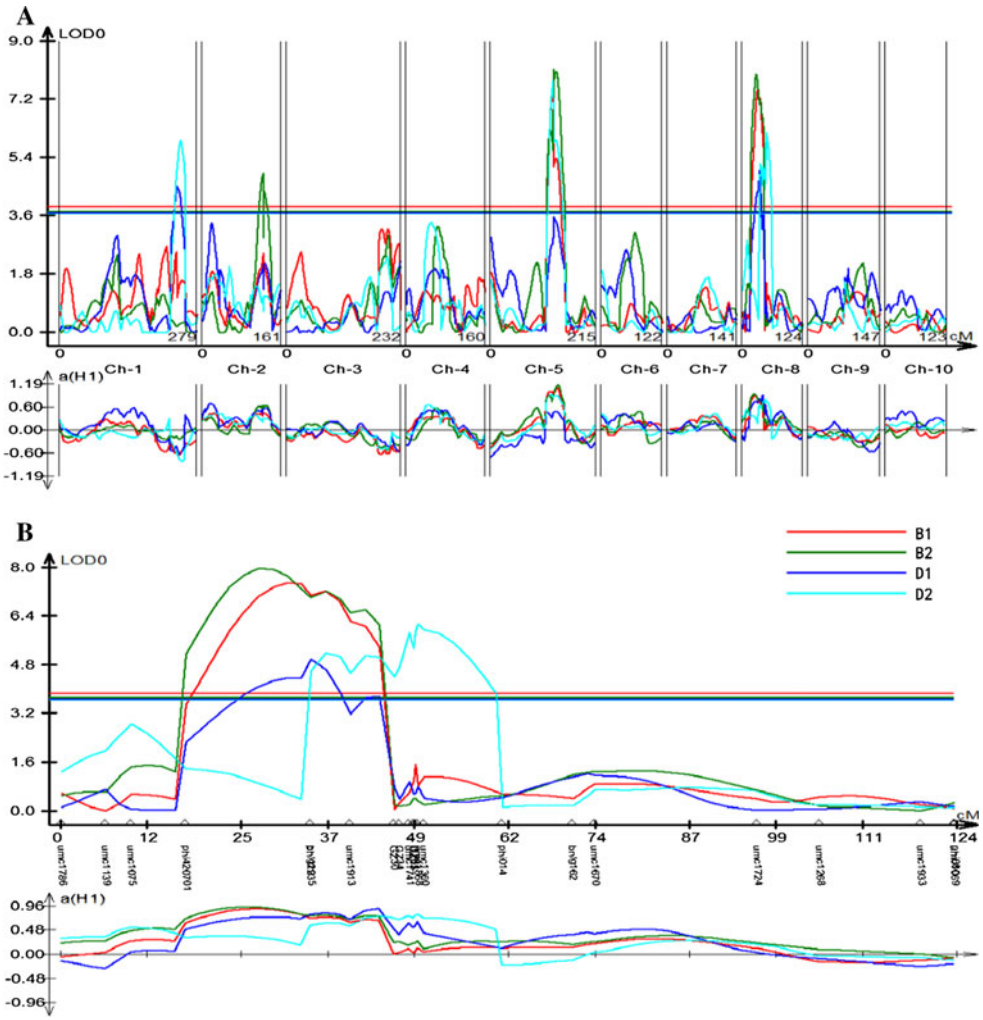


Fig. 4 Detection of GLS resistance QTL in four replicate plots. **a** Log of odd (LOD) profiles and additive genetic effects of the QTL for GLS resistance. QTL detection was conducted based on data collected from the 161 $F_{2:3}$ families which were grown in Baoshan (*B1* and *B2*) and Dehong (*D1* and *D2*) in 2010. **B** Log of odd (LOD) profiles and additive genetic effects of the major QTL-*qRgls1*. The QTL-*qRgls1* was commonly present in four replications based on data collected from the 161 $F_{2:3}$ families in Baoshan (*B1* and *B2*) and Dehong (*D1* and *D2*) in 2010

IDP5 rendered recombinants resistance/susceptible to GLS disease. Thus, the *qRgls1* locus was narrowed down to a region flanked by the markers GZ204 and IDP5 with the physical distance of 1.4 Mb according to the B73 reference sequence. The genetic effect of QTL-*qRgls1* could be calculated by subtracting the resistance percentage of homozygous genotype from that of heterozygous genotype, and the result showed that *qRgls1* could enhance the resistance percentage by 19.70–61.28 %.

