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Genetic mapping of northern corn leaf blight-resistant quantitative trait loci in maize

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Abstract

Northern corn leaf blight (NCLB), a corn disease infected by *Exserohilum turcicum*, can cause loss of harvest and economy. Identification or evaluation of NCLB-resistant quantitative trait loci (QTL) and genes could improve maize breeds. This study aimed to identify novel QTLs for NCLB-resistance.

Two maize strains (BB and BC) were utilized to generate B73 × B97 and B73 × CML322 and constructed the genetic linkage using high-throughput single nucleotide polymorphism (SNP) linkage map analysis of 170 (BB) and 163(BC) recombinant inbred line (RIL) genomic DNA samples. NCLB-resistant QTL was associated with phenotypic data from the field trial of 170 BB and 163 BC strains over two years using these 1100 SNPs to identify high-density NCLB-resistant QTLs.

In BB, QTL of the NCLB resistance was on chromosome 1 and 3 (LOD scores between 2.74 and 5.44); in BC, QTL of NCLB resistance was on chromosome 1, 2, 4, 8, and 9 (LOD scores between 2.52 and 8.53). A number of genes or genetic information related to NCLB resistance in both BB and BC were identified with the maximum number of genes/NCLB resistance-related QTL on chromosome 3 for BB and on chromosome 1 for BC.

This study successfully mapped and identified NCLB-resistant QTL and genes for these 2 different maize strains, which provides insightful information for future study of NCLB-resistance and selection of NCLB-resistant maize variants.

Abbreviations: CIMMYT = wheat improvement center in Mexico, NAM = nested association mapping, NCLB = northern corn leaf blight, QTL = quantitative trait loci, RIL = recombinant inbred line, SNP = single nucleotide polymorphism.

Keywords: NCLB-resistant genes, northern corn leaf blight, quantitative trait loci, single nucleotide polymorphisms

1. Introduction

Maize (*Zea mays* L.), also known as corn, is one of the major crops serving as a staple food for humans or animals in many parts of the world.[1] Recently, maize has been used as industrial material and bio-fuel in various countries, like the United States.[1] It has always been an eternal goal and a challenge for genetic breeders to pursue the continuous increase in corn production. However, diseases, especially major critical diseases, such as northern corn leaf blight (NCLB), could pose a significant threat to reduce the corn harvest because NCLB can cause over 30% loss of production[1,2]; for example, a most recent study showed increase in NCLB severity and incidence in Ontario, Canada, possibly due to the appearance of new races.[3] Thus, research for increase in the production of maize and generating disease-resistant strains is biologically and economically significant.[3] In order to generate disease-resistant strains for maize, an effective solution is to crossbreed and select resistant varieties and this would depend on scientific exploration and our better understanding of the genetic basis of disease resistance.[3,4] Accumulated evidence suggests that NCLB, one of the major maize diseases, is caused by different strains of *Exserohilum turcicum* pathogens that could carry different physiological properties and some newly formed strains of this pathogen are always capable of overcoming the naturally or genetically modified resistance of maize to the disease.[5] In this regard, identification or evaluation of NCLB-resistant quantitative trait loci (QTL) and genes could improve maize breeds to resist NCLB; for example, genome-wide association mapping could also reveal novel sources of resistance to NCLB resistance in maize.[6]

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The authors declare that there is no conflict of interest in this work.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Genetically, a QTL is defined as a chromosomal region or locus to correlate with a variation in a phenotype or quantitative trait. At the gene level, QTLs could typically carry or contain a gene or genes that control the phenotype or quantitative trait of a plant. In Maize, previous studies identified a number of QTLs to associate with disease resistance.[7] Before year 2000, NCLB resistance-related QTLs were studied by simple sequence repeat (SSR) and/or restriction fragment length polymorphism (RFLP) markers; however, such techniques revealed their disadvantage and inability to precisely map or linkage map QTLs at the gene level. For example, the linkage maps constructed by SSR and RFLP markers have an average distance of 10 to 20 cM, which shows a low density with many large spacer sections not covered. In addition, SSR and RFLP analysis procedures are complicated and time-consuming with a low degree of automation, and only process a limited number of samples at a given period of time. With the rapid development of research on human single nucleotide polymorphisms (SNPs), cDNA microarray and DNA sequencing technology, 3rd generation SNP markers[8] with a higher degree of automation have quickly replaced SSR, RFLP and other traditional markers. These markers have been utilized in the study of model animals, plants and major crops. This technique has a number of advantages, for example, full automation in sequence detection and data analysis, high density, strong representation and high reliability, leading SNP markers to be widely used in many fields of research, including plant genetic linkage mapping, species origin, phylogenetic relationship, and genetic polymorphisms.[8] In 2009, Buckler et al first constructed a nested association mapping (NAM) population containing 5000 recombinant inbred lines (RILs), through the hybridization of 25 inbred lines with their common parent B73. Their data indicated that NAM populations relative to the individual are more efficient in QTL detection with more reliable results. In this study, we first performed a field trial using 2 maize strains (BB and BC) to generate B73 × B97 and B73 × CML322 through crossbreeding (B73 is NCLB-susceptible and CML322 is an inbred population highly susceptible to NCLB, whereas B97 is NCBL-resistant,). We then constructed the genetic linkage maps using the high-throughput SNP linkage map analysis of genomic DNA samples from 170 BB and 163 BC RILs. After that, we utilized these 1100 identified SNPs to associate NCLB-resistant QTL with phenotypic data from the field trial of 170 BB and 163 BC strains conducted for over two years and identified high-density NCLB-resistant QTLs and genes. We expected to identify novel QTLs for NCLB-resistance, which could be used for the future molecular-based breeding of NCLB-resistant strains.

