High-density Mapping for Gray Leaf Spot Resistance using Two Related Tropical Maize RIL Populations

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Abstract

The identification of QTL/genes to resist gray leaf spot (GLS) caused by *Cercospora zeae-maydis* or *Cercospora Zeina* plays an urgent role in improving GLS resistance in maize breeding practice. In our study, two groups of recombinant inbred line (RIL) populations derived from CML373×Ye107 (176 RILs) and Chang7-2×Ye107 (190 RILs) were generated and subjected to genotyping-by-sequencing (GBS). GBS technology was used for large-scale single nucleotide polymorphism (SNP) discovery and simultaneous genotyping of all F_7 lines from two related RIL populations in order to identify quantitative trait loci (QTL) associated with GLS resistance under natural conditions of disease occurrence. A total of 1929222287 reads in CML373×Ye107 (RIL-YCML) and 2585728312 reads in Chang7-2×Ye107 (RIL-YChang), with an average of 10961490 (RIL-YCML) and 13609096 (RIL-YChang) reads per individual, were got, which was roughly equal to 0.70-fold and 0.87-fold coverage of the maize B73 RefGen_V4 genome for each F_7 individual, respectively. 6418 and 5139 SNP markers were extracted to construct two high-density genetic maps. Comparative analysis using these physically mapped marker loci demonstrated a satisfactory colinear relationship with the reference genome. Eleven GLS-resistant QTL have been detected. The individual QTL accounted for 2.05-24.00% of the phenotypic variance explained (PVE). The new consensus QTL (q*YCM-DS3-3*/ *qYCM-LT3-1*/ *qYCM-LT3-2*) with the largest effect was located in chromosome bin 3.05, with an interval of 2.7 Mb, representing 13.08 to 24.00% of the PVE. Further gene annotation indicated that there were four candidate genes (*GRMZM2G032384*, *GRMZM2G041415*, *GRMZM2G041544*, and *GRMZM2G035992*) for *qYCM-LT3-1*, which may be related to GLS resistance.

Introduction

Gray leaf spot (GLS), caused by the polycyclic pathogens *Cercospora zeae-maydis* or *Cercospora Zeina*, is one of the most important foliar diseases of maize (*Zea mays* L.) worldwide [1]. The incidence of GLS has been rising in both severity and distribution in maize-growing regions, largely because of the reduced- or no-tillage practice, which allows fungal inocula to survive in crop residues and infect crops in early stages of development [2]. The yield losses attributed to GLS are as high as 70–100% because of associated severe blighting, stalk deterioration and lodging under severe epidemics [3]. This disease has also been commonly found in Yunnan province, China, since 2003 because of a shortage of resistant maize germplasm and the suitable geographical, climatic conditions that are optimal for the GLS epidemic [4]. Thus, GLS control in maize breeding programs has become more and more urgent.

The formation of rectangular lesions on leaves is the typical symptom caused by GLS, which are usually parallel to leaf veins [5]. A range of sub-phenotypes, including reduced total number of infections and reduced expansion, can also be observed at the macroscopic level [6]. There was a significant positive correlation between disease development and the distance between major leaf veins, indicating that lesions seem to be limited by major veins [7, 8].

Compared with conventional strategies for disease control through agricultural chemicals, different cultivation measures (crop rotation, field sanitation and so on), there is an urgent need for breeding and growing GLS-resistant varieties, as it is a green, safe, eco-friendly and sustainable strategy to reduce yield losses [9]. Therefore, it is very important to deeply understand the genetic mechanism of resistance and to mine resistance genes for maize yield improvement and disease management. Transferring resistance genes/ quantitative trait loci (QTL) from donors to elite maize germplasm recipients is a feasible way to improve the disease resistance of germplasm resources [10]. Most previous researches show that GLS tolerance of maize is a complex quantitative trait and remarkably subject to the environment [7, 11–13]. So, it is considerably desirable to discover molecular markers related to QTL containing GLS-resistant fragment to accelerate the breeding and development of disease-resistant germplasm resources through marker-assisted selection (MAS).

The main traditional molecular markers currently used for linkage map construction are simple sequence repeats (SSRs), amplified fragment length polymorphisms, restriction fragment length polymorphisms, expressed sequence tags, and sequence-tagged sites; however, these simple polymerase chain reaction (PCR) -based molecular markers has the weak points of low-density, time-consuming, hard-sledding, less-richness and incomplete coverage of the genome, which limit the application of the markers in the study of maize genetic variation [14]. The development of next-generation sequencing (NGS) platforms and the decrease in cost provide new strategies for large-scale SNP marker development. As the latest application of NGS technology, Genotyping-by-sequencing (GBS) is a popular method for reducing the complexity of the genome with low data volume, simple operation and cost saving [15]; maize, rice and other species have successfully used GBS technology to construct high-density genetic maps, laying the foundation for further targeted trait mapping, MAS and map-based cloning of important agronomic trait genes [16, 17].

Linkage and association studies have been successfully used for the genetic dissection of GLS in different resistant germplasms under various environments and many QTL-mapping studies for GLS resistance were mainly based on bi-parental crosses, except natural populations [13, 18–22]. For instance, Nine GLS resistant QTL were detected in the RIL population derived from inbreds Qi319 and Ye 478, which could explain 22.94–80.02% of the phenotypic variation and the interval containing the main QTL *qGLS1.02* included 12 candidate genes [21]. QTL mapping of a maize RIL population identified seven GLS-resistance QTL from five sites, and each QTL explained more than 11% of the phenotypic variation [19]. Four GLS-resistance QTL were detected on chromosomes 1, 2, 5, 8 using a tropical Y32 × Q11 population with 161 $F_{2:3}$ families, each of which could explain 2.53–23.90% of the total phenotypic variation and the QTL *qRgls1* and *qRgls2* with the largest effects was fine-mapped to a 1.4 Mb, 1Mb region, respectively; and there were 15 predicted genes in the mapped *qRgls2* region [11, 18]. Recently, a major resistance QTL *qRgls1.06* was fine-mapped to an interval of 2.38 Mb, and introduction of *qRgls1.06* through marker-assisted backcrossing have improved several GLS susceptible elite inbred lines [22]. A major QTL, *qGLS_YZ-1*, in an interval of 2.4 Mb of chromosome 2, was identified using two doubled haploid populations sharing a common resistant parent, which were planted in areas with high incidence of GLS in Southwest China [13]. The maize NAM population was used to identify three QTL that caused disease reductions of greater than 10% [7]. Based on genome-wide association study (GWAS) conducted with 355972 SNP markers in 157 tropical maize inbred lines, seven SNPs were significantly associated with GLS, and three gene models are related with plant defense pathway [20]. These identified QTL laid the foundation for further fine mapping and cloning of resistance genes.