Discussion

Considering that there was no standard method for artificial inoculation of maize GLS and artificial inoculation was usually unreliable in eliciting uniform infection across plants, we chose natural infection in Baoshan and Dehong, Yunnan province, where GLS was prevalent due to high humidity and moderate temperatures. According to resistant performance in years 2010 and 2011, the susceptible parent Q11 was highly infected by *Cercospora zeae-maydis* and the resistant parental line Y32 showed no GLS symptom. Moreover, the genetic effect of the QTL-*qRgls1* could be steadily observed across years 2010 and 2011. It could be concluded that the natural infection method was feasible and our visual scoring system works well in QTL analysis of GLS disease resistance. To our surprise, the phenotypic data based on scanning the GLS lesions seemed less valuable in QTL analysis. The scan method and the software should be no problem to assess the percentage of the GLS lesions, the reason why this method did not work can be attributed to the way we picked up leaves. Since we only picked up one leaf at ear node from each plant, in many cases a single leaf was not sufficient to represent the performance of a whole plant. Surely, the phenotypic data would be more reliable if all leaves of a plant are subjected to scanning. However, this could not happen considering enormous work and the negative impacts on normal plant growth. Above all, the visual disease scoring was very simple and straightforward, moreover, it could represent the performance of a plant for GLS resistance.

GLS resistance was previously reported to be moderate to highly heritable, controlled predominantly by additive