2. Materials and methods

2.1. Ethical statement

This study was not involved any human subjects or animal work; thus, our study was exempted from the IRB and IACUC reviews.

2.2. Materials

B73, B97, and CML322 were originally obtained from the Maize Research Group in the Institute of Crop Sciences at the China Agricultural Academy of Sciences. B73 was derived from an Iowa Stiff Stalk Synthetic (BSSS) variety after recurrently and selectively breeding for high-intensity stems with excellent lodging resistance, B97 inbred line originated from the US NSS group, and CML322 was a subtropical inbred line developed at the International Maize and Wheat Improvement Center in Mexico (CIMMYT). In this study, a field trial was performed to utilize BB (crossbred from B73 × B97) and BC (crossbred from B73 × CML322), which had 170 lines and 163 lines, respectively. NCLB pathogens were provided by the Department of Pathology and Agronomy, Jilin Agricultural University; which is officially used for diagnosing NCLB pathogens in Jilin, China. These pathogens comprise of a mixture of multiple physiological strains and were stored at −4°C.

2.3. Field trials and phenotypic data collection

We conducted the field trials between April 2013 and April 2014 at the Agricultural Experimental Station, Jilin Agricultural University (Jilin, China). These trials included BB (170 RILs) and BC (163 RILs), as well as three parent populations (B73, B97 and CML322; as controls). The experimental sites were located in a flat and uniform fertilization area. The trials were randomized at 2 sites with a 2 × 2 block design. The maize was planted as a single zone (3 m in length, 0.6 m row space, and 0.25 m seedling space), with two seedlings per planting hole; and planting density was 4500 per Chinese acre. The maize field was managed according to maize production standards.

In order to prepare the inoculation of NCLB pathogens to the maize, NCLB pathogens were grown in sorghums grains for 10 days. After washing off the pili, the pathogens were cultured for another two days to produce spores for subsequent inoculation. At the bell (6–7 leaves) and leaf peak stages, sorghums grains carrying the NCLB pathogen were inoculated onto the heart leaves of maize plants at approximately 4:00 PM in a day with previous rainfall or a day with possible rainfall. Plants were sprayed with water after inoculation if rainfall did not occur, to ensure the humidity necessary for NCLB pathogens to grow. On day 25 of plant spinning, leaf blight incidence was assessed. In case further inoculation was needed at the adult stage of maize plants, the pathogen strains were spread on the surface of the maize plant. The leaf blight disease was surveyed in each maize plant at the maize flowering stage, according to the nine-grade evaluation system (Fig. 1).

The data on each NCLB phenotypic character were summarized as mean ± standard deviation (SD) of duplicate plants, according to the grading system shown in Figure 1. Data were recorded in Microsoft Excel 2007 (Microsoft, Inc., Beijing, China), and statistically analyzed using SAS software (SAS, Cary, NC).

2.4. Construction of the genetic linkage map

Genomic DNA samples were collected from 170 RILs of BB, 163 RILs of BC, and 3 parental populations (B73, B97 and CML322; as controls); and were analyzed for SNP distribution in Illumina Maize SNP 500G Bead Chips (Illumina, San Diego, CA), according to manufacturer's standard operational protocols provided by the Life Sciences Core Laboratory Center (Institute of Genomic Diversity, Cornell University, Ithaca, NY). Then, SNP data results were compared to the maize genome database (www.panzea.org and www.maizeGDB.org) and genomically analyzed according to a previous study reported by Yan et al[9] The partition of linkage maps, calculation of SNP orders and positions within each linkage map, and linkage map illustration were generated using HighMap software (http://www.mybiosoftware.com/highmap-construction-and-analysis-of-high-density-linkage-map.html); in which maximum likelihood and smooth algorithm were utilized to analyze ten thousand markers, in order to make the map distance more stable.

