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Full Length Article

Quantitative Trait Loci Mapping for Resistance to Curvularia Leaf Spot in Maize

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Abstract

Curvularia leaf spot is a chronic and persistent disease of maize (*Zea mays* L.) in China. This disease is caused by the fungus *Curvularia lunata.* The objectives of this study were to map genomic regions and locate consistent quantitative trait loci (QTL) related to resistance to Curvularia Leaf Spot in maize. This information can then be used to identify disease resistance genes and to develop markers for molecular marker-assisted breeding. We used 239 $F_{2:3}$ families (F_3 generation derived from selfed F_2 individuals) of a cross between Ma 664 (resistant) and H4074 (susceptible), and conducted field trials using a randomized complete block design with three replications. Data from 112 simple sequence repeat markers were used for linkage analysis. The locations of the QTLs on the linkage groups were determined using composite interval mapping. Eleven significant QTL were detected for resistance to *C. lunata*. Of them, three were major QTL (at Bin3.08, Bin503, and Bin10.04) with contribution rates higher than 10%. The major QTL at Bin10.04 was detected in three environments, which suggests that this region may contain a major gene for resistance to *C. lunata.* © 2020 Friends Science Publishers

Keywords: *Curvularia lunata*; Maize genetic linkage map; QTL mapping; Simple Sequence Repeats molecular marker

Introduction

Curvularia leaf spot is a significant foliar disease of maize that occurs in all maize cultivation regions (Liu *et al.* 2015a), but especially in north and northeast China, the main maize cultivation regions in China. This disease is caused by the fungus *Curvularia lunata*, and its severity depends on whether the climatic conditions are favorable for fungus development. The fungus can overwinter in the debris of diseased corn plants left on the soil surface, and conidia produced in the following spring can be spread by wind or splashing of droplets during rain events. The disease is prevalent in areas where dewy mornings are followed by hot humid afternoons and relatively cool nights. The disease occurs mainly during the maize reproductive period and damages the whole plant, which decreases quality and yield. In 1999, an outbreak of maize Curvularia leaf spot in Liaoning Province resulted in the infection of nearly 17,500 hectares of maize, which decreased maize production by 250 million kg (Li *et al.* 2002).

The development of maize cultivars resistant to Curvularia leaf spot through conventional breeding is one way to control the disease and ensure the security of corn production in China. However, conventional breeding of Curvularia leaf spot resistant cultivars has been difficult because of the complexity of the resistance trait. Although Curvularia leaf spot resistance is a highly heritable trait, it is controlled by many minor quantitative trait loci (QTLs) (Wang *et al.* 2014; Dong *et al.* 2015). Improvements in cultivation methods (Li and Mo 2015) and field management strategies have effectively reduced the incidence of Curvularia leaf spot in maize (Zhao *et al.* 2001). Although Curvularia leaf spot is now well contained in China, the combined effects of climate change, increasing areas of corn monocultures, and the narrow North Chinese resistant germplasm means that the disease still poses a serious threat to Chinese corn production.

Previous studies on the pathogenic mechanisms of *C. lunata* have identified and characterized some of the important genes and determined which pathways are involved in pathogenesis (Liu *et al.* 2016; Gao *et al.* 2017). However, relatively few studies have focused on the location of resistance genes in maize, and the mechanisms of maize disease resistance. Several previous studies have identified QTLs associated with resistance to Curvularia leaf spot, but research on their effects has obtained inconsistent results (Liu *et al.* 2009; Hou *et al.* 2013; Liu *et al.* 2015b). To date, no conserved QTL regions associated with

Curvularia leaf spot resistance under different environments have been found.

In this study, to identify consistent QTL intervals related to Curvularia leaf spot resistance, we obtained phenotypic data for maize in three environments (Gongzhuling; Changchun) across two years (2015 and 2016). The results of this study lay the foundation for the identification of resistance genes and the development of markers for molecular-assisted breeding.

