Fine-mapping the recently discovered QTL qMrdd2 that confers resistance to maize rough dwarf disease

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Research article

Abstract

Background: Maize rough dwarf disease (MRDD) is a disease caused by a virus that seriously affects maize yield and quality worldwide. Rice black streaked dwarf virus (RBSDV) in the Fijivirus genus in the Reoviridae family causes MRDD in maize. Typical MRDD symptoms of include severe dwarfing of plants, shortening of internodes. MRDD resistance is a complex trait that is quantitatively inherited and is controlled by several quantitative trait loci (QTL). MRDD is most efficiently controlled by the cultivation of disease-resistant corn hybrids. Results: Disease resistance in the MRDD-resistant Qi319 and -susceptible Ye478 parental inbred lines and the 314 recombinant inbred lines (RILs) that were derived from a cross between them was evaluated across three environments. A stable resistance QTL, qMrdd2 , which explained 8.64 to 11.02% of the total phenotypic variance in MRDD resistance, was identified repeatedly and was mapped using BLUP values to a 0.55-Mb region between the markers MK807 and MK811 on chromosome 2. We validated the effect of qMrdd2 using a chromosome segment substitution line population that were derived from a cross between maize inbred Qi319 as the resistance donor and Ye478 as the recipient. The disease-severity index (DSI) of CSSL haplotype II harboring qMrdd2 was significantly lower than the DSI of susceptible parent Ye478 ($P < 0.05$). Mapping results using CSSLs were consistent with localization interval determined using RILs. The qMrdd2 locus acted with an additive effect but no significant dominant gene action in conferring MRDD resistance. We fine-mapped qMrdd2 locus into a 315-kb region flanked by the markers RD81 and RD87 by testing recombinant-derived progeny using selfed backcrossed families. Conclusions: qMrdd2 is a recently discovered QTL from Qi319 for resistance to MRDD with an additive effect but no significant dominant gene action for MRDD resistance. qMrdd2 was fine-mapped to a 315-kb interval on maize chromosome 2. Introgression of the MRDD resistance allele at the qMrdd2 locus of CSSL haplotype 2 using linked markers umc1824 and bnlg125 will be useful for maize breeding to reduce yield losses caused by MRDD. Keywords: Maize, Maize rough dwarf disease, QTL, Fine-mapping, RIL CSSL.

Background

Maize is an important food, feed, and energy crop with an essential role in agricultural production and economic development. Various diseases of maize have been discovered that seriously affect the yield and quality of maize. MRDD is a major viral disease that adversely affects maize production. In 1949, corn shrinkage disease due to MRDD was first reported in Italy, followed by Argentina, France, Spain, Iran, and Greece [1−4]. Since the 1990s, changes in climate and crop rotation patterns have resulted in widespread MRDD in the Huanghuaihai corn production area of China [5]. Rice black-streaked dwarf virus (RBSDV), the pathogen that causes MRDD, is a member of the Fijivirus genus in the Reoviridae, a family of viruses that also includes maize rough dwarf virus (MRDV), Mal de Río Cuarto virus (MRCV), and southern rice black-streaked dwarf virus (SRBSDV) [6−9]. In China, the small brown planthopper (SBPH) vector, Laodelphax striatellus transmits RBSDV, which causes MRDD by its. The typical symptoms of MRDD are severe dwarfing of plants; shortening of internodes; thickened, short, and stiff green leaves, slow development of roots, and malformed or underdeveloped male and female ears [10−12]. Plants with severe disease will die early, which results in decreased yield. In addition, the disease can also harm crops such as barley, wheat, oats, sorghum, and millet, with similar symptoms [13−14]. The occurrence of MRDD is affected by many factors, such as the number of vectors, the presence of host weeds, field layout, crop rotation, environmental conditions, and the disease resistance of plant varieties. At present, the adjustment of sowing dates and the use of chemical agents for preventing and controlling disease are common approaches for

limiting MRDD. Appropriate adjustment of the sowing period to allow corn seedlings to avoid the peak period of inoculation with RBSDV by adult planthoppers can reduce the MRDD incidence rate [15]. However, with their disadvantages of high risk and low efficiency, the use of these methods can still result in significantly decreased maize yield. Chemical controls increase the costs of economic inputs and can cause environmental pollution. Therefore, MRDD is most efficiently controlled by cultivating excellent disease-resistant corn hybrids, which is also safer for the environment than the use of pesticides [16–18].