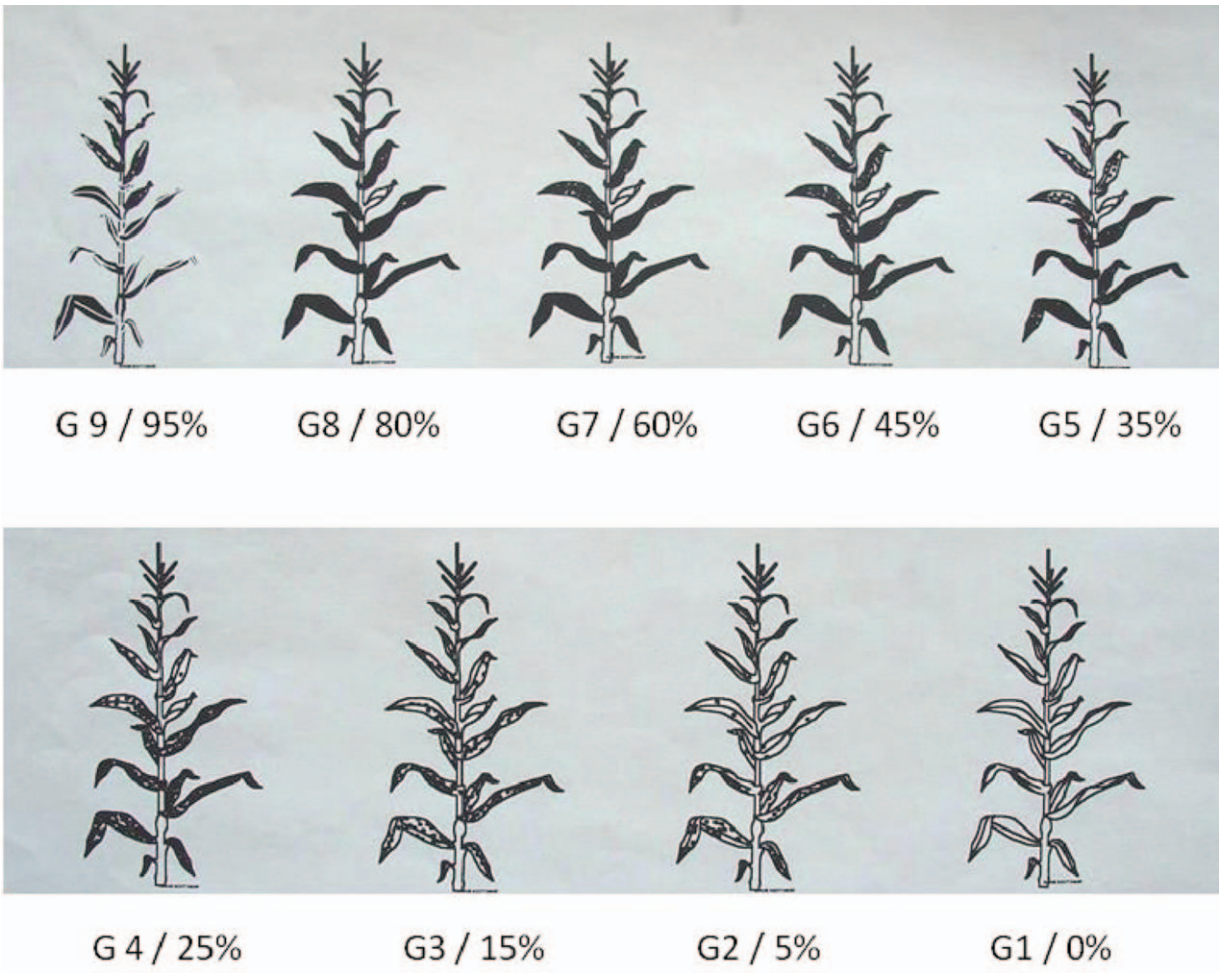

Figure 1. Grade of northern corn leaf blight disease-infected maize leaves. Imagines were collected from field trials and illustrate the grades of *Exserohilum turcicum*-infected maize leaves.

2.5. Assessment of NCLB-resistant QTLs and genes

The SNP data on the 170 RILs of BB, 163 RILs of BC, and three parental populations (B73, B97, and CML322 as controls) were summarized as mean ± SD. These data were used for QTL mapping assessment through the inclusive composite interval mapping (ICIM) method with the QTL loci Mapping software (Version 3.2; Integrated Breeding Platform, https://www.integratedbreeding.net/), according to a previous study.[10] In the QTL mapping process, each individual trait under each environment was evaluated using the 1000-randomly sampling test to determine the QTL logarithm of the odds ratio (LOD score) threshold. Other parameters were also included for QTL mapping, including a default window size of 10 cM, a background labeling of 5, background control by forward-backward stepwise regression, and step setting at 1 cM. After that, QTL mapping was assessed based on the threshold of each trait. LOD threshold was set to 2.50. When the actual LOD value was greater than this threshold (2.5), it was considered that there was a QTL in the chromosome region. In addition, QTLs were also estimated for the additive effect value and contribution rate; thus, a QTL was named according to the principle of McCouch et al. For example, a QTL is named by a capital letter for the environment initial, plus a capital letter for the population abbreviation, and the letter 'q'. This is followed by the abbreviation of the trait description, and the number of the chromosome that QTL localized; while numbers 1, 2, 3 . . . were used to represent multiple QTLs in case they were localized at the same chromosome.

2.6. Statistical analysis

The phenotypic data were summarized as mean ± SD and statistically analyzed using SAS software. Gene linkage and SNPs association with QTL and genes were analyzed using the ICIM method in the QTL loci Mapping software (Version 3.2). A P value $\leq .05$ was considered statistically significant.