Materials and Methods

Plant materials and field trials

The maize cultivar Ma 664 was selected by the breeder Yi-yong Ma (Jilin Agricultural University). This cultivar is strongly resistant to *C. lunata* (the disease showed the lowest grade, grade I, in a field trial in 2014). The cultivar H4074 was selected by the breeder Shu-yan Guan (Jilin Agricultural University). This cultivar is highly susceptible to *C. lunata* (disease grade 7 in the field trial). Therefore, a mapping population of 239 F_2 individuals was derived from the cross between Ma664 and H4074. In 2015, the 239 F_2 individuals, the two parent lines, and the F_1 generation were planted in Changchun, Jilin Province. In 2016, the $F_{2:3}$ families derived from selfed F_2 individuals were planted in Gongzhuling and Changchun, Jilin Province. Hereafter, the three groups constructed above are abbreviated as follows: F2, Rep1, and Rep2. At each location, the field experiment had a randomized complete block design with three replications for each genotype. Maize was planted with row lengths of 5 m, inter-plant spacing of 25 cm, and row spacing of 60 cm. There was a protective line around each plot, and conventional field management was employed.

Trait evaluation

The F_2 population and two $F_{2:3}$ family populations (Rep1, Rep2) at the 12–13 leaf stage were inoculated with *C. lunata* (provided by the Jilin Academy of Agricultural Sciences). The spore concentration of the inoculum was adjusted to 10–15 spores per field of view under 100x magnification. The inoculum was sprayed onto both sides of whole maize leaves until the solution dripped from the leaves. The evaluation criteria for the disease level were those specified by Hou *et al.* (2013). The incidence of different disease levels is shown in Fig. 1, and the different disease levels are described in Table 1.

Phenotypic data analysis

Pairwise comparisons of means of the parents' disease grades were tested for significance with t-tests implemented in SPSS 25 software (http://www.ibm.com/legal/copytrade.shtml). The phenotypic data of the F_2 population and populations of the two $F_{2:3}$ families were tested by Descriptive Statistics of S.P.S.S. 25 for normal distribution, where absolute values of kurtosis and skewness of less than 1 confirmed normal distribution.

Molecular data collection and linkage map construction

The DNA was extracted from the plant materials as described by Mu *et al.* (2010). Simple sequence repeat (SSR) markers covering the entire genome were selected from the maize genome database (http://www.maizegdb.org/) and screened to identify those that were polymorphic between the two parents. These markers were used to genotype the mapping population (F_2 population). Marker linkage analysis and construction of linkage maps were conducted using Ici Mapping 4.0. A limit of detection (LOD) threshold of 2.5 was used to assign markers to the same linkage group. The observed frequencies at each marker were tested against the expected Mendelian segregation ratio of 1:2:1 using a K^2 test for goodness of fit.

QTL analysis

The DNA extracted from members of the F_2 generation was subjected to PCR amplification and capillary electrophoresis detection using the selected markers. This procedure was used to genotype all members of the F_2 generation. The phenotypic data for the $F_{2:3}$ populations and the SSR marker molecular linkage map information were used to identify QTLs using IciMapping 4.0. We used the inclusive composite interval mapping (ICIM) method for single-environment QTL mapping of traits. A QTL was considered to be significantly correlated with resistance to Curvularia Leaf Spot when the LOD score was greater than 2.5. The genetic effects and phenotype contribution rates were analyzed. Each QTL was scored according to its dominance ratio (DR; DR = | d | / | a |). Thus, when DR < 0.2, the QTL was additive; 0.2 < DR < 0.8, the QTL was partially dominant; 0.8 < DR < 1.2, the QTL was dominant; and DR > 1.2, the QTL was super-dominant. Epistatic effects of QTLs were analyzed using the ICIM with epistatic interactions (ICIM-EPI) method.

Results

Genotype analysis and construction of maize genetic linkage map

We tested 650 SSR primer pairs and found that 150 pairs (23.08%) were sufficiently polymorphic between the two parents. The genotype data for the F_2 population were analyzed using SPSS software. The separation of 74.7% SSR markers in the F_2 population was consistent with a 1:2:1 segregation ratio. Of the 150 SSR polymorphic markers, 38 (25.3%) showed segregation distortion. Of those, 20 markers (52.6%) were biased to the female parent,