The identification of maize germplasm that is resistant to MRDD is the basis for the cloning MRDD resistance genes and the breeding of resistant maize varieties. Many studies conducted in China have screened for MRDD-resistant corn germplasm under conditions of natural MRDD infection. At present, many studies have effectively combined disease-resistant maize inbred lines with breeding results based on heterosis groupings (BSSS, PA, Lancaster, Lvda Red Cob, Sipingtou, and PB) to produce disease-resistant hybrid maize [19–22]. The most disease-resistant heterotic subgroup, PB, exhibits a disease incidence rate of 1.6% and some of the highest rates of disease resistance. Resistance to MRDD in China was inherited from US maize hybrid P78599 and the MRDD-resistant lines derived from it include Qi319, Shen137, SH15, 89 – 1, Golden 59, X178, and P138 [19–22].

MRDD resistance is a complex quantitative trait that is controlled by many genes with small effects [23–30]. $F_{2:3}$ QTL mapping has allowed detection of two QTL that together explained 36.2% of the phenotypic variance in resistance to MRDD in $F_{2:3}$ progeny of the MRDD-resistant inbred BLS14 and the susceptible inbred Mo17 [25]. In the F_2 population of a cross between resistant inbred 90110 and susceptible inbred Ye478, three QTL located in chromosome bins 6.02, 7.02, and 8.07 [26] together explained 20% of the phenotypic variance in MRDD resistance. A similar study using RILs derived from 90110 × Ye478 identified five QTLs, one of which was a major QTL in chromosome bin 8.07 that explained 28.9% of phenotypic variance in resistance to MRDD [27]. In 2011, a major QTL from the resistant inbred line X178 detected in chromosome bin 8.03 explained between 24.6% and 37.3% of the phenotypic variance in MRDD resistance [28]. The major recessive QTL that could explain 24.6 to 37.3% of the phenotypic variance in resistance to MRDD, qMrdd1, which originated from resistant inbred X178, was identified in a 1.2-Mb region of bin 8.03 [29]. Subsequently, qMrdd8 was fine-mapped into a 347-kb interval in which one SNP and two InDels were significantly associated with MRDD resistance [30].

In the present study, the genetic effects of qMrdd2, which confers MRDD resistance, were analyzed using the RIL and CSSL populations derived from a cross between Ye478 and Qi319 to identify and verify regions of the maize genome containing this QTL. qMrdd2 was then fine mapped by performing recombinant-derived progeny testing with self-pollinated families. The objectives of the present study were to identify, verify, and fine-map the qMrdd2, which confers resistance to MRDD in Qi319; to identify markers that are tightly linked to the qMrdd2 region, and to develop these markers for subsequent marker-assisted selection (MAS) of MRDD resistance.

Results

Phenotypic evaluation of resistance to MRDD in parents and RILs

The MRDD resistance of parental inbred lines Qi319 (MRDD-resistant) and Ye478 (MRDD-susceptible) and 314 RILs derived from a cross between them was evaluated at Xuzhou in 2015 and 2016 and at Xinxiang in 2016. Descriptive statistics for MRDD resistance in the three environments are shown in Table 1, and in Figure 2A and 2B. ANOVA revealed highly significant differences ($P < 0.01$) between the resistant inbred line Qi319, with an