3. Results

3.1. Phenotypic characterization of NCLB disease in BB and BC

In this study, we first performed a field trial using BB and BC to generate B73 × B97 and B73 × CML322. We found that the parental phenotypes of B73 were quite different from the other

Table 1

Phenotypes of 2 recombinant inbred lines (RIL) and their parents.

Trait	Year	Parents		B73 × B97				
		B73	B97	Mean	Range	*CV (%)*	Skewness	Kurtosis
	2013	5	6	5.917 ± 1.39	2.5–9	23.61	−0.195	−0.479
NCLB	2014	5.67	7.5	6.170 ± 1.4	3–9	22.80	−0.177	−0.915
Trait	**Year**	**Parent**		**B73 × CML322**				
		B73	**CML322**	**Mean**	**Range**	***CV (%)***	**Skewness**	**Kurtosis**
	2013	6	2	4.877 ± 1.72	2–9	35.44	0.383	−0.930
NCLB	2014	7.5	2	5.113 ± 1.66	2–9	32.64	0.130	−0.923

two parental strains of B97 and CML322 for their target traits (Table 1). Absolute values of the skewness and peak of NCLB in BB and BC were less than one under both environments, indicating normal distribution with QTL mapping. The correlation analysis of traits in different environments was significant for BB and BC (Table 2), indicating that phenotypic variations of BB and BC were attributed with genetic bases at a certain degree.

3.2. Genetic linkage mapping identification of SNPs in BB and BC

After that, we constructed the genetic linkage maps using high-throughput SNP linkage analysis of 170 (BB) and 163 (BC) recombinant inbred line (RIL) genomic DNA samples and obtained a total of 1100 SNP markers. These data demonstrated the same pattern in these 2 linkage maps of their 10 chromosomes (Table 3). Specifically, there were 174, 126, 130, 111, 137, 78, 78, 105, 84, and 77 markers on chromosomes 1–10, respectively, in both BB and BC (Fig. 2), which covered the genome with an entire length of 729.28 cM and an average distance of 0.66 cM for BB, and the genome with an entire length of 884.67 cM with an average distance of 0.80 cM for BC. In the BB linkage map, chromosome 1 carried the highest number (174) of SNP markers, while chromosome 10 had the least number (77) of SNP markers. The longest map distance was in chromosome 1 (115.01 cM), whereas the shortest map distance was in the tenth chromosome (40.98 cM). The longest average distance was in chromosome 2 (0.84 cM), while the shortest average distance was in the third and fifth chromosomes (0.52 cM).

Similarly, in the BC, the linkage map revealed that chromosome 1 carried the highest number (174) of SNP markers and chromosome 10 had the least number (77) of SNP markers. The longest map distance was 138.05 cM in chromosome 1, while the shortest map distance was 65.78 cM in chromosome 9. The longest average distance was 0.94 cM in chromosome 6, while the shortest average distance was 0.67 cM in chromosome 5. Detailed information for the SNP distribution is listed in Table 3.

Table 2

Traits of 2 recombinant inbred lines in different environments (year).

Line	Leaf blight	Leaf blight
	2013	2014
B73 × B97		0.86*
B73 × CML322		0.85*

* Significant difference at $P = .01$.

3.3. Mapping of NCLB-resistant QTL in BB and BC

Next, NCLB-resistant QTL in BB and BC were mapped using these SNPs. The complete interval mapping (ICIM) analysis and the data are listed in Table 4 and Figure 3. Briefly, in BB, the QTL of NCLB resistance was detected on chromosomes 1 and 3 with LOD scores ranging between 2.74 and 5.44; while in BC, the QTL of NCLB resistance was on chromosomes 1, 2, 4, 8, and 9 with LOD scores ranging between 2.52 and 8.53. The QTL of NCLB resistance was found in chromosome 1 for both BB and BC, but the QTL genome localization was different; that is, the position of 97.46 in BB vs. the position of 101.81 in BC.

Furthermore, the number of significant markers was identified for QTL-related NCLB resistance, and is shown in Tables 5 and 6. In brief, a great number of genes or genetic information was found to relate to NCLB resistance in BB and BC. The genes or genetic information were also precisely localized within the QTL in BB and BC (Supplementary Digital Content, Fig. 1, http://links.lww.com/MD/E612 and 2, http://links.lww.com/MD/E612). The maximum number of genes associated with NCLB resistance-related QTL was on chromosome 3 in BB (Table 5 and Supplementary Digital Content, Fig. 1, http://links.lww.com/MD/E612), which matched with the largest LOD scores for NCLB resistance on this chromosome, as described in Table 4. Similarly, in BC, the maximum number of genes associated with NCLB resistance-related QTL was on chromosome 1 (Table 6 and Supplementary Digital Content, Fig. 2, http://links.lww.com/MD/E612), which matched with the largest LOD scores (Table 4).