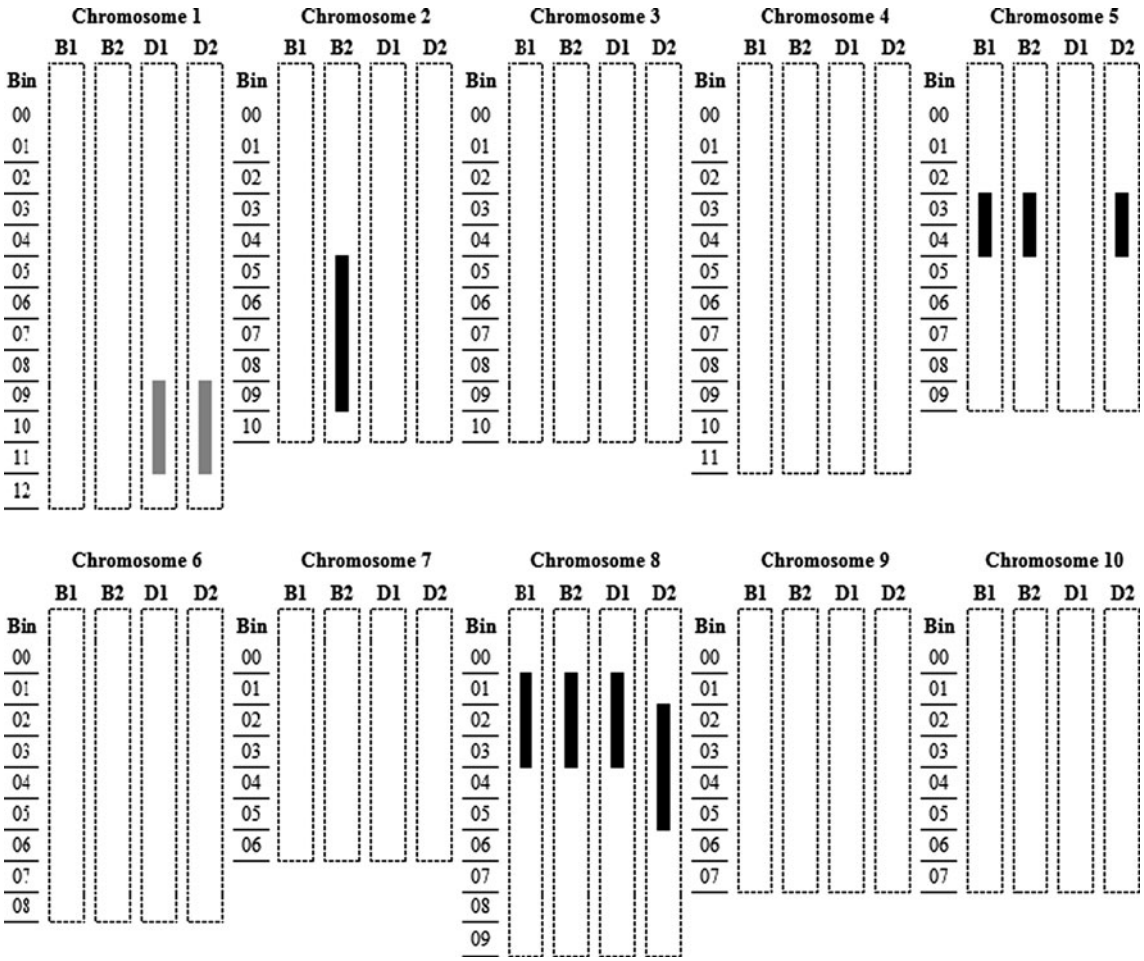


Fig. 5 Distribution and positions of all QTL for GLS resistance detected in four replicate plots. The *black bars* represent those resistance QTL derived from the resistant parent Y32; while the *gray bars* correspond to the resistance QTL derived from the susceptible parent Q11. The *bar lengths* correspond to the confidence intervals

Table 2 Parameters associated with quantitative trait loci (QTL) for GLS resistance identified in the F_2 population

Name	Chr.	Bins	R	Position (cM)	Flanking markers	Support interval (cM)	LOD	Additive	Dominant	SRA	R^2 (%)
qRgls1	8	8.01–8.03	B1	31.2	phi420701-GZ34	17.2–46.8	7.51	0.8667	−0.0751	Y32	18.01
		8.01–8.03	B2	27.2	phi420701-GZ34	17.2–46.8	7.98	0.9495	−0.2960		20.34
		8.01–8.03	D1	34.5	phi420701-GZ34	17.2–46.8	5.00	0.8105	−0.0307		10.87
		8.02–8.05	D2	49.3	bnlg2235-phi014	34.5–61.0	6.17	0.8055	−0.1247		14.54
qRgls2	5	5.03–5.04	B1	125.7	umc1171-bnlg1046	112.8–151.9	7.74	1.0083	−0.4010	Y32	23.90
		5.03–5.04	B2	127.4	umc1171-bnlg1046	112.8–151.9	8.15	0.8852	−0.0851		18.93
		5.03–5.04	D2	125.7	umc1171-bnlg1046	112.8–151.9	7.78	0.9341	−0.1910		20.24
qRgls3	1	1.09–1.11	D1	34.8	umc1500-bnlg1720	20.8–53.4	4.52	−0.4006	−0.5513	Q11	2.53
		1.09–1.11	D2	28.8	umc1500-bnlg1720	20.8–53.4	5.92	−0.7388	−0.2279		10.73
qRgls4	2	2.05–2.09	B2	122.0	umc2253-umc1551	96.9–140.9	4.93	0.6513	−0.3674	Y32	11.86

R^2 percentage of the phenotypic variance explained by the QTL, *SRA* source of resistance allele, *R* replication

gene action (Clements et al. 2000; Gordon et al. 2006). In accordance with these findings, the broad-sense heritability for GLS resistance was estimated to be as high as 0.85 and the additive gene action dominated GLS resistance for all QTL in the current study. In the initial QTL mapping, a significant threshold to declare a putative QTL for each data set was obtained from 1,000 permutations at $P < 0.05$, and this automatically generated the threshold LOD value