Table 1: Evaluation criteria of Curvularia Leaf Spot disease grades

Disease grade	Description of corresponding disease grade	Resistance level
1	No lesions or only sporadic lesions, area of lesions accounts for less than 5% of leaf area	Highly resistant (HR)
3	A few lesions, area of the lesions accounts for 6%–10% of the leaf area.	Resistant (R)
5	More lesions, accounting for 11%–30% of the leaf area	Middle resistant (MR)
7	Many lesions, some connected, accounting for 31%–50% of the leaf area.	Susceptible (S)
9	Whole plant is covered with disease spots, lesions are connected and account for more than 50% of the leaf area. Leaves die in late stages of the disease.	Highly susceptible (HS)

Table 2: Lengths, numbers, and distances between SSR markers for each maize linkage group

	Linkage groups										Average	Total
	1	2	3	4	5	6	7	8	9	10		
Chain length (CM)	243.0	199.5	180.5	187.5	175.9	131.2	162.0	144.5	139.6	140.0	170.4	1703.7
Number of linkage groups	15	13	10	11	13	10	11	10	10	9	11.2	112
Average spacing (CM)	16.2	15.3	18.1	17.0	13.5	13.1	14.7	14.5	14.0	15.6	15.2	

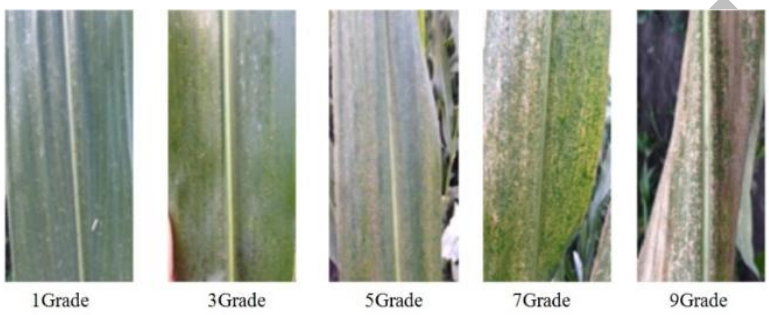

Fig. 1: Disease grades of Curvularia Leaf Spot in maize.

11 (28.9%) were biased to the male parent, and 7 (18.4%) were biased to the F1 generation.

The markers were unevenly distributed among linkage groups, and the distances between markers ranged from 6.25 and 29.70 cM, with no large gaps Fig. 4. The relative order of the markers was consistent with that on the genetic linkage map at the Maize GDB database, which indicates that our data conformed to QTL positioning requirements. The lengths, numbers, and distances between SSR markers are shown in Table 2.

Phenotype analysis

The incidence of resistance was significantly different between the two parental populations, as determined by independent samples t-tests (Table 3). This confirmed that these parents were suitable for the construction of populations for mapping QTLs related to resistance to Curvularia leaf spot.

The distribution of disease grades in the F2 and $F_{2:3}$ families is shown in Table 4. The disease grades were distributed evenly (1, 3, 5, 7 and 9), indicative of a continuous distribution of the resistance phenotype. This confirmed that the resistance of maize to Curvularia leaf spot is a quantitative trait controlled by multiple genes.

A descriptive statistical analysis of the F_2 and two $F_{2:3}$ populations was conducted. These analyses (Table 4 and 5) showed that 90% of the F_2 population had a disease level between those of the parents [average value of 5.52, skewness of 0.155, peak of 0.062, and standard deviation (SD) of 1.78]. The overall disease incidence in the population was consistent with a normal distribution (Fig. 2A). For the $F_{2:3}$ family Rep1 group, 98% of the population had a disease level between those of the parents (average 4.85, skewness 0.062, kurtosis 0.614, and SD 1.49). The overall disease incidence in the population showed a normal distribution (Fig. 2B). For the $F_{2:3}$ family Rep2 group, 98% of the population had a disease level between those of the parents (average 4.90, skewness 0.143, kurtosis −0.037, and SD 1.50). The overall disease incidence in the population showed a normal distribution (Fig. 2C). These results showed that resistance to Curvularia Leaf Spot in maize is a quantitative trait under polygene control, and confirmed that the population was suitable for QTL mapping.