average DSI of 13.75, and the susceptible inbred line Ye478, with an average DSI of 71.35 in the three environments. The parental lines Qi319 and Ye478 consistently exhibited either MRDD resistance or susceptibility in each year and location (Figure 2A and 2B). The average DSI of each RIL was used to represent the disease resistance of each line. The continuous, normal distributions of phenotypes in the field from highly MRDD resistant to completely susceptible reflected the quantitative control of MRDD resistance in maize (Figure 2C). The broad-sense heritability (H^2) of resistance to MRDD ranged from 76.98 % to 81.67 % in the RILs (Table 1). Meanwhile, the parents Qi319, Ye478, and their F_1 were also mock-inoculated by virus-free SBPH as a control. The numbers of surviving virus-free SBPH did not differ significantly ($P > 0.05$) among Qi319, Ye478, and their F_1 from the first day to the seventh day (Figure 2D), which implied that the QTL controls resistance to MRDD, not to the SBPH pest.

Identification of QTL for MRDD resistance

In the present study, a stable QTL for resistance to MRDD that explained 8.17 to 11.02 % of the total phenotypic variation in MRDD resistance across three environments was identified on chromosome 2 using genotypes and phenotypic values in the 314 RILs (Table 2 and Figure 3A). Alleles for MRDD resistance came from the resistant parent Qi319 and increased phenotypic values for MRDD resistance in all three environments. In 2015, the significant QTL *qMrdd2* was identified between MK806 and MK811 on chromosome 2 (LOD score = 5.83) and explained 8.17% of the phenotypic variation in MRDD resistance (Table 2). In 2016, another significant QTL for resistance to MRDD was detected in the same region and explained 8.64% and 11.02% of the total phenotypic variance at Xuzhou and Xinxiang, respectively. The CIs for these two QTL covered average physical distances of 0.15 Mb on the B73 RefGen_v3 reference genome (Table 2). The stable resistance QTL, designated *qMrdd2*, was identified repeatedly and was mapped using BLUP values into a 0.55-Mb region between the bin markers MK807 and MK811 on the B73 RefGen_v3 genome.

Validation the effect of *qMrdd2* in CSSL populations

The effect of *qMrdd2* was investigated in a CSSL population derived from a cross between Qi319 and Ye478 that carried different haplotypes for the segments covering chromosome 2. The average background recovery rates by molecular MAS (BC_5F_2) of these eight CSSLs ranged from 91.45% to 99.62% (Table 4). Combined with the DSI (%) field phenotypic values for plants in three different environments, we found that CSSL haplotype II, which carries the introgressed segment including *qMrdd2* flanked by linked markers umc1824 and bnlg125, was associated with MRDD resistance, with DSIs of 20.01%, 56.73%, and 51.71%, respectively (Table 4, Figure 3B and 3C). The DSI of CSSL haplotype II harboring *qMrdd2* was significantly lower than that of the MRDD-susceptible parent Ye478 ($P < 0.05$) in three different environments (Figure 3B and 3C). The length of the introgressed *qMrdd2* fragment in CSSL haplotype II was about 7.0 Mb, according to B73 RefGen_v3 (Table 4). These mapping results were consistent with the mapping of this interval using RILs and indicate that *qMrdd2* could be used to improve resistance to MRDD. Therefore, we knew that the introgression of CSSL haplotype II using the linked markers umc1824 and bnlg125 could be performed to construct secondary backcross populations or selfed segregating populations for fine-mapping of *qMrdd2*.

Fine-mapping of *qMrdd2*

To fine-map *qMrdd2*, we designed 150 InDel primer pairs within the *qMrdd2* region based on 30× genome sequencing of the resistant parent Qi319 and the susceptible parent Ye478, and detected 13 polymorphic InDel markers between Qi319 and Ye478, as shown in Table 3. The flanking SSR markers umc1824a and bnlg125 were first used to identify recombinants from 6000 BC_1F_2 plants and 6000 F_3 plants in the winter nursery of Hainan in 2016. The recombinants were self-pollinated to generate BC_1F_3 and F_4 families and their genotypes were resolved using the 13 polymorphic InDel markers distributed throughout the *qMrdd2* region. Finally, a total of 16 recombinant haplotypes were detected (Figure 4).