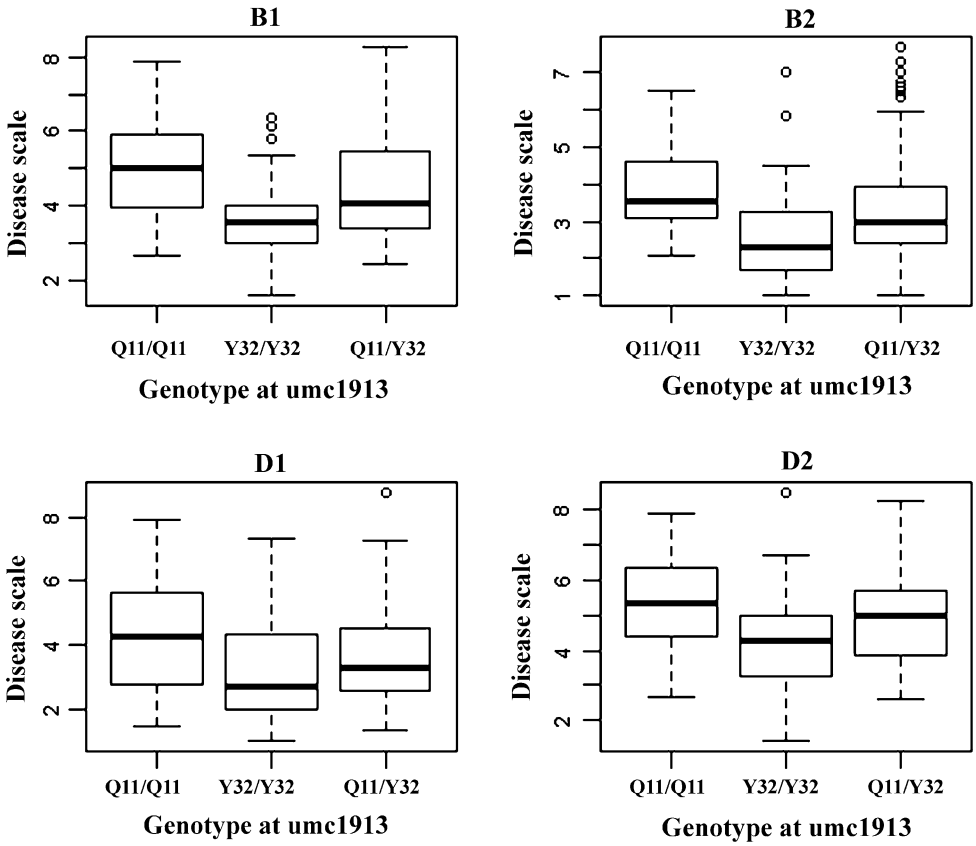


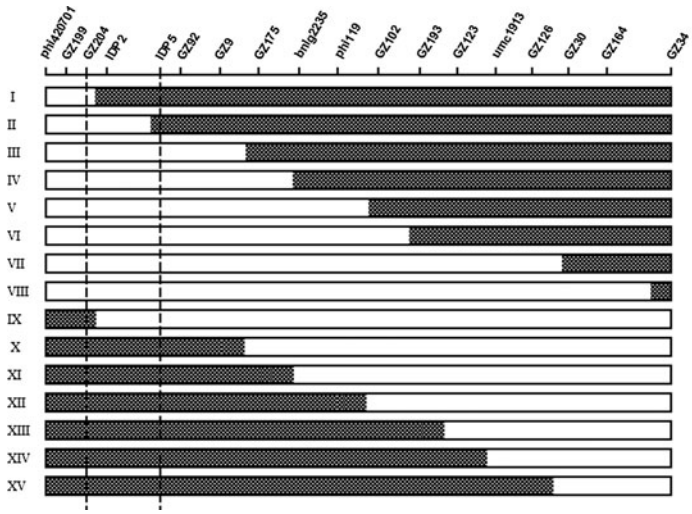
Fig. 6 *Box-and-whisker plots* of GLS disease scale at the major QTL-*qRgls1* in the F_2 population. The F_2 individuals were genotyped at the marker umc1913 that is closely linked to *qRgls1* and the distributions and mean values of disease scales were depicted for the three genotypes: homozygous Q11/Q11, heterozygous Q11/Y32, and homozygous Y32/Y32, in four replicate plots

Table 3 The newly developed molecular markers in the *qRgls1* region on chromosome 8