QTL positioning

Four QTL loci were detected by genome-wide scanning of the F_2 population, based on a combination of genotypic and morbidity data. Single QTLs were located on chromosomes 1, 3, 8, and 10 (Fig. 3). The whole genome was analyzed by ICIM-EPI with LOD > 2.5 as the unit. Epistatic interactions among QTLs were not detected. The names, numbers, and effects of QTLs related to leaf spot resistance in the F_2 population are shown in Table 6. The detected QTLs accounted for 40.33% of the phenotypic variation in

Table 3: T-tests of disease incidence levels between parents

Year	Levene's Test for Equality of Variances		T-test for Equality of Means	
	F	Sig	T	Sig
2015	0.924	0.345	-19.008	0.000
2016	7.338	0.011	-16.310	0.000

Table 4: Disease grade distribution in maize parents, and F_1, F_2 and F_2-derived populations

Year	Generation	Disease Grade					Total Plants	Mean ± SE
		1	3	5	7	9		
	P_1	12	3	0	0	0	15	1.40 ± 0.214
2015	P_2	0	0	3	13	0	15	6.73 ± 0.182
	F_1	0	5	10	0	0	15	4.33 ± 0.252
	F_2	5	32	122	56	24	239	5.52 ± 0.115
	P_1	13	2	0	0	0	15	1.27 ± 0.182
2016	P_2	0	0	5	10	0	15	6.33 ± 0.252
	F_1	0	6	9	0	0	15	4.20 ± 0.262
	$F_{2:3}$rep1	6	53	138	37	5	239	4.85 ± 0.966
	$F_{2:3}$rep2	3	60	126	46	4	239	4.90 ± 0.969

Table 5: Descriptive statistics of F_2 population and its two derived $F_{2:3}$ families

Segregation population	Mean ± SE	Standard deviation	Skewness ± SE	Kurtosis ± SE
F2	5.52 ± 0.115	1.78	0.155 ± 0.157	0.062 ± 0.314
$F_{2:3}$rep1	4.85 ± 0.966	1.49	0.062 ± 0.157	0.614 ± 0.314
$F_{2:3}$rep2	4.90 ± 0.969	1.50	0.143 ± 0.157	-0.037 ± 0.314

Table 6: Results of QTL mapping for resistance to *Curvularia lunata* (ICIM LOD>2.5)

Population	Name	Bin	Position	Distance to markers	Marker interval		LOD score	PVE(%)	Additive effect	Dominant effect	Gene action
					Left	Right					
F2	qCLS1.10	1.10	212.00	0.22-11.75	bnlg1347a	umc1862	3.65	4.87	-0.26	-0.68	OD
	qCLS3.08	3.08	130.00	26.86-2.84	umc2269	bnlg1108	8.78	14.00	-0.92	0.24	PD
	qCLS8.01	8.01/8.02	26.00	7.12-11.29	umc1483	umc1913	4.29	7.05	-0.67	-0.04	A
	qCLS10.04	10.04	75.00	0.15-19.77	mmp121	umc1506	10.48	14.42	-0.90	-0.40	PD
Rep1	qCLS1.02	1.02	29.00	13.02-3.96	bnlg1014	umc1467	4.64	8.90	-0.58	0.34	PD
	qCLS5.07	5.07	139.00	0.28-10.83	umc1375	umc2013	4.40	6.69	-0.55	0.11	A
	qCLS7.01	7.01/7.02	38.00	5.73-10.43	umc1270	bnlg1247	2.51	4.55	0.45	-0.03	A
	qCLS10.04	10.04	75.00	0.15-19.77	mmp121	umc1506	7.53	11.60	-0.68	-0.29	PD
Rep2	qCLS2.10	2.10	193.00	7.54-6.53	bnlg1893	umc2214	3.97	5.21	-0.48	-0.06	A
	qCLS5.03	5.03	62.00	0.44-15.54	umc1705	bnlg1902	8.56	10.62	-0.68	-0.19	PD
	qCLS6.05	6.05	72.00	2.05-16.45	umc2141	bnlg1732	3.68	4.60	-0.17	-0.58	OD
	qCLS9.01	9.01	7.00	7.00-3.11	umc1957	umc1867	4.67	6.03	-0.45	0.36	PD
	qCLS10.04	10.04	75.00	0.15-19.77	mmp121	umc1506	14.75	19.00	-0.90	-0.21	PD