In 2017, the recombinant-derived families represented 16 haplotypes, 10 of which were homozygous, were selected and planted for fine-mapping of *qMrdd2* in three locations. The MRDD resistance of each of the 10 homozygous recombinant-derived families (Haplotypes VII−XVI) were evaluated for under conditions of natural infection and planted with Ye478 in a 1:1 ratio (Figure 4). Most of the haplotypes VII−XI were highly susceptible to MRDD and most of the haplotypes XII−XVI were highly resistant to MRDD regardless of genotype in three locations. The exceptions were haplotype X in Xuzhou, for which the DSI did not significantly differ from Ye478 (Student's t-test, $P > 0.05$), and haplotypes XII−XVI, for which DSIs differed significantly from Ye478 (Student's t-test, $P < 0.05$) (Fig. 4). These results indicated that *qMrdd2* was located between RD56 and RD114. The DSIs of haplotype X in Xuzhou differed significantly from that of Ye478. At the same time, 1079 BC_1F_3 individuals derived from 28 recombinants including six haplotypes I−Ⅵ were selected and planted in Xinxiang, Xuzhou, and Jining. Within the heterozygous region of *qMrdd2*, the three genotypes of the BC_1F_3 individuals were Qi319/Qi319, Ye478/Ye478, or heterozygous Qi319/Ye478. The DSIs of these three genotypes were evaluated separately under conditions of natural infection and calculated for each BC_1F_3 family. Analyzing the combined genotypic and phenotypic data revealed significant differences in DSI among the three genotypes for haplotype I at any field location. This result implied that *qMrdd2* could be located within the heterozygous region but not in the homozygous Qi319 region. The remaining haplotypes (II-VI) were resistant to MRDD as no significant differences were detected among the three genotypes according to one-way ANOVA ($P > 0.05$). This result implied that *qMrdd2* could be located within the homozygous Qi319 region but not in the heterozygous region. Based the above results, the *qMrdd2* was fine-mapped into a 315-kb interval between the markers RD81 and RD87.

Model of gene action for MRDD resistance controlled by *qMrdd2*

We examined the nature of the gene action controlling resistance to MRDD by *qMrdd2* in F_2 families categorized according to their genotypes within *qMrdd2* region between markers RD81 and RD87, Qi319/Qi319, Qi319/Ye478, and Ye478/Ye478. The DSI of plants that were homozygous (Qi319/Qi319) for the Qi319 allele in the *qMrdd2* region was significantly lower than in the plants with homozygous Ye478 genotypes (Ye478/Ye478). The DSI for heterozygotes was intermediate between those of the homozygous Qi319 and Ye478 genotypes (Figure 5). In Jining, 750 F_2 plants were evaluated for resistance to MRDD. the plants with the homozygous genotype Ye478/Ye478 (DSI 88.65%) had a higher DSI ($P < 0.05$) than those with the homozygous genotype Qi319/Qi319 (DSI 50.02%). Furthermore, the DSI of plants carrying the heterozygous genotype Qi319/Ye478 (DSI 74.20%) was close to the mid-parent value of the homozygous genotypes Qi319/Qi319 and Ye478/Ye478. In Xinxiang, 500 F_2 plants were evaluated for resistance to MRDD and a similar model for the gene action of *qMrdd2* was found. DSIs were 55.19%, 82.92%, and 61.04% for plants carrying homozygous Qi319/Qi319,

Ye478/Ye478, and heterozygous Qi319/Ye478 alleles, respectively. These results indicate that *qMrdd2* exerts an additive effect but no significant dominant gene action on MRDD resistance.