Location (Mb)	BAC name	Markers	Forward primer (5′–3′)	Reverse primer (5′–3′)	Type
8.47	AC187287	GZ199	CCGACCATGAGGGAGTAC	TTGACGCAAGGAGAAACG	SSR
8.61	AC197020	GZ204	ACGAAGTGGGAAGGGAGA	GTGCCTGTGACAGCAACC	SSR
8.88	AC199563	IDP2	ACCAGATGGCAGTTACCTTA	GTAGATGCAACCTCGCTTT	IDP
10.07	AC195361	IDP5	GAGACAATGAAGGCAGAT	TTGTGGACCAACTATGAG	IDP
10.29	AC186894	GZ92	TCAAACGTGGCCTCGGTATA	TGAACGGGCTTCACTAGGAG	SSR
11.39	AC214754	GZ9	TTCATCGCCAAGAAACTCAA	CGGAAACAATTCTCCTCCAC	SSR
12.93	AC214105	GZ175	TTAGGAGTTCCGCCTGACCG	TGGTAGCCGAAGCTGTTGACC	SSR
15.20	AC212454	GZ102	TGCTTGTATCTGAATGTCGTCGTG	CTTGCTGTGCTGCGTGTTGC	SSR
16.77	AC211474	GZ193	CTCTGACGGCAGTGGTACTAAA	ATGTCTGCAATATGCCCAAC	SSR
17.24	AC200500	GZ123	GCAGCCAGCAGTAGCCAACA	TGCAAGGCAGTGACCGAAAA	SSR
19.31	AC215231	GZ126	CTGGATCAAGGCAAGGAACA	CGCCCAAACCTACTACTAGAACTG	SSR
20.95	AC187096	GZ30	TTGTTGTCAGCAAGGTCGTG	GTCGCAGCTAACCTGGTCGA	SSR
21.86	AC194455	GZ164	CTACCAACAAGAAGATGCGTGAA	AGCGGCAAGGCAGGAAGTGA	SSR
23.77	AC198503	GZ34	GTCGGCTTCTTCTCCATCCTTT	GTCGCCCTTCGTTCACCTCT	SSR
37.04	AC195985	GZ55	ATTTATAGGACACCAACGCTAC	CTGAACTATGCGAGATTTGC	SSR
63.27	AC215498	GZ81	GCACGCCACAAGGATCAAGC	CACCGAGGAGTCACGCCAGT	SSR

Location (Mb): the physical location according to the B73 reference genome sequence

at 3.6. Based on this criterion, the QTL-*qRgls2* was detected in three replications B1, B2, and D2, but not in D1, however, a peak at LOD value of 3.4 did appear in D1 between markers umc1171 and bnlg1046. If we set the LOD value at 2.5 as threshold, the QTL-*qRgls2* can be detected in all the four replications, which further support



Deduced phenotype	No. of recombinants	Marker	Resistance percentage (%) Homozygote	Heterozygote	p-Value
R	2	GZ92	41.31±3.67	82.56±4.45	3.43E-06
S	1	GZ92	63.33	66.00	0.78
S	1	GZ175	96.55	90.00	0.16
S	2	bnlg2235	51.57±11.05	59.22±19.10	0.18
S	1	GZ102	39.53	44.83	0.59
S	2	GZ193	66.57±1.66	75.73±3.39	0.63
S	4	GZ30	36.10±33.70	37.80±31.77	0.12
S	1	GZ34	96.23	91.30	0.79
S	2	phi420701	7.03±5.78	10.94±15.47	0.94
R	2	GZ9	15.96±11.57	64.27±24.34	1.32E-09
R	3	GZ175	24.47±15.68	77.14±5.60	2.49E-16
R	3	phi420701	40.95±30.90	60.33±21.50	2.54E-03
R	1	phi420701	75.00	89.74	0.03
R	1	phi420701	9.52	39.29	2.38E-04
R	1	phi420701	43.18	72.34	8.40E-03

Fig. 7 Fine mapping of the major QTL-*qRgls1* based on recombinant-derived progeny. The 27 F_4 recombinants were fallen into 15 genotypes based on their Y32 donor regions. The genetic structure for each genotype is depicted as *black filled* and *white rectangles*, corresponding to heterozygous Q11/Y32 alleles and homozygous Q11/Q11 alleles, respectively. The resistance percentages of heterozygotes and homozygotes in progeny are listed in the *table* (on the *right*). Significant difference ($P < 0.05$) between the two genotypes indicates the presence of *qRgls1* in the donor region, and thus their parental recombinant(s) are deduced to be resistant (*R*). No significant difference ($P > 0.05$) between the two genotypes suggests a lack of *qRgls1* in the donor region, and thereby, their parental recombinant(s) is deemed to be susceptible (*S*). Analysis of both the donor regions and the phenotypes for all recombinant types enable to narrow *qRgls1* down to 1.4 Mb between the markers GZ204 and IDP5

the existence of QTL-*qRgls2* on chromosome 5. Considering it was more accurate to use the threshold automatically generated from 1,000 permutations at $P < 0.05$ to detect authentic QTL, we simply gave up setting 2.5 as the threshold LOD value. The presence of the QTL-*qRgls2* was also supported by other studies. The QTL for GLS resistance in bin 5.03 has been recognized as a consensus QTL using meta-analysis approach (Shi et al. 2007).