Note: Bin indicates the corresponding Bin interval on the MaizeGDB map of the QTL; Position indicates QTL position on chromosome (CM); "a-b" in the "Distance to markers" column indicates that the distances of the QTL locus to left and right marks is a and b respectively(CM) ;"Left" and "Right" of the Marker interval indicates the left and right markers of the QTL interval; LOD indicates log10 of the likelihood odds ratio; PVE（%）represents the phenotypic variance percentage that owe to the corresponding QTL.A, D, PD and OD in the "Gene action" column represent additive effects, dominant effects, partial dominance effects and super-dominant effects, respective

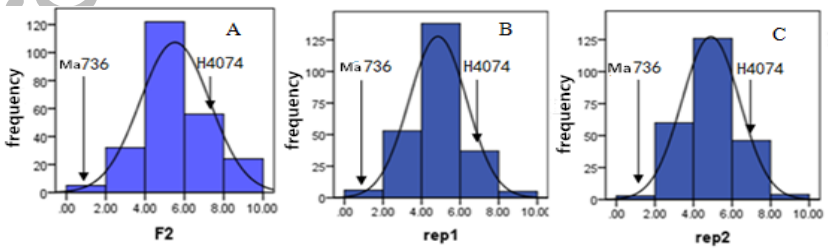

Fig. 2: Disease grade distribution in F_2 population and its $F_{2:3}$ families.

resistance. The QTLs in the intervals umc2269–bnlg1108 (on chromosome 3) and mmp121–umc1506 (on chromosome 10) made the largest contributions to phenotypic variance (14.00% and 14.42%, respectively). The QTLs associated with the bnlg1347a–umc1862 interval accounted for 8.77% of phenotypic variation (LOD 3.65). The QTL located on umc1843–umc1913 on chromosome 8 accounted for 7.05% of phenotypic variation (LOD 4.29). The additive effect of each QTL was negative, which indicates that all the QTLs related to low disease incidence