Discussion

A recently discovered QTL conferring MRDD resistance in maize

The genetic basis for quantitative genetic traits often involves multiple QTL, including those with large and small effects [28-32]. Relative to traits controlled by one or a few genes, quantitative genetic traits controlled by QTL, especially major QTL, are vital genetic resources for improving crop traits. Because MRDD is a complex viral disease, it was important to choose a suitable resistant parent for identifying disease resistance in segregating progeny as the basis for QTL mapping. Several MRDD-resistance loci have so far been reported in the maize genome. Several of these candidate genes for disease resistance loci have been fine-mapped or cloned. For example, as mentioned above, a major recessive QTL, *qMrdd1*, explaining 24.6 to 37.3 % of the phenotypic variance in resistance to MRDD, originated from the MRDD-resistant inbred X178 and was identified in a 1.2-Mb region of bin 8.03 [29]. Later, *qMrdd8* was fine-mapped into a 347-kb interval in which one SNP and two InDels were significantly associated with MRDD resistance [30]. Five other QTL located on chromosome 2 (bin 2.02), 6 (bin 6.02), 7 (bin 7.02), 8 (bin 8.07), and 10 (bin 10.05) that explained 11.9 to 34.8 % of the phenotypic variance in MRDD resistance, were identified using F_2 and BC_1 populations combined with bulked segregate analysis (BSA) [27]. Many screens for MRDD resistance in maize germplasm have been conducted in China under natural MRDD infection conditions. The major sources of MRDD resistance currently used in China have been derived from the US maize hybrid P78599 and include Shen137, SH15, 89-1, Golden 59, X178, and P138 [19-22]. In our study, we consistently detected a stable resistance QTL, derived from the inbred Qi319 in the PB heterotic group and mapped *qMrdd2* using BLUP values (Table 2) to a 0.55-Mb region between the bin markers MK807 and MK811, according to the B73 RefGen_v3 genome. The PB group includes tropical maize germplasm from the Americas. However, the haplotypes carrying the resistance gene *qMrdd8* derived from X178 are different from the haplotypes carrying the resistance gene *qMrdd2* derived from Qi319, which suggests that these two MRDD-resistant materials (X178 and Qi319) might possess different mechanisms of resistance to MRDD [30]. These results further suggest that Qi319 could serve as a parent for breeding new disease-resistant hybrids. Future experiments using lines derived from X178 and Qi319 should also help clarify the genetic mechanisms of MRDD resistance and will have important theoretical and applied consequences for breeding maize varieties resistant to MRDD.

Validation of the genetic action of *qMrdd2* in both RILs and CSSLs

Both genetic background and the environment can influence the resistance of plants to viruses [33, 34]. Choosing a suitable mapping population for phenotyping disease resistance is essential for QTL mapping. Compared with traditional mapping populations, the number of markers used to construct genetic linkage maps is relatively small, so their resolution is low and the confidence intervals for targeted QTL are typically between 10 and 20 cM. Such large intervals make molecular marker-assisted breeding difficult and imprecise and also limits the deep analysis of genes conferring disease resistance in maize. Genotyping-by-sequencing (GBS) has become a popular new method for acquiring dense genome-wide markers and has been successfully used for genetic studies in a variety of species [35, 36]. In two studies, an F_2 and the US-NAM population were subjected to GBS to increase marker densities to 6533 and 5296 markers, respectively [37, 38]. A high-density linkage map with 4183

bin markers and an average marker interval of 0.37 cM was constructed to map QTL for flowering and plant architecture-related traits in a maize RIL population [39]. In the present study, a stable QTL on chromosome 2 that explained 8.17 to 11.02% of the total phenotypic variation in MRDD resistance was identified in three environments using genotypes and phenotypic BLUP values in 314 RILs (Table 2 and Fig. 3A). The studies have fully demonstrated the feasibility, accuracy, and efficiency of GBS technology for the construction of genetic maps in maize.