4. Discussion

Identification of disease-resistant or sensitive QTLs could provide a novel strategy to generate disease-resistant strains of crops, including maize. To date, the SNP-based linkage map is technically sound and useful to precisely map the phenotype of a plant through genetic linkage, such as QTL to a chromosomal region or locus, and to help researchers estimate or investigate the function of the chromosomal region or locus at gene levels.[11] A previous study demonstrated that the documentation and evaluation of stable or environment-specific QTL could result in the improvement of plant breeding[8] because the gene-level mapping of disease-related or resistant QTL can be utilized for the genetic dissection of quantitative disease resistance. In other words, the utilization of genetic markers could link a particular QTL to the phenotype of the disease resistance. Thus, the SNP marker-assisted mapping could help identify valuable quantitative traits, while better understanding of these traits would provide necessary genetic information to

Table 3

The numbers of SNPs in these 2 recombinant inbred lines.

	B73 × B97				B73 × CML322			
	#SNPs	Total distance	Average distance	Longest distance	#SNPs	Total distance	Average distance	Longest distance
Chr. 1	174	115.01	0.66	6.81	174	138.05	0.80	10.92
Chr. 2	126	104.98	0.84	9.61	126	110.77	0.89	9.41
Chr. 3	130	67.49	0.52	10.31	130	92.86	0.72	6.81
Chr. 4	111	87.60	0.80	8.41	111	98.19	0.89	8.28
Chr. 5	137	70.64	0.52	9.19	137	91.62	0.67	10.80
Chr. 6	78	43.59	0.57	5.86	78	72.72	0.94	9.79
Chr. 7	78	56.23	0.73	10.71	78	69.66	0.90	11.61
Chr. 8	105	83.63	0.80	11.94	105	78.85	0.76	11.25
Chr. 9	84	59.13	0.71	10.80	84	65.78	0.79	8.87
Chr. 10	77	40.98	0.54	6.08	77	66.17	0.87	22.27
Total	1100	729.28	0.66	11.94	1100	884.67	0.80	22.27

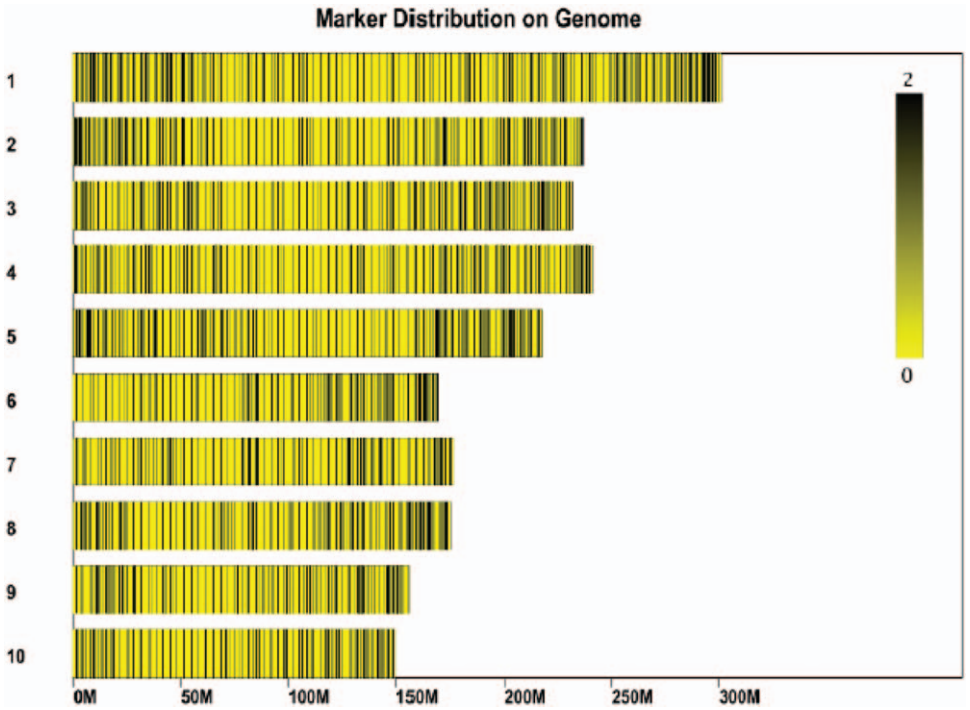

Figure 2. Distribution of SNP markers at different chromosomes. The X-axis illustrates length of different chromosomes. The yellow bar indicates the number of chromosomes. The genome was divided into 1 M units. A darker color on the chromosome indicates more numbers of SNP markers.

Table 4

Genetic mapping of NCLB-related quantitative trait loci in different environments.

QTL	Chr.	Position	Left marker	Right marker	LOD LOD peak	A	R2 (%)
BB line							
Leafblight	1	24.83	PZA02278.1	PZB00008.1	2.74	0.21	2.77
Leafblight	1	36.35	PZA03064.6	PHM4926.16	2.98	0.23	3.05
Leafblight	1	63.80	PZA03531.1	PZA02191.1	2.68	0.22	2.56
Leafblight	1	97.46	PZA01652.1	PZA00887.1	5.17	0.32	5.29
Leafblight	3	46.38	PZA02427.1	PHM4145.18	5.44	-0.33	5.53
BC line							
Leafblight	1	1.60	PZA02032.1	PHM2244.142	3.67	0.31	3.45
Leafblight	1	44.82	PZA01135.1	PZA03240.1_2	2.52	0.25	2.33
Leafblight	1	101.81	kip1.3	PZA01588.1	8.53	0.50	8.83
Leafblight	2	23.05	PHM5822.15	zfl2.9	3.43	0.31	3.25
Leafblight	4	26.65	PZB01461.1	PZA00344.10	6.28	0.43	6.36
Leafblight	8	34.12	PHM14152.18	PZA03612.2_1	4.27	0.34	4.17
Leafblight	9	32.88	PZA00060.2	PZA00213.19	2.62	0.26	2.44