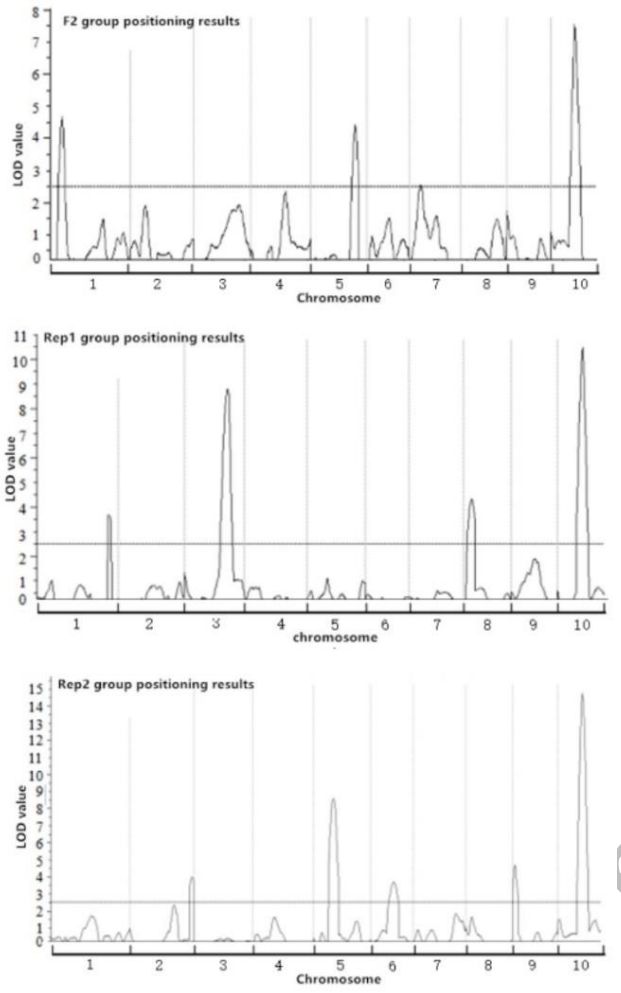

Fig. 3: QTL mapping results for each maize population

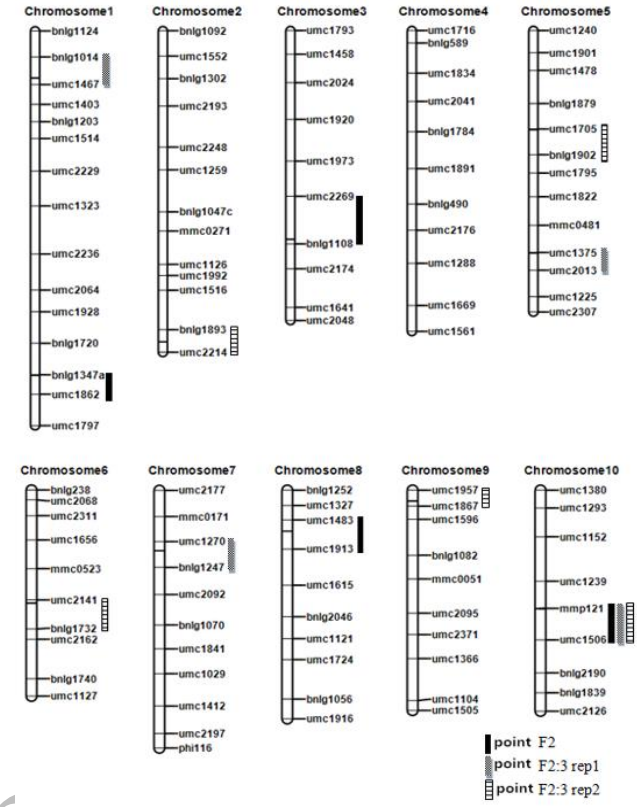

Fig. 4: Maize genetic linkage map showing distribution of QTLs for resistance to Curvularia Leaf Spot in maize

were from the resistant parent Ma 664. According to the DR ratio proposed by Stuber *et al*. (1992), qCLS1.10 (DR = 2.62), qCLS3.08 (DR = 0.26), qCLS8.01 (DR = 0.06) and qCLS10. 04 (DR = 0.44) were super-dominant, partially dominant, additive, and partially dominant, respectively.

Four QTL loci (Fig. 3) located on chromosomes 1, 5, 7, and 10 were detected in the $F_{2:3}$ Rep1 population and accounted for 31.74% of the phenotypic variation in resistance. As shown in Table 6, except for qCLS7.01 (in the umc1270–bnlg1247 interval on chromosome 7), the other QTLs had negative additive effects, which indicates that they were derived from the disease-resistant parent Ma 664, while qCLS7.01 was inherited from the female parent H4074. The QTLs qCLS10.04 (DR = 0.43) and qCLS1.02 (DR = 0.59) were partially dominant, and accounted for 8.9% and 11.60% of the phenotypic variation in resistance, respectively. The qCLS7.01 (DR = 0.07) locus located at umc1270–umc2013 in the umc1375–umc2013 interval and the umc1270–bnlg1247 interval on chromosome 7 showed additive effects, and accounted for 6.69% and 4.55% of the phenotypic variation in resistance, respectively. Among the QTLs, qCLS10.04 on chromosome 10 was detected in the $F_{2:3}$ Rep1 and F_2 populations, and was partially dominant. The IGEM-EPI algorithm was used to analyze the epistatic interactions of the whole genome, and no epistatic interactions were detected among these QTLs.

Five QTLs (on chromosomes 2, 5, 6, 9, and 10) were detected in the $F_{2:3}$ Rep2 population (Fig. 3) and accounted for 45.46% of the phenotypic variation in resistance. As shown in Table 6, all of the QTLs had negative additive effects, which indicates that the QTLs from the male parent reduced disease incidence and improved resistance. The qS2.10 locus on chromosome 2 was an additive effect QTL (DR = 0.13) located at bnlg1893–umc2214 (LOD 3.97) that accounted for 5.21% of the phenotypic variation. The qCLS6.05 locus on chromosome 6 was super-dominant (DR = 3.41), was located at umc2141–bnlg1732 (LOD 3.68), and accounted for 4.60% of the phenotypic variation. There are still major obstacles for using super-dominant QTLs in crop breeding. Therefore, this QTL needs to be further investigated. The QTLs qCLS9.01 (DR = 0.8) and qCLS10.04 (DR = 0.23) on chromosomes 9 and 10, respectively, were partially dominant. They were located at umc1957–umc1867 and mmp121–umc1506, respectively (LOD 4.67 and 14.75, respectively) and accounted for

6.03% and 19.00% of the phenotypic variation, respectively. qCLS10.04 on chromosome 10 was detected in the F_2 and $F_{2:3}$ Rep1 populations and had the same genetic effect in both populations. It had high LOD values and phenotypic variation contribution rates; therefore, we consider that this is a stable QTL related to resistance to Curvularia Leaf Spot in maize. This QTL can serve as the starting point to identify candidate resistance genes through fine positioning mapping. The IGEM-EPI algorithm was used to analyze the epistatic interactions of the whole genome, and no epistatic interactions were detected among the analyzed QTLs.