The QTL-*qRgls1* on chromosome 8 was initially mapped in four replications, in which *qRgls1* in B1, B2, and D1 shared the same confidence interval, and overlapped with that in D2 (Fig. 4b). In fine-mapping step, *qRgls1* was narrowed down to an interval of 1.4 Mb flanked by the markers GZ204 and IDP5. Intriguingly, the fine-mapped region was no longer overlapped with the *qRgls1* confidence region in D2. These findings suggested that the *qRgls1* interval from the initial QTL mapping in D2 was inaccurate. Since the genotypic data were identical across four replications, the wrong location in D2 may be due to inaccurate phenotype. This also reminds us that accurate phenotype is critical to successful QTL detection, and fine-mapping process is indispensable to declare an authentic QTL.

The QTL-*qRgls4* on chromosome 2 was only detected in B2, covering bins 2.05–2.09, where a consensus QTL for GLS resistance has previously been identified in bin 2.06 (Shi et al. 2007). The QTL-*qRgls3* on chromosome 1 was detected in D1 and D2, and the resistance allele was derived from the susceptible parent Q11. It is common that the susceptible parent harbors resistance alleles at some QTL loci, however, the genetic effect is normally weak for these resistance alleles, and the accumulative effects are not powerful enough to curb the disease.

The success toward fine mapping of a QTL mainly depends on high-density markers, sufficient recombinants, and accurate phenotype (Yang et al. 2012). In the current study, to obtain an accurate phenotype was proved to be the most difficult issue in QTL analysis. In the initial mapping, we used an average resistance scale of each $F_{2:3}$ family as a whole to represent its parental F_2 plant. And this proved to be preferred method for obtaining accurate phenotype for its parental line, as the major resistance QTL could be consistently detected in different replications. In the fine mapping process, we backcrossed the F_4 recombinants to Q11 to generate backcross progeny. Statistically significant difference in GLS resistance between heterozygous and homozygous genotypes in backcross progeny was determined by a *t* test to declare the presence of a resistance QTL in their parental line. This fine-mapping strategy was very powerful to narrow down the resistance QTL locus until the candidate genes underlying the target QTL were identified.

The resistance QTL-*qRgls1* could enhance the resistance percentage by 19.70–61.28 %, when all progeny of the 27 recombinants were taken into consideration in 2011. However, there existed substantial variations in resistance percentages for some recombinants. For example, the standard deviation (SD) value of resistance percentage

among four recombinants in VII type and three recombinants in XII type were as high as 31 %. This may result from different genetic backgrounds, considering that the recombinants which had the similar donor regions were derived from different $F_{2:3}$ families which substantially differed in genetic backgrounds. Nevertheless, the SD value of resistance percentage would gradually decrease with continuously backcrossing to increase the recovery rate of the recurrent parent. In the present study, the QTL-*qRgls1* has been restricted to $\sim$1.4 Mb region, in which a number of high-density markers could be further developed to narrow down *qRgls1* into a few candidate genes for both map-based cloning and MAS to improve maize resistance to GLS.

In general, quantitative disease resistance could decrease disease severity by two ways: (1) to detect and counter pathogen attack; (2) to block pathogen progression after the initial infection. In the current study, plants differed substantially in lesion numbers, but not lesion sizes, suggesting that maize resistance to GLS occurs mainly in initial pathogen invasion, but not in pathogen progression. This can also be confirmed that the tiny chlorotic spots that were frequently observed in resistant plants resulted from hypersensitive response (HR). Anyway, this speculation needs to be confirmed when the GLS resistance gene(s) is validated and pathogen–host interaction is comprehensively investigated.

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