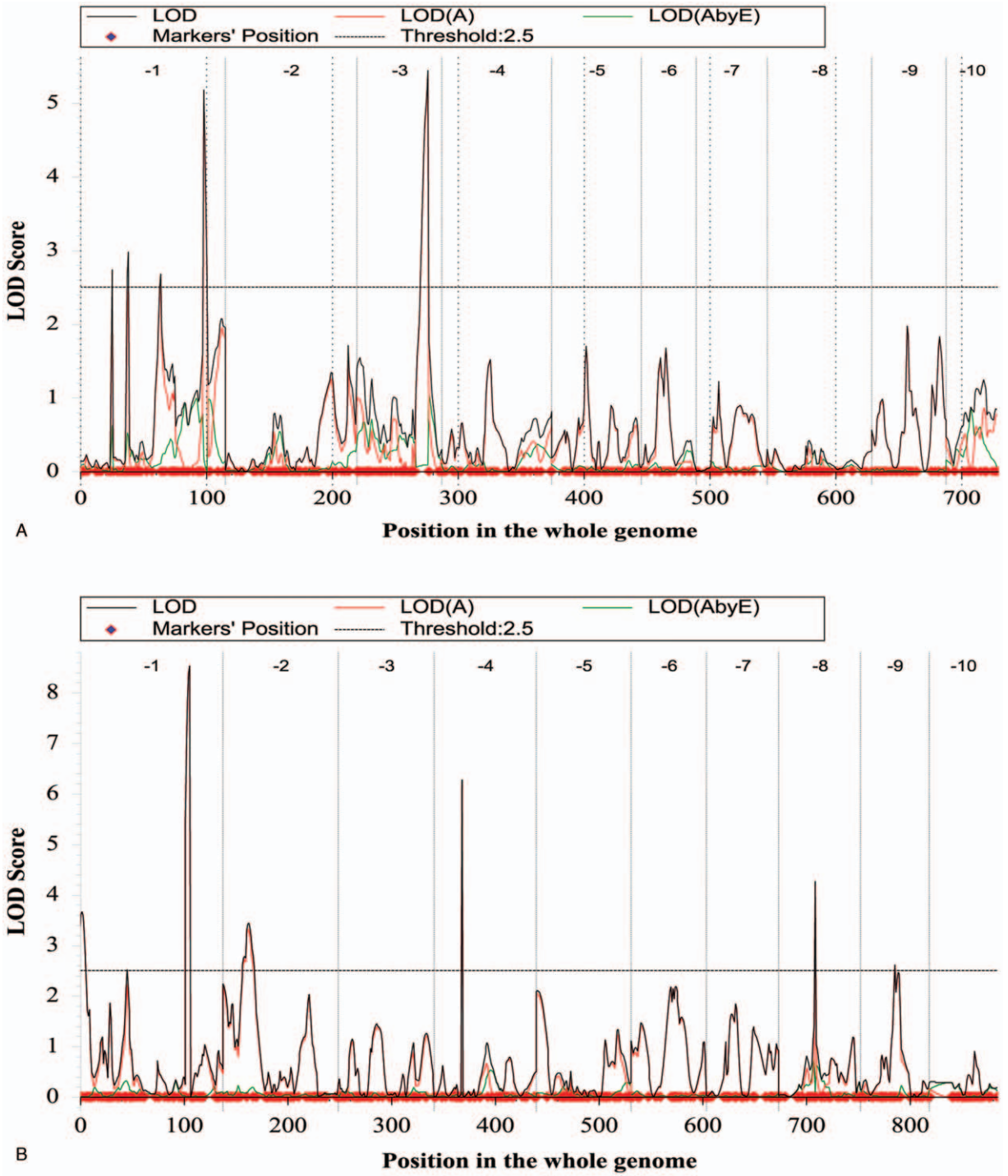

Figure 3. Mapping of northern corn leaf blight disease QTL. (A) BB population. QTLs were based on the complete interval mapping of SNP markers in BB maize. (B) BC population. QTLs were based on the complete interval mapping of SNP markers in the BC population.

specifically select disease-resistant strains in the future. For a long period of time, cost and throughput rate of large sample sizes were the major bottleneck for marker-assisted selection breeding. Thus, the current SNP-based genetic linkage mapping provides

(i) a high-throughput genotyping platform, and
(ii) the precise mapping of QTLs in a large amount of samples.

Indeed, a recent study showed that multiple loci-association mapping with candidate genes could help to identify the known plant disease-resistance pathways and found 49 SLB and 48 NLB resistance-related unique QTLs in such linkage mapping.[12]

ICIM is an additive, dominant, epigenetic and statistic approach that can be applied to map QTL for populations in a single environment through bi-parental crossbreeding. A

Table 5

Genes and genetic information of a candidate NCLB in the BB line.