Although there are many putative QTL located on different chromosomal loci in maize, none of the genes underlying them have been cloned. Maize lines originated in Suwan 1 (tropical germplasms) show good combining ability with Reid or non-Reid (temperate germplasms) heterotic groups and adopting these good combiners can remarkably improve maize breeding efficiency, especially for grain yield in southwestern China [23, 24], tropical GLS-resistant germplasm resources have better application value and mean in breeding under abiotic and biotic stress [10, 25]. Thus, screening additional germplasm, especially tropical maize germplasm, is of great significance for mining GLS resistance QTL. In this study, the inbred line Ye107 was used as common parent in crosses to two tropical and temperate inbred lines to develop two related RIL populations for the identification of GLS resistance QTL. The major objectives were to: (1) identify tag SNP markers from high-throughput GBS data in two related RILs, (2) construct two high-density linkage maps according to these SNP markers, (3) map QTL for disease scale (DS), lesion type (LT), and further predict candidate genes for the identified QTL possessing the largest effects through Maize Genetics and Genomics Database (MaizeGDB). The work will be not only helpful in understanding the genetic mechanism of GLS resistance, but also lay the foundation for MAS and map-based cloning in further studies.

Materials And Methods

Genetic materials and experimental design

Two groups of RIL populations were chosen as samples in this study, of which one F_7 RIL was composed of 176 lines, which were got from a cross between CML373 and Ye107 lines. In addition, the second RIL population contained 190 F_7 lines, which came from a cross between Chang7-2 and Ye107. Both RILs were abtained by the single-seed descent method. The common parent Ye107, used as female, derived from American maize hybrid DeKalb XL80 and is one parent of the once predominant hybrid cultivar Yedan2 in China. CML373 is a tropical inbred line developed by CIMMYT, and Chang7-2 is one of the parental lines of the hybrid Zhengdan958, an elite hybrid that was widely grown on approximately 500 hectares between 2001 and 2015 in China, and is still a competitive variety in the plains of North China [26].

The GLS resistance of two populations and three parents were estimated under natural infection in three replicates at two locations, Baoshan (BS) and Dehong (DH), in Yunnan province in 2018 and 2019. Both the locations are GLS hotspots and experience severe GLS epidemics almost every year. Disease screening trials were performed using a randomized complete-block design. Plots were planted as single rows with a row spacing of 0.7 m and row length of 4.0 m. Ten seeds per entry were planted in each plot, distance between two adjacent plants was 0.4 m and the population density was approximately 60,000 plants/ha. Trials were managed in accordance with standard local practices.

Evaluation of phenotyping in the field

GLS resistance data were represented by DS and LT. All data for GLS resistance were recorded beginning one leaf below the primary ear and as the average value for the row excluding the first and the last plant in the row. Disease phenotyping was observed at 4 weeks after flowering. DS was evaluated using disease index and disease severity on a scale of 1, 3, 5, 7, and 9, which was based on the percentage of infected area on the entire leaf of 0-100%, and infected leaves corresponding to different scales are depicted in Fig. 1a. A scoring system with one scale (1, 2, 3, 4, and 5) based on lesion size, chlorotic defense response, and presence of necrotic or dry tissue was used to evaluate LT, where 1 = no lesion or chlorotic tissue, 2 = leaf chlorosis, but no necrotic tissue, 3 = leaf chlorosis, little necrotic tissue, 4 = necrotic tissue, small lesions, and 5 = necrotic tissue, large lesions with tissue drying between adjacent lesions (Fig. 1b). Phenotypic data were subjected to analysis of variance using PROC GLM of SAS 9.1 software (SAS Institute Inc. 2004). The model for variance analysis was $Y = \mu + \beta_G + \gamma_L + (\gamma\beta)_{LG} + \varepsilon_{LGR}$, where β_G represents the effect of the Gth RIL line, γ_L is the effect of the Lth environment, $(\gamma\beta)_{LG}$ is the corresponding interaction effect, and ε_{LGR} is the residual effect. All effects were considered to be random.

DNA extraction and genotyping by sequencing

From both RIL populations and their parents, young leaf tissues were gathered, and snap frozen in liquid nitrogen, subsequently transferred to a -80℃ freezer. On the basis of the manufacturer's protocols with the Plant Genomic DNA Kit (TIANGEN, Beijing, China), total DNA of each parental and RIL leaf sample was extracted. DNA quality and concentration were measured using the method described by Zhou [27].

1.5 μg genomic DNA from each of the three parents was used to construct a sequencing library following the method described by Cheng [28]. An Illumina Novaseq 6000 sequencer sequenced Paired-end sequencing libraries with an insert size of approximately 350 bp.

The genomic DNA from each line of both RIL populations was incubated with *MseI* (New England Biolabs, Ipswitch, MA), T4 DNA ligase (NEB), ATP, and the Y-adapted N-containing barcode at 37°C. Restriction-ligation reactions were heat-inactivated at 65℃, and then digested using additional restriction enzymes *HaeIII* (NEB) and *EcoRI* (NEB) at 37°C. The restriction ligation samples were purified using Agencourt AMPure XP (Beckman). PCR amplifications were carried out in a single tube using purified samples and Phusion Master Mix (NEB) after adding universal primer and index primer to each sample. Using Agencourt AMPure XP (Beckman) purified PCR productions and pooled them, then these productions run out on a 2% agarose gel. Fragments of 350 to 400 bp (with indexes and adaptors) in size were isolated using a Gel extraction Kit (Qiagen, Valencia, CA). These fragments were then purified using the Agencourt AMPure XP (Beckman), then diluted for sequencing. Finally, paired-end sequencing was performed using an Illumina Novaseq 6000 sequencer (Illumina, USA) at Novogene Bioinformatics Institute, Beijing, China [16, 27].

Sequence data grouping and SNP identification

The sequences of each F_7 individual were sorted and recognized according to the barcoded adapters, and then the raw data generated by sequencing was filtered and preprocessed to obtain clean data for subsequent downstream analyses using the specific processing steps described by [16, 27].

The high-quality clean reads of each F_7 individual were mapped to the B73 reference genome using BWA (Version: 0.7.13) [29]. SAMtools software was used to convert alignment files into the BAM format [30]. The variants were called for all samples using the Genome Analysis Toolkit (GATK, version: 3.8.1) [31]. SNPs or InDels were aligned and annotated using the software tool ANNOVAR [32] based on the GFF3 files from the Zea_mays.AGPv4 sequence (ftp://ftp.ensemblgenomes.org/pub/release-40/plants/fasta/zea_mays/dna/Zea_mays.AGPv4.dna.toplevel.fa.gz). The segregation pattern aa × bb for SNP markers between the parents was used for further analysis.