Discussion

A consistent environment is required to accurately assess the potential of plant genotypes to resist the onset and progress of Curvularia leaf spot, and to determine the magnitude of the genetic factors that contribute to resistance. This is because the development of Curvularia leaf spot is extremely sensitive to environmental conditions. In this QTL mapping study, we obtained phenotypic data for Curvularia leaf spot resistance of maize in two years (2015 and 2016) at sites in Changchun and Gongzhuling. In both years, the summer was humid and relatively hot. These environmental conditions made it possible to assess the level of Curvularia leaf spot resistance in the segregating populations.

We conducted interval mapping at the LOD threshold of 2.5, and detected 11 QTLs related to resistance to Curvularia leaf spot. Of the three main QTLs (at Bin3.08, Bin5.03, and Bin10.04), the QTL on chromosome 10 was consistently detected in three environments. In another study, a stable QTL for resistance to Curvularia leaf spot was detected at the same site (Bin10.04) in analyses of an $F_{2:3}$ family population of Shen 137×Huangzao 4 (Hou *et al.* 2013). The results of that study and our study indicate that Bin10.04 on chromosome 10 is a stable main QTL for resistance to Curvularia leaf spot.

The additive and dominant effects of QTLs can differ among various genetic backgrounds and/or among the same materials in different years. The additive and dominant effects of the consistent QTL located in Bin10.04 differed between the two years and the three environments, but this QTL was inherited dominantly, which is consistent with the findings of Hou *et al.* (2013). All of the QTLs detected in this study had different additive and dominant effects, but were predominantly additive and partially dominant. Zhao *et al.* (2002) studied the inheritance of resistance to Curvularia leaf spot using the ADAA model and found that the resistance of maize was mainly additive and dominant.

When we searched the Maize-GDB database, we did not find any Curvularia leaf spot resistance-related QTLs in the marker interval corresponding to the QTL loci located in this study. However, we found that qCLS1.10 (bnlg1347a–umc1862), located in this study, is located in a sugarcane borer resistance QTL region (bnl8.29a–umc106a), and the umc2269–bnlg1108 interval of qCLS3.08 partially overlaps with igc3b–umc63a, a QTL associated with resistance to the European corn borer. In addition, qCLS5.03's umc1705–bnlg1902 interval contains a gray leaf spot resistance-related QTL (near umc43). Thus, this area may represent a large QTL marker interval that includes many different QTL or the loci targeted in this study, which may be multi-effect QTL.

In this study, the F_2 population and $F_{2:3}$ families were used as locating groups, and F_2 was used as the mapping population. The phenotypic values of several individuals were substituted for the F_2 representative values, thereby reducing the effects of environmental factors on plant traits and enabling the repeated trial of multiple points. Hou *et al.* (2013) noted that self-crossing of F_2 individuals yields $F_{2:3}$ families with reduced heterozygous genotypes, resulting in low estimates of QTL-associated dominant effects. Here, we compared the genetic effects of the consistent QTL in Bin10.04 in the F_2 population and two $F_{2:3}$ families, and we reached the same conclusion. In studies on maize QTLs, the $F_{2:3}$ phenotypic mean has often been used instead of the F_2 phenotype to account for the shortcomings of the F_2 generation (Lu *et al*. 2002; Park *et al*. 2013; Hou *et al*. 2015; Liu *et al*. 2016). Those studies identified genetic loci associated with important agronomic traits of maize using $F_{2:3}$ families as the locating populations and successfully mapped stable QTLs. Here, we located QTLs for Curvularia leaf spot resistance in maize that stably exists in different environments. This confirmed the feasibility of using $F_{2:3}$ families as QTL-locating populations.