Trait	#SNP	Chr ID	Start	End	#Gene	COG	GO	KEGG	Swissport	N
Leafblight	2	1	269,324,476	270,965,149	47	10	24	7	21	35
Leafblight	2	1	241,222,820	249,417,407	290	66	129	36	124	176
Leafblight	2	1	182,538,809	184,243,434	76	17	34	8	36	49
Leafblight	4	1	10,096,431	14,959,203	257	54	121	20	112	157
Leafblight	5	3	9,806,074	24,353,398	514	93	234	50	235	327

previous study has proven that ICIM is an efficient mapping tool with a high detection power, as well as a low false discovery rate and few biased estimates of QTLs.[13] In the current study, we utilized ICIM to perform a QTL mapping analysis of our SNP data, to assess the environment interaction between phenotypic data and genetic linkage during the 2-year period (year 2013 and 2014). Moreover, disease severity was typically used as a reliable phenotypic evaluation for the QTL mapping of NCLB resistance. Our present study assessed NCLB severity using the composite index, which included at least 7 components, such as lesion size, infection efficiency of inoculation dose, incubation or latency period, lesion expansion rate and number, or spore sporulation rate, according to previous studies.[14,15] Then, we separately identified unique QTLs for each of these 2 strains (BB and BC) using NCLB scores, and found significant differences in both positions and effects of these QTLs between these 2 strains. The identified QTLs at the different chromosomes of these 2 maize stains could be useful targets for the further mechanistic study of NCLB resistance in maize, after precise gene mapping and cloning procedures.

Furthermore, numerous QTLs linked to NCLB resistance in maize have been previously reported in literature. For example, 2 major QTLs, including one QTL at chromosome 1 (qNLB1.06) reported by Chung et al[16,17] and Jamann et al[18] and another QTL at chromosome 8 (qNLB8.06) reported by Chung et al,[16,17] were closely linked and functionally related to Ht2. These were precisely mapped and narrowed to 3.6 Mb and 0.46 Mb in chromosomes 1 and 8, respectively. To date, these QTLs have covered 89% of the maize genome,[19] and such data indicate the fairly low resolution of QTL mapping.[15] As such, use of high-density SNP markers in our current study could significantly contribute to improving the resolution of disease-associated QTLs. In fact, prior to this study, few recent studies have been conducted using high-density SNP markers and revealed the resolution of QTL mapping of NCLB resistance.[6,15] The QTL qNCLB5.04 localized at chromosome 5 was significantly associated with NCLB scores and lesion width.[15] Four QTLs associated with NCLB resistance was found in this chromosomal region and was previously detected in the NAM population[20]; and a candidate gene, GRMZM2G024612, in this QTL was also identified by subsequent association mapping. Another genomic region important for NCLB resistance was identified in chromosome 8, in which 2 associated genes (Ht2 and Htn1) were identified.[21–23] The gene Ht2 has been further delimited to a region of up to 0.46 Mb via precise mapping, and other candidate genes were predicted and annotated according to the reference genome sequences of B73 parental strain.[16,17] Except for chromosomes 5 and 8, there appears to be a relative paucity of NCLB resistance-related QTLs at other chromosomes. In the present study, we identified each unique QTL to associate with leaf blight resistance at chromosomes 1, 2, 3, 4, 8, and 9 respectively. To the best of our knowledge, these have never been reported before. We also found that two regions at chromosome 1 and one region at chromosome 3 might have a significant association with NCLB resistance-related QTL. In addition, the present study also provided the prediction of genes in each identified QTL region in these BB and BC strains. Future studies would investigate and identify particular genes and their functions in these identified QTL to understand the molecular mechanisms responsible for NCLB resistance in maize. A recent study revealed a durable wheat disease resistance gene Lr34 could confer common rust and NCLB resistance in maize, which is coded by a rare SNP of an ATP-binding cassette (ABC) transporter,[24] while a previous study revealed that Histatin-1 (Htn1), as a maize disease resistance gene against NCLB, encodes a wall-associated receptor-like kinase for resistance to adapted pathogens.[19]

In conclusion, in the present study, we built 10 linkage maps for these two strains (BB and BC) using the 1100 SNP markers that we identified. BB has a total of map distance of 729.28 cM with 0.66 cM as the average distance, while BC has a total map distance of 884.67 cM with 0.80 cM as the average distance. In analyzing these 10 linkage maps with their sub-type data and these 2 quantitative phenotypic values, we found a trait of NCLB-resistant locus in both BB and BC. Genes within the NCLB-

Table 6

Genes and genetic information of a candidate NCLB in the BC line.