Linkage map construction

To ensure the quality of genetic map, the polymorphic SNPs were filtered according to the following rules: 1) filtering SNPs with sequencing depths of < 10-fold between both parents, 2) screening genotypes covering at least 70% of all offspring, 3) filtering the SNP markers with segregation distortion test $P < 0.01$ by Chi-square test. In the end, genetic linkage maps were constructed from the SNP genotypes in both RILs using the JoinMap software version 4.0.

QTL analysis

QTL for GLS disease severity in the two related RILs were identified for each field trial based on the genetic maps and applying the Composite Interval Mapping (CIM) model using the *R/qtl* package. To determine the logarithm of odds (LOD) threshold, each trait in each environment was tested separately by permutation tests 1000 times.

Results

Resequencing parental lines and GBS of two related RILs

Three parents Ye107, CML373, and Chang7-2 in this study were respectively re-sequenced at effective sequencing depths of 10.4, 12.06, and 9.77, resulting in 151321997, 185545807, and 152687930 reads mapped to the B73 RefGen_V4 genome, respectively; and subsequently a total of 17862110, 17683437, and 17368518 SNPs for Ye107, CML373, and Chang7-2 were identified. Furthermore, 5358642 and 5017118 homozygous polymorphic SNPs between the Ye107/CML373 and Ye107/Chang7-2 parental genotypes were obtained (Table S1, Fig. 2).

A total of 1929222287 and 2585728312 reads were generated for RIL-YCML and RIL-YChang, respectively. The average number of reads for each individual was 10961490 for RIL-YCML and 13609096 for RIL-YChang, which amount to about 0.70-fold and 0.87-fold coverage of the maize genome, respectively. Meanwhile, the further analysis showed about 43.68% and 44.14% overall GC content, and about 95.85% and 96.31% Q20 scores for RIL-YCML and RIL-YChang, respectively (Table S1). Only 5358642 and 5017118 homozygous polymorphic SNPs respectively fell into the aa × bb segregation pattern for the two RILs according to the genotype characteristics of homozygous inbred line. Finally, 6481 and 5139 SNPs passed the relatively strict screening criterion and were utilized to construct genetic maps for RIL-YCML and RIL-YChang, respectively.

Linkage map construction

The 6481 SNPs successfully mapped to 10 linkage groups (LGs) was used to construct a high-density linkage map for the RIL-YCML (Fig. 3a), in which 1436.6 cM with LG9 (114.53 cM) being the smallest and LG1 (178.56 cM) being the largest was the total length of the linkage map and 0.22 cM was determined as the average distance between adjacent markers. Each linkage group was labeled with 391 (LG9) to 831 (LG4) markers, with an average of 641.8 markers. Two gaps > 5 cM in length was present and the largest gap of 5.91 cM was located at chromosome 6 (Table 1). As for RIL-YChang, a high-density genetic linkage map was generated based on 5139 SNP markers (Fig. 3b). This linkage map spanned 1546.6 cM, with an average genetic distance of 0.3 cM and all gaps were less than 5 cM. The SNP markers ranged from 229 to 997 in number having an average of 513.9 markers in each linkage group (Table 1). Above maps demonstrated a satisfactory co-linear relationship (Spearman coefficient > 0.98) with the reference genome (Table 1, Fig. 4). In brief, two high-density and high-quality genetic maps were constructed, which were fit for QTL mapping analysis.

Table 1
Characteristics of the high-density genetic map

mapping population	Linkage group	Total markers	Gap ≤ 5 cM (%)	Max gap (cM)	Total distance (cM)	Average distance (cM)	Spearman coefficient [a]
RIL-YCML	1	587	100.0%	3.84	178.6	0.30	0.999
	2	682	100.0%	2.80	147.6	0.22	0.999
	3	709	100.0%	2.47	145.3	0.20	0.999
	4	831	100.0%	2.14	131.1	0.16	0.999
	5	751	100.0%	2.31	137.5	0.18	0.999
	6	782	99.9%	5.91	148.1	0.19	0.995
	7	504	100.0%	4.57	134.5	0.27	0.995
	8	510	100.0%	2.80	135.6	0.27	0.997
	9	391	99.7%	5.32	114.5	0.29	0.999
	10	671	100.0%	3.67	163.8	0.24	0.999
	Total	6418	-	-	1436.6	0.22	-
RIL-YChang	1	997	100.0%	2.89	137.7	0.14	0.999
	2	366	100.0%	3.43	174.8	0.48	0.999
	3	616	100.0%	1.68	90.3	0.15	0.999
	4	716	100.0%	2.12	93.6	0.13	0.999
	5	639	100.0%	3.05	174.4	0.27	0.999
	6	294	100.0%	3.07	195.5	0.66	0.999
	7	229	100.0%	2.13	136.0	0.59	0.999
	8	358	100.0%	2.30	148.5	0.41	0.999
	9	351	100.0%	3.09	198.7	0.57	0.999
	10	573	100.0%	4.53	197.2	0.34	0.981
	Total	5139	-	-	1546.6	0.30	-

[a]Spearman coefficient: the rank correlation between genetic map and reference genome. This parameter is used to test the SNP order of the genetic map

Phenotype evaluation of the parental lines and mapping populations

Phenotyping data were collected for DS and LT in BS and DH in 2018 and 2019 for the two related RILs as well as for the three parental lines. GLS disease data from 2018 to 2019 showed no significant differences; the mean value of GLS disease data from both years were then utilized for subsequent analyses (Table 2). The comparison of resistance of three parental lines displayed that CML373 was most resistant to GLS (BS DS:3, LT:1, DH DS:1, LT:1), followed by Ye107 (BS DS:6.3, LT:3, DH DS:5, LT:3) and Chang7-2 (BS DS:9, LT:5, DH DS:9, LT:5). Significant differences for two traits were observed among parental lines within and among populations. In Table 3, we summarized the descriptive statistics of the traits. There was a strong correlation between DS and LT (BS: $r = 0.57$ to 0.82, DH: $r = 0.58$ to 0.80; $p < 0.01$) for the two RIL populations, which showed a uniform trend across three replicates in BS, but not in DH, indicating that environmental factors significantly affected the phenotypic variances (Fig. 5). Variance for GLS resistance was also analyzed in order to explore the sources of phenotypic variation. Both genetic and environmental factors obviously affected phenotypic variances (Table 2). The QTL for GLS resistance in each environment were considered owing to the environmental effect. Moreover, the loci detected in common or those that overlapped under different environments were defined as consistent QTL.