Segregation distortion skews the genotypic frequencies from their Mendelian expectations (Lu *et al.* 2002). In this study, 38 (25.3%) of 150 of polymorphic markers showed segregation distortion in the F_2 population. Of these 38 markers, 20 (52.6%) were biased towards the female parent, 11 (28.9%) were biased towards the male parent, and seven (18.4%) were biased toward the F_1. This is consistent with the results reported by Lu *et al*. (2002). Since Mangelsdorf and Jones (1926) first reported segregation distortion in maize, many researchers have detected this phenomenon when studying maize linkage maps (Bentolila *et al*. 1992; Gardiner *et al*. 1993; Murigneux *et al*. 1993; Pereira and Lee 1995). Liu and Yang (2015) constructed a maize genetic linkage map and found that 12 (33.3%) out of 31 polymorphic markers showed segregation distortion; one (8.33%) was biased to the male parent, two (16.67%) were biased towards the heterozygote, and five (41.67%) were unbiased. There are many reasons for the segregation distortion of molecular markers. Lu *et al.* (2002) studied the segregation of SSR molecular markers in maize and found that most chromosomes have functional genes that cause segregation distortion of markers. This affects the normal separation of alleles and determines the direction of segregation distortion. The ratios of marker segregation distortion are positively correlated with the generation of the population.

This is because there is unequal selection between male and female gametes in the process of meiosis and combination of gametes. Molecular markers that show segregation distortion are located in particular regions of chromosomes. In this study, markers on all chromosomes showed segregation distortion, and those showing segregation distortion were located in certain hot spots on chromosomes. The segregation distortion of molecular markers leads to inconsistencies between the marker recombination rate and the genetic distance of the marker, which reduces the accuracy of the genetic linkage map. Some studies have reported that segregation distortion of molecular markers has little effect on the location of loci (Hackett and Broadfoot 2003; Zhang *et al*. 2010). However, we found that the segregation distortion of markers introduced errors into the genetic linkage map, so that the relative map position of the molecular marker was different from that in Maize-GDB. Therefore, when this occurred, the marker was removed from the genetic linkage map to reduce mapping errors.

The construction of a high-density and precise genetic linkage map is a prerequisite for the accurate detection of QTLs. In this study, we detected stable differences between parents and the separation of F_2 populations in accordance with the 1:2:1 genotypic ratio of 112 SSR markers, which were divided into 10 linkage groups. A linkage map of maize molecular markers was constructed. The relative sequences of the markers were consistent with the genetic linkage map at the Maize-GDB database. The total length of the map was 1,703.7 cM and the average density was 15.2 cM, in accordance with QTL-locating requirements. With the application of single nucleotide (SNP) molecular marker technology in maize gene mapping, more precise genetic linkage maps have been constructed. These maps have made it easier to fine-map maize QTLs (Zou and Song 2003; Pan *et al*. 2011; Warburton *et al*. 2015; Song *et al*. 2017).

Molecular marker-assisted screening of stress-resistant crops is extremely important and is a very effective breeding method. The QTL detected in Bin10.04 in this study has potential uses in marker-assisted selection in breeding, but further research is required to identify the gene(s) responsible for resistance. It can be risky to use QTL-linked markers for breeding when their effects on other agronomic traits are unknown, or when the mechanism of the genetic effect is unclear (Tanksley and Hewitt 1988). Epistatic effects can also influence molecular marker-assisted selection. Vasal *et al*. (1970) found that epistatic interactions, especially those among dominant genes, are major genetic effects related to leaf spot resistance. Epistatic interactions among QTLs were not detected in this study. Appropriate combinations of populations and genetic mating design are essential to accurately detect the presence of epistatic effects among QTLs. Therefore, further research to analyze the genetic effects of QTL at Bin10.04 and the interaction with the environment are of great significance for the molecular marker-assisted selection of maize lines resistant to Curvularia leaf spot.

Conclusion

We detected three main QTLs, one each on chromosomes 3, 5, and 10 (Bin3.08, Bin5.03, and Bin10.04). The QTL on chromosome 10 was detected in three environments. This locus may contain a stable resistance gene. Further research is required to identify and characterize this gene.

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Author Contributions

Jian-Bo Fei and Zhao-Xu Dong planted maize populations; Zhi-Bo Liu and Dong-Liang Jin collected phenotypic data; Jing Qu, Si-Yan Liu, Yi-yong Ma, and Shu-Yan Guan obtained genotype data, Jian-bo Fei wrote the manuscript with contributions from Zhao-Xu.

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