Trait	#SNP	Chr ID	Start	End	#Gene	COG	GO	KEGG	Swissport	N
Leafblight	2	1	4,446,791	5,589,651	86	16	35	10	35	44
Leafblight	2	1	83,780,725	88,342,158	131	24	56	11	54	83
Leafblight	7	1	256,489,030	262,168,307	242	47	114	28	103	154
Leafblight	2	2	10,516,660	12,644,166	148	19	78	13	74	87
Leafblight	2	4	187,355,764	188,385,744	46	8	21	6	21	28
Leafblight	4	8	129,959,468	132,879,659	110	16	42	9	47	59
Leafblight	3	9	128,457,261	130,918,999	110	21	53	16	44	65

resistant chromosome loci were further predicted and functionally annotated. Thus, future studies would investigate whether QTLs of NCLB resistance in BB and BC can be identified in other environments with similar causal agents of NCLB. Moreover, future study would further identify candidate genes that are directly linked to the QTL of NCLB-resistance identified in the present study.

Author contributions

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Formal analysis: Yan Gao, Shi Lu.
Funding acquisition: Tianyu Wang.
Investigation: Mo Zhang.
Supervision: Piwu Wang, Tianyu Wang.
Writing – original draft: Haifeng Xia.
Writing – review & editing: Wei Gao.

References

[1] Smith A. The Oxford encyclopedia of food and drink in America (2 ed.). Oxford University Press, USA. 2013.
[2] Weems JD, Bradley CA. Exserohilum turcicum Race Population Distribution in the North Central United States. Plant Dis 2018;102:292–9.
[3] Jindal KK, Tenuta AU, Woldemariam T, et al. Occurrence and Distribution of Physiological Races of Exserohilum turcicum in Ontario, Canada. Plant Dis 2019;103:1450–7.
[4] Lopez-Zuniga LO, Wolters P, Davis S, et al. Using maize chromosome segment substitution line populations for the identification of loci associated with multiple disease resistance 2019;9: 189–201.
[5] Chang H, Fan K. Comparative studies on some biology and pathology of corn and broom corn isolates of Exserohilum turcicum (Pass) Leonard & Suggs. Bot Bull Acad Sinica 1986;27:18.
[6] Ding J, Ali F, Chen G, et al. Genome-wide association mapping reveals novel sources of resistance to northern corn leaf blight in maize. BMC Plant Biol 2015;15:206.
[7] Welz H, Geiger H. Genes for resistance to northern corn leaf blight in diverse maize populations. Plant Breeding 2000;119:1–4.
[8] Collard B, Jahufer M, Brouwer J, et al. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 2005;142: 169–96.
[9] Yan J, Shah T, Warburton ML, et al. Genetic characterization and linkage disequilibrium estimation of a global maize collection using SNP markers. PLoS One 2009;4:e8451.
[10] Buckler ES, Holland JB, Bradbury PJ, et al. The genetic architecture of maize flowering time. Science 2009;325:714–8.
[11] St Clair DA. Quantitative disease resistance and quantitative resistance Loci in breeding. Annu Rev Phytopathol 2010;48:247–68.
[12] Li YX, Chen L, Li C, et al. Increased experimental conditions and marker densities identified more genetic loci associated with southern and northern leaf blight resistance in maize. Sci Rep 2018;8:6848.
[13] Li S, Wang J, Zhang L. Inclusive composite interval mapping of QTL by environment interactions in biparental populations. PLoS One 2015;10: e0132414.
[14] Brewter V, Wicks Carson M Z. Mapping components of partial resistance to Northern leaf blight of Maize using reciprocal translocations. Phytopathology 1992;82:225–9.
[15] Chen G, Wang X, Long S, et al. Mapping of QTL conferring resistance to northern corn leaf blight using high-density SNPs in maize. Mol Breeding 2016;36:1–9.
[16] Chung CL, Longfellow JM, Walsh EK, et al. Resistance loci affecting distinct stages of fungal pathogenesis: use of introgression lines for QTL mapping and characterization in the maize–Setosphaeria turcica pathosystem. BMC Plant Biol 2010;10:103.
[17] Chung CL, Jamann T, Longfellow J, et al. Characterization and fine-mapping of a resistance locus for northern leaf blight in maize bin 8.06. Theor Appl Genet 2010;121:205–27.
[18] Jamann TM, Poland JA, Kolkman JM, et al. Unraveling genomic complexity at a quantitative disease resistance locus in maize 2014;198:333–44.
[19] Wisser RJ, Balint-Kurti PJ, Nelson RJ. The genetic architecture of disease resistance in maize: a synthesis of published studies. Phytopathology 2006;96:120–9.
[20] Poland JA, Bradbury PJ, Buckler ES, et al. Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. Proc Natl Acad Sci U S A 2011;108:6893–8.
[21] Zaitlin D, DeMars S, Gupta M. Linkage of a second gene for NCLB resistance to molecular markers in maize. Maize Genet Coop Newsl 1992;66:69–70.
[22] Simcox KD, Bennetzen JL. The use of molecular markers to study Setosphaeria turcica resistance in maize. Phytopathology 1993;83:1326–30.
[23] Hurni S, Scheuermann D, Krattinger SG, et al. The maize disease resistance gene Htn1 against northern corn leaf blight encodes a wall-associated receptor-like kinase. Proc Natl Acad Sci U S A 2015;112: 8780–5.
[24] Sucher J, Boni R, Yang P, et al. The durable wheat disease resistance gene Lr34 confers common rust and northern corn leaf blight resistance in maize. Plant Biotechnol J 2017;15:489–96.