Table 2
Analysis of variances for GLS resistance[a]

		DS					LT				
Population	Source	DF	SS	MS	*F*	Pr > *F*	DF	SS	MS	*F*	Pr > *F*
RIL-YCML	Genotype(G)	175	2562.43	14.64	5.19	< 0.0001**	175	489.64	2.79	4.34	< 0.0001**
	Environment(E)	1	122.48	122.48	43.41	< 0.0001**	1	2.87	2.87	4.44	0.0352*
	Year	1	6.44	6.44	2.28	0.1312	1	1.24	1.24	1.92	0.1659
	G*E	175	1955.84	11.18	3.96	< 0.0001**	175	309.79	1.77	2.75	< 0.0001**
	Error	1408	3972.92	2.82			1408	907.88	0.645		
RIL-YChang	Genotype(G)	189	2705.08	14.31	5.84	< 0.0001**	189	718.47	3.81	5.93	< 0.0001**
	Environment(E)	1	129.79	129.79	52.99	< 0.0001**	1	892.29	892.29	1391.11	< 0.0001**
	Year	1	3.39	3.39	1.39	0.2391	1	0.25	0.25	0.3829	0.5361
	G*E	189	978.21	5.18	2.11	< 0.0001**	189	323.84	1.71	2.6712	< 0.0001**
	Error	1520	3722.67	2.45			1520	974.96	0.64		

[a] DF, degree of freedom; SS, sum of squares; MS, mean squares; * and ** indicate significance at 5 and 1% probability levels, respectively.

Table 3
Phenotypes of the parental lines and RIL populations in Baoshan and Dehong locations

		Parents			RIL-YCML					RIL-YChang				
Trait[a]	Env.[b]	CML373	Ye107	Chang7-2	Range	Mean ± SD[c]	Skewness	Kurtosis	CV[d]	Range	Mean ± SD	Skewness	Kurtosis	CV
DS	BS	3.0	6.3	9.0	1.00–9.00	4.49 ± 1.77	-0.12	-0.34	0.39	1.00–9.00	6.49 ± 1.51	-0.36	-0.38	0.23
	DH	1.0	5.0	9.0	1.00–9.00	4.61 ± 1.64	-0.07	0.21	0.36	1.00–9.00	5.92 ± 1.57	-0.29	-0.13	0.30
LT	BS	1.0	3.0	5.0	1.00–5.00	2.60 ± 0.91	0.65	-0.33	0.35	1.67-5.00	4.15 ± 0.72	-0.94	0.61	0.17
	DH	1.0	3.0	5.0	1.00-4.50	2.33 ± 0.65	0.58	0.38	0.28	1.50-5.00	3.34 ± 0.76	0.72	0.10	0.22

[a]Trait is the name of gray leaf spot and flowering time, respectively; IA, infection area; HR, hypersensitive reaction; LT, lesion type

[b]Env., the specific environment: BS is Baoshan, DH is Dehong

[c]SD, standard deviation

[d]CV, coefficient of variation

QTL identification in the RIL-YCML population

Ten QTL for GLS resistance were found in the RIL-YCML population by the CIM method (Table 4, Fig. S1), and each of the two GLS-related traits had its own corresponding QTL: 8 of them influenced DS and were distributed on chromosomes 1, 3, 4 and 8; 2 of them influenced LT and were distributed on chromosome 3. The resistance alleles at *qYCM-DS1-1, qYCM-DS1-2, qYCM-DS3-1, qYCM-DS3-3, qYCM-LT3-1*, and *qYCM-LT3-2* were derived from the resistant parent CML373; whereas the resistance allele at *qYCM-DS3-2, qYCM-DS3-4, qYCM-DS4*, and *qYCM-DS8* originated from the susceptible parent Ye107. The QTL (*qYCM-LT3-1*) with the largest effect was located in chromosome bin 3.05, representing 24.00% of the phenotypic variance explained (PVE) in BS. The second largest-effect QTL, *qYCM-DS3-3*, which accounted for 20.37% of the PVE in DH, was also located in chromosome bin 3.05. Eight additional QTL were detected in bins 1.05, 1.06, 3.04, 3.05, 3.09, 3.09, 4.08, and 8.03, respectively, which explained 2.05–17.20% of the variation in GLS disease scores.

Table 4
QTL for GLS resistance detected in the RIL-YCML population

Trait[a]	QTL	Effect[b]	chr.[c]	start	end	bins	LOD[d]	A[e]	PVE[f]
DS	*qYCM-DS1-1*	BS	1	93.09	93.38	1.06	3.39	0.29	2.05
	qYCM-DS3-1	BS	3	45.75	46.04	3.04	10.51	0.85	17.20
	qYCM-DS3-2	BS	3	138.24	138.53	3.09	3.93	-0.41	4.03
	qYCM-DS8	BS	8	33.81	33.81	8.03	3.17	-0.50	5.94
	qYCM-DS1-2	DH	1	89.30	89.88	1.05	2.73	0.22	1.53
	qYCM-DS3-3	DH	3	58.01	58.59	3.05	12.31	0.81	20.37
	qYCM-DS3-4	DH	3	138.24	138.53	3.09	3.70	-0.27	2.24
	qYCM-DS4	DH	4	101.48	101.48	4.08	2.55	-0.36	3.99
LT	*qYCM-LT3-1*	BS	3	56.82	58.01	3.05	8.61	0.49	24.00
	qYCM-LT3-2	DH	3	58.30	59.48	3.05	5.46	0.16	13.08

[a]Trait is the name of the component of GLS resistance: DS, disease scale; LT, lesion type

[b]The effect of each QTL in a particular environment: BS is Baoshan; DH is Dehong

[c]Chr., chromosome

[d]LOD, the logarithm of odds score

[e]A, the additive effect value

[f]PVE, the phenotypic variance explained by individual QTL

QTL identification in the RIL-YChang population

Four QTL for GLS resistance were found in the RIL-YChang population by the CIM method (Table 5, Fig. S2). There were two QTL each for DS and LT. The relatively larger effect QTL, located in chromosomes bins 5.03 and 9.04, accounted for 12.26% and 10.03% of the PVE, which came from Ye107; whereas the remainder were from Chang7-2 (bin 1.11) and Ye107 (bin 9.04), which explained 6.55% and 6.24% of the phenotypic variation, respectively.

Table 5
QTL for GLS resistance detected in the RIL-YChang population

Trait[a]	QTL	Effect[b]	chr.[c]	start	end	bins	LOD[d]	A[e]	PVE[f]
DS	*qYCh-DS5*	BS	5	60.39	60.66	5.03	6.22	-7.45	12.26
	qYCh-DS1	DH	1	125.51	125.78	1.11	3.01	3.94	6.55
LT	*qYCh-LT9-1*	BS	9	92.34	92.74	9.04	4.76	-0.26	10.03
	qYCh-LT9-2	DH	9	69.68	69.82	9.04	3.20	-0.21	6.24

[a]Trait is the name of the component of GLS resistance: DS, disease scale; LT, lesion type

[b]The effect of each QTL in a particular environment: BS is Baoshan; DH is Dehong

[c]Chr., chromosome

[d]LOD, the logarithm of odds score

[e]A, the additive effect value

[f]PVE, the phenotypic variance explained by individual QTL

Comparison of QTL between the two GLS resistance sub-phenotypes in the two related RILs

A total of 14 QTL (10 in RIL-YCML and 4 in RIL-YChang) for GLS resistance were identified in this study. Due to the influence of LOD threshold size and small differences in the phenotypic estimates, QTL were significantly correlated with some traits, but not for others. This could be why these correlated traits are often mapped at different positions. However, two QTL corresponding to DS (*qYCM-DS3-2, qYCM-DS3-4*) were identified in the same region in the two environments. There was also one pair of QTL (*qYCh-LT9-1/ qYCh-LT2-2*), of which the confidence intervals for a single trait were in the bin 9.04. Three QTL

(*qYCM-DS3-3, qYCM-LT3-1, qYCM-LT3-2*) regions assigned as one stable consistent QTL were consistently detected and overlapped in BS and DH. One (*qYCM-DS3-3*) of them influenced DS, while two (*qYCM-LT3-1, qYCM-LT3-2*) of them influenced LT.

Candidate gene analysis

According to the Maize Genetics and Genomics Database accessible at MaizeGDB (https://www.maizegdb.org/), the physical interval (139,130,610 – 141,837,160) of *qYCM-LT3-1* with the largest effect encompassed 49 protein-coding genes (Table S2). In recent study, the transcriptome and proteome analyses of two commercial maize lines, Yayu889 and Zhenghong532 has shown that two candidate GLS resistance proteins including calmodulin-like protein and leucine-rich repeat receptor-like protein kinase were identified [33]. Meanwhile, the increase of SA, carotenoids and reactive oxygen species has positive correlation with the defense response [33]. Studies by *de novo* assembly of transcriptomes of B73 containing a GLS resistance QTL derived from CML444 have identified several likely candidate genes, including a lectin receptor-like kinase, a wall-associated kinase, two glutathione s-transferases [34]. Armadillo (ARM) repeat proteins also response to biotic and abiotic stress [35], one gene *GRMZE2G476902* linked to a significantly associated SNP which was associated with GLS encodes an ARM repeat protein [20]. MYB transcription factors are a large family involved in regulating plant responses to various biotic and abiotic stresses; observations in Arabidopsis indicate that ABA signal mediated by MYB96 can induce the biosynthesis of salicylic acid, thus improving plant disease resistance [36]. Among the candidate genes in the interval of *qYCM-LT3-1*, *GRMZM2G032384* is a Leucine-rich repeat kinase family protein gene, *GRMZM2G041415* is a MYB transcription factor gene, *GRMZM2G041544* is a wall associated kinase gene, and *GRMZM2G035992* is an ARM repeat superfamily protein gene.

Discussion

In present study, two paternal lines CML373 and Chang7-2 respectively displayed high resistance and high susceptibility, whereas the common maternal line Ye107 exhibited susceptibility. The GLS phenotypes in the two environments showed continuous distribution from high resistance to high susceptibility, which was consistent with a quantitatively inherited trait. Some lines even exhibited phenotypic characteristics of transgressive segregation, suggesting that both parents contributed to the expression of the phenotype (Fig. S3). The GLS results showed that the three parents might each contain specific resistance genes. In the present study, QTL mapping of two related RIL populations from Ye107, CML373, and Chang7-2, of which Ye107 was a common female parent, identified eleven GLS resistance QTL, and the individual QTL here accounted for 2.05-24.00% of the PVE. Prior to our study, there were many reports of QTL for GLS resistance that were identified under field conditions and some GLS resistance QTL hotspots were noted. Balint-Kurti [37] identified Two GLS QTL hotspots in bins 1.05–1.06 and 2.03–2.05 by summarizing GLS QTL detected in seven populations. Visual inspection of the data for QTL reported in three populations revealed GLS QTL hotspots in bin 1.05/1.06, bin 2.05/2.06, bin 4.08, bin 5.03, and bin 5.05 [38]. Shi [9] also projected QTL from the same studies onto the IBM2005 map, and noted seven “consensus QTL” (bin 1.06, bin 2.06, bin 3.04, bin 4.06, bin 4.08, bin 5.03, and bin 8.06). Berger [19] extended the analyses of Wisser et al. and Shi et al., and identified QTL hotspots in bin 1.05/1.06, bin 2.03/2.04, bin 4.08, bin 5.03/5.04 and bin 7.02/7.03. Among the QTL we detected, bin 1.05 (*qYCM-DS1-2*), bin 1.06 (*qYCM-DS1-1*), bin 3.04 (*qYCM-DS3-1*), bin 4.08 (*qYCM-DS4*), and bin 5.03 (*qYCh-DS5*) GLS QTL overlapped with hotspots mentioned above, *qYCM-DS8* (bin 8.03) overlapped with *qRgls1* and *qYCh-LT9-1/ qYCh-LT9-2* (bin 9.04) was in the same bin as QTL *9A1-GLS* observed in at least two environments in previous studies [11, 19]. The remainder three QTL (*qYCM-LT3-1/qYCM-LT3-2/ qYCM-DS3-3, qYCM-DS3-2*, and *qYCh-DS1*) appeared to be unique and might be new GLS resistance sources for use in maize breeding programs. Especially for *qYCM-LT3-1* with the largest effect, we have further compared physical positions of previously reported QTL with it. The QTL (*qYCM-LT3-1*) located in bin 3.05 at between 139,130,610 and 141,837,160 bp did not overlap with previously identified intervals for GLS resistance [20, 39]. Shi et al. detected a QTL located in bin 3.05 at between 157,101,755 and 161,035,765 bp, which explained 7.6% of the genetic variation, and Kuki et al. detected a SNP in bin 3.05 at 129,109,843 bp which were not located within the confidence interval of *qYCM-LT3-1*, which explained 1.48% of the genetic variation [20, 39]. *qYCM-LT3-1* is distinguished from these earlier minor QTL as it could also explain up to 24.0% of phenotypic variation in GLS resistance and had not previously been detected. To confirm the genetic effect of *qYCM-LT3-1*, we chose the strongest linkage marker, marker3008829, and genotyped it: homozygous CML373/CML373 and Ye107/Ye107 in the F_7 population, and calculated disease resistance for these two genotypes (Fig. S4). In both the environments, the homozygous genotype CML373/CML373 showed high resistance to GLS, and the homozygous genotype Ye107/Ye107 displayed low GLS resistance. The result clearly demonstrated there was a real and effective resistance QTL in bin3.05. The SNP marker 3008829 associated with GLS resistance in chromosome 3.05 may be used for developing molecular markers closely linked with the target trait, enhancing breeding efficiency by MAS.

Previous studies have shown that a highly susceptible parent also has the potential for enhancing offspring disease resistance [40]. The 10.73% of total phenotypic variation could be explained by a GLS resistance QTL (*qRgls3*) which was derived from the susceptible parent Q11 through a set of 161 progenies from the cross of Q11/Y32 at Dehong [11]. Another susceptible parent Ye478 was selected to hybridize with the same resistant parentY32 to construct an $F_{2:3}$ population, the results also indicated that some GLS-resistant QTL came from the susceptible parent [12]. Using a genetic map containing 199 SSR markers based on the bi-parental RIL population (Qi319×Ye478), one QTL on chromosome 3 was detected from Ye478 [21]. There were also three GLS resistance alleles that originated from the susceptible parent SC Malawi in an RIL F_7 population derived from a cross between CML444 and SC Malawi [19]. In this study, seven GLS resistance QTL (*qYCM-DS3-2/qYCM-DS3-4, qYCM-DS4, qYCM-DS8, qYCh-DS5, qYCh-LT9-1, qYCh-LT9-2* in Ye107 and *qYCh-DS1* in Chang7-2) were derived from susceptible parents. Interestingly, these QTL derived from the common parent Ye107 were detected only in individual population. This further demonstrated that the inheritance to GLS is complex and is affected by both environments and genetic backgrounds, and some valuable resistance QTL/genes which can improve disease resistance may be present in susceptible plants, such as maize [41, 42] and rice [43]. Thus, in breeding practice, we should completely consider the genetic background of susceptible parents, which will have value to breeders for improving resistance to GLS through resistance gene pyramiding.

Detection of GLS resistance QTL has shown its variability based on different seasons and sites [41]. Our previous study identified seven QTL related to GLS disease scores from an $F_{2:3}$ population, and two QTL were consistent across sites, but the other five QTL were not [12]. Environmental effects and

interactions between environment and genotype might lead to lack of consistency in the map position of QTL identified in each environment. In light of this, the high purity of genotypes for the two RIL populations in this study contributes to testing in multiple environments later, which will further give evidence in the detected QTL.

Although many quantitative disease resistance QTL have been detected in the maize genome, the underlying genes have been identified in only a few cases [44]. A major GLS resistance QTL qRgls2 in bin 5.03-04 was mapped to a ~ 1Mb region with advanced backcross populations and the final mapped interval contained 15 predicted genes according to the B73 reference genome [18]. Association mapping in 253 maize inbred lines identified a SNP in a glutathione *S*-transferase (GST) gene that were correlated with resistance to GLS, northern leaf blight, and southern leaf blight. [45]. A QTL *qGLS1.04* for resistance to GLS was identified through nested association mapping and near-isogenic line analysis, the expression of a candidate flavin-monooxygenase (FMO) gene underlying *qGLS1.04* was up-regulated under the treatment of *C. zeae-maydis* toxin cercosporin [7]. In this study, the physical interval of *qYCM-LT3-1* contained four candidate genes underlying phenotypes of interest. Further studies using GBS in large RIL populations together with NAM populations are going on to verify and resolve QTL to underlying causal genes or sequence variants.

GBS representing a low-cost but high-yield application of the NGS platforms has been developed for targeting a reduced representation of the full genome space [46]. The rapid and high-throughput sequencing technology of GBS can yield large amounts of SNP markers with removal of ascertainment bias which has been used for a series of studies including genetic mapping of agronomically genes for MAS [47–49]. In our study, a wealth of SNPs had been identified by GBS and simultaneously been used to genotype two maize genetic mapping populations. The average effective depth of re-sequencing of the three parents was approximately 10.74-fold, ensuring the high reliability and quality of GBS data. Based on this result, the accuracy of genotyping was relatively insensitive to even the relatively high error rates of low-coverage sequence data and the inferior SNP calling could be found and filtered out in their progenies [50]. Total average valid bases data size was 330.17Gb, of which the proportion of bases with a coverage depth of not less than 5-fold in the *Zea mays* reference genome AGPv4 was 3.78%, including 5358642 homozygous polymorphic SNPs in RIL-YCML, and 5017118 homozygous polymorphic SNPs in RIL-YChang. The final high-density genetic map contained 6418 and 5139 SNP markers for the two RIL populations after a relatively strict screening criterion, respectively. Therefore, on the basis of the above advantages, GBS will have broad application prospects in genome-wide molecular marker development and MAS breeding in the future.

Declarations

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Authors' Contributions

LC analyzed phenotype and genotype of RIL progeny, constructed linkage map and mapped QTL, and wrote the draft of the manuscript. LL provided advice on experimental design. ZWL contributed to construction of the RIL populations. YDZ and MSK carried out language editing. YYW and XMF designed the whole work and provided finance for this project. All authors agreed with the publication of manuscript.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

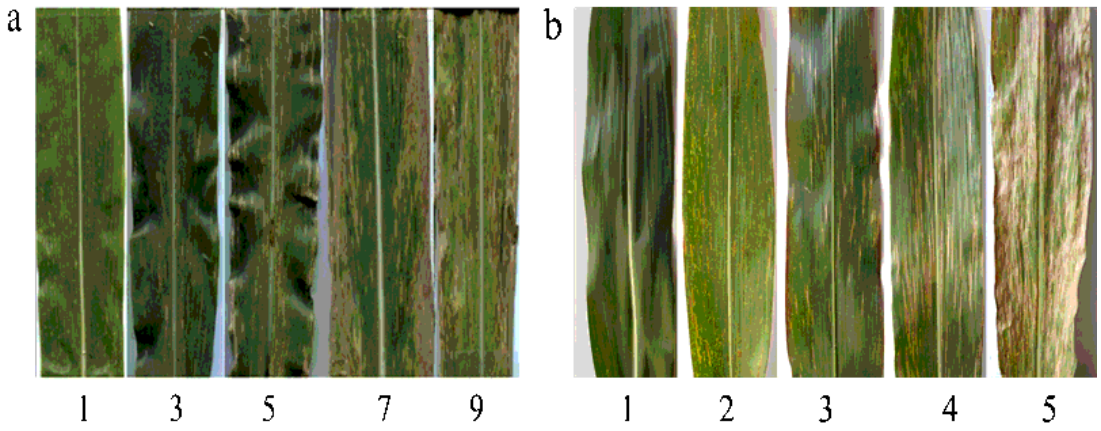


Figure 1

Sub-phenotypes of GLS. a, Symptoms of GLS with disease scales 1-9, the criteria of scales for GLS disease rating is based on the percentage of infected area in the entire leaf of 0-100%: scale 1 0-5 %, scale 3 6-10 %, scale 5 11-30 %, scale 7 31-70 %, scale 9 71-100 % (Liu et al. 2016). b, Symptoms of 1-5 scales for lesion type.

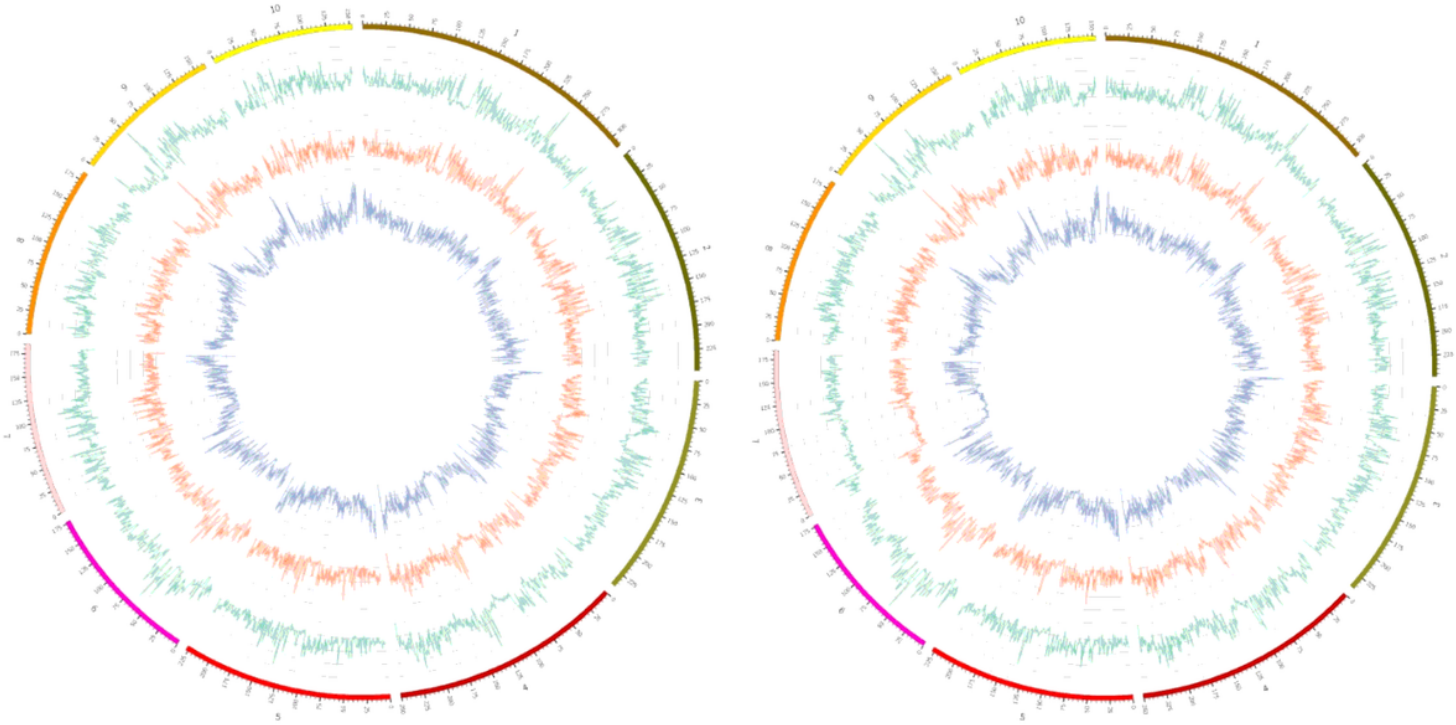


Figure 2

Features of the distribution of SNPs and genetic variants throughout the Ye107/CML373 (left) and Ye107/Chang7-2 (right) genomes, respectively. The outermost circle with scale represents the ten maize chromosomes. Tracks (outer to inner circles) indicate the following: track1) the density of SNPs that are polymorphic between Ye107/CML373 (left) and Ye107/Chang7-2 (right) genomes, respectively; track2) the density of polymorphic SNPs with coding sequences between Ye107/CML373 (left) and Ye107/Chang7-2 (right) genomes, respectively; track3) the density of insertions or deletions (indels) between Ye107/CML373 (left) and Ye107/Chang7-2 (right) genomes, respectively. Histograms of different colors represent different tracks.

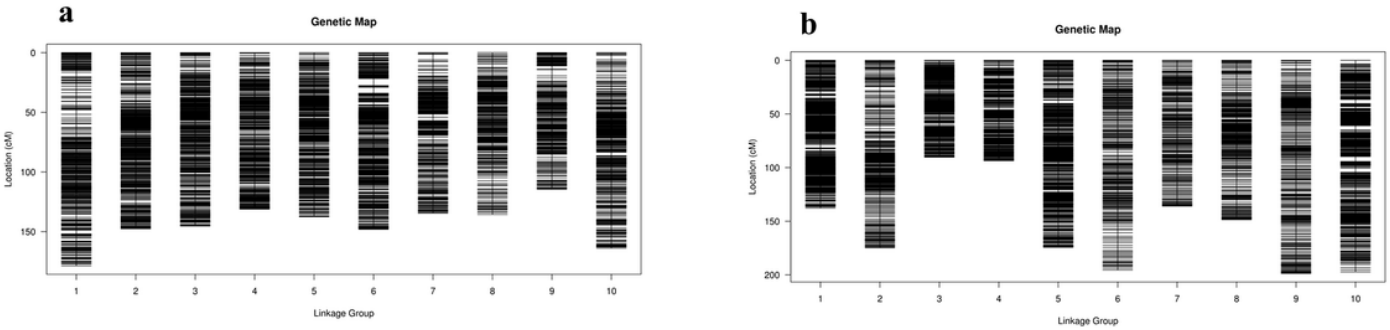


Figure 3

a High-density SNP-based genetic map derived from a cross between Ye107 and CML373. b High-density SNP-based genetic map derived from a cross between Ye107 and Chang7-2. A black bar indicates an SNP marker, the x-axis and y-axis indicate linkage group number and genetic distance (centimorgan as unit), respectively

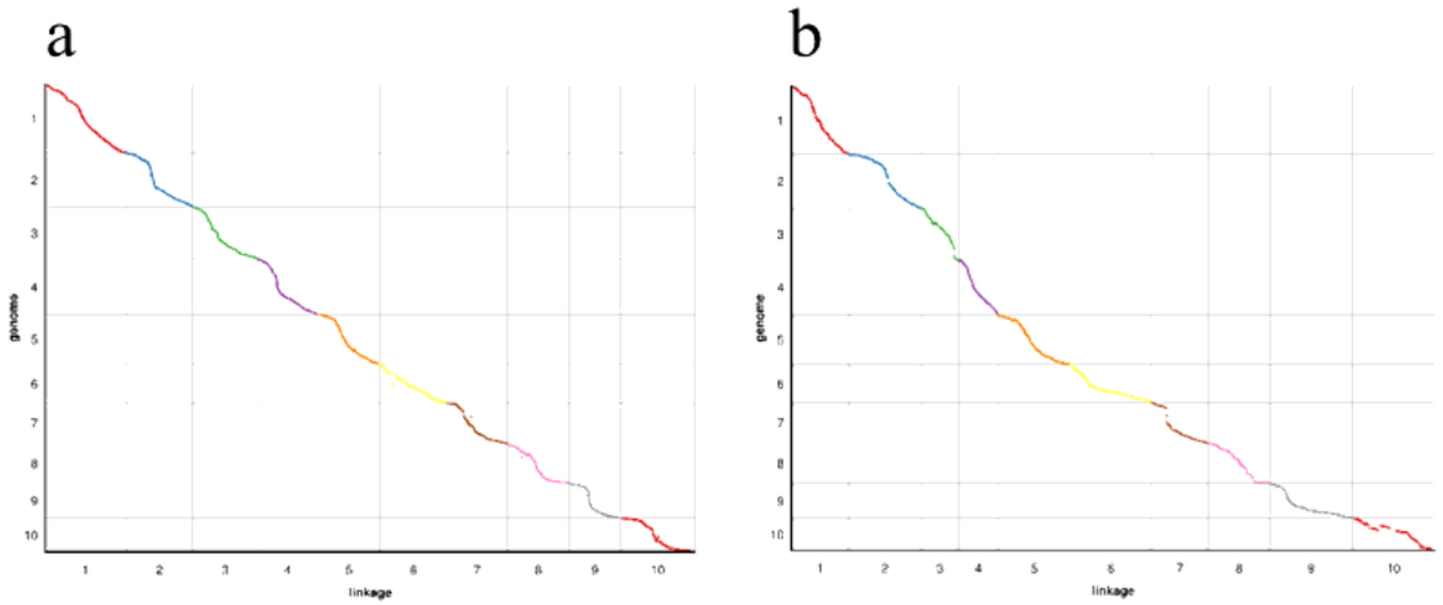
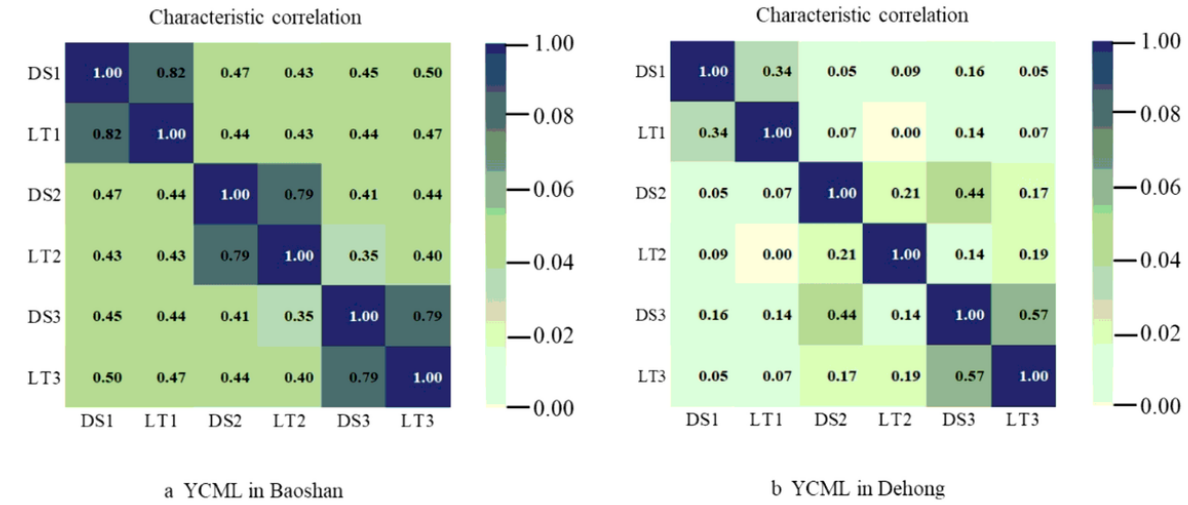


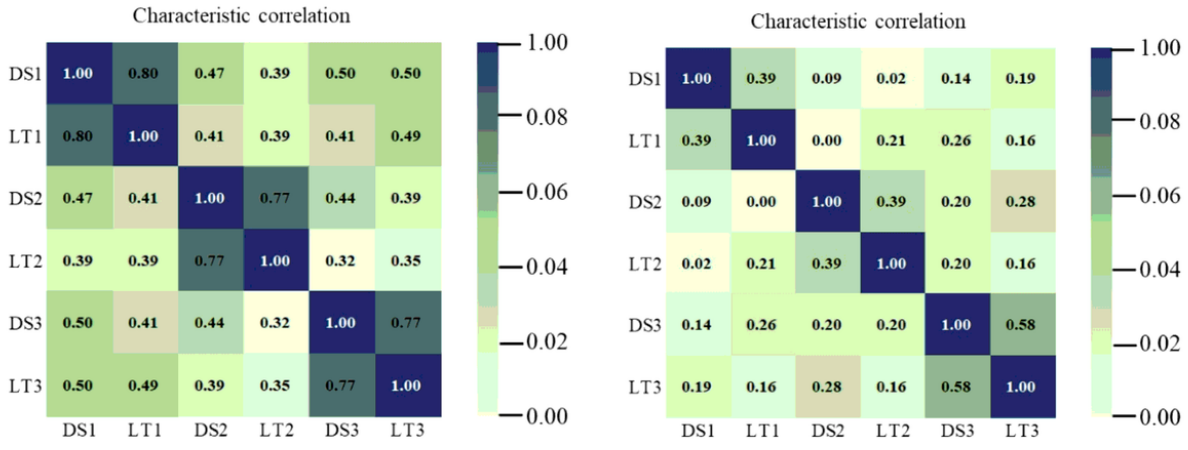
Figure 4

a Scattered colinear map between the genetic map and the reference genome for the RIL-YCML population. b Scattered colinear map between the genetic map and the reference genome for the RIL-YChang population. The X-axis is the genetic distance of each linkage group and the Y-axis is the physical length of each linkage group. Scatter points represent marker collinearity between genome and genetic map. The more diagonal the marker, the better the collinearity between the transferred map and genome



a YCML in Baoshan

b YCML in Dehong



c YChang in Baoshan

d YChang in Dehong

Figure 5

Correlations between variation in DS and LT. DS, disease scale; DS1, DS2, and DS3 means replicate 1, replicate 2, and replicate 3, respectively, and the rules apply to LT.

Supplementary Files

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