The phenotypic differences between CSSLs and their recurrent parents can be considered to be due to the introduction of the fragment [40]. Therefore, a QTL located in the chromosome segment carried by the introduced fragment can be identified by analyzing the phenotypic differences between a CSSL and its recurrent parent, and provides material for the exploration of the QTL controlling a target trait [40]. Because CSSLs can eliminate most of the interference from the genetic background the gene/QTL introduced by the fragment can be regarded as a single Mendelian factor in a near-isogenic line, which lays the foundation for fine mapping of the gene responsible for the QTL [41-43]. CSSLs allow genetic effects to be more accurately estimated in particular backgrounds [40]. Stepwise regression in data from 130 CSSLs was used to detect 11 QTL for kernel row number (KRN) in three environments, that explained from 9.87 to 19.44% of phenotypic variation in KRN [43]. In the present study, eight CSSLs covering chromosome 2 were identified to detect and validate the genetic effects of *qMrdd2* on MRDD. The DSI of CSSL haplotype II harboring *qMrdd2.02* differed significantly from that of the susceptible parent Ye478 ($P < 0.05$) in three environments (Figure.3B and 3C). The consistent results for QTL mapping in our study between RILs and the CSSLs further verify the presence of *qMrdd2* in the segment and demonstrate the feasibility of identifying QTL for resistance to diseases using a combination of RILs and CSSLs.

Application of QTL for resistance to MRDD in maize breeding

By using molecular MAS technology, the breeding of maize for MRDD resistance could be accomplished quickly by searching for molecular markers closely linked to the target gene and then screening for maize plants carrying the target fragment. Despite the great successes in identifying QTL for different traits in many different species and identifying the functional genetic variants behind these QTL, the success of molecular MAS has sometimes been limited [44]. When MAS was used to introgress the major QTL *qHSR*1 for resistance to head smut into 10 smut-susceptible maize inbreds, the head smut resistance of all 10 of these converted inbreds and their hybrids was improved substantially, while other agronomic traits were mostly unchanged [45]. The gibberella stalk rot resistance of elite maize *ZmCCT* haplotypes without transposable elements in their promoters was enhanced and yield-related traits were also improved, without changes in flowering time [46]. The QTL *qMrdd8* from the donor parent X178 was introgressed into seven elite inbred lines from three maize heterotic groups using multi-generation backcrossing and MAS. The seven converted inbred lines and five converted hybrids exhibited enhanced resistance to MRDD across different environments, while other agronomic traits were not affected under non-pathogenic stress conditions [16]. A wheat line carrying the gene for resistance to powdery mildew, *Pm21*, as donor parent was backcrossed to three spring wheat varieties by combining evaluation for disease resistance with selection using molecular markers. After five backcrosses and four generations of self-pollination, nine converted lines with high resistance to PM and good agronomic traits were developed [47]. As mentioned above, here, the DSI of CSSL haplotype II harboring *qMrdd2* significantly differed from that of the susceptible parent Ye478 ($P < 0.05$) in three different environments (Fig.3B and 3C). This result was consistent with localization *qMrdd2* the RIL population and indicated that *qMrdd2* could improve resistance to MRDD. Thus, introgression of the MRDD resistance allele at the *qMrdd2* locus or the introgression of CSSL haplotype II using

linked markers umc1824 and bnlg125 will be useful for improving the MRDD resistance of hybrid maize adapted to growing regions in China.

Conclusions

MRDD has some of the most significant negative economic impacts among diseases affecting maize in China. Most commercially cultivated maize varieties are susceptible to MRDD. So far, breeding maize hybrids for MRDD resistance has efficiently minimized losses caused by MRDD. The *qMrdd2* locus has recently been identified as an effective MRDD resistance QTL from Qi319, with an additive effect but no significant dominant gene action for resistance to MRDD, as shown by experiments in multiple environments using 314 RILs. Using advanced backcross and self-pollinated populations derived from CSSLs, we fine-mapped *qMrdd2* into a 315-kb interval containing the tightly linked marker RD-26. Meanwhile, the DSI of CSSL haplotype II, which harbors *qMrdd2.02*, was significantly different from that of the susceptible parent Ye478 or the other CSSLs. The introgression of the MRDD resistance allele at the *qMrdd2* locus or CSSL haplotype II using the linked markers umc1824 and bnlg125 into appropriate backgrounds will help reduce MRDD-related maize yield losses. These results provide new insights into the genetic basis of MRDD resistance and have potential value for enhancing maize grain yield.

Methods

Plant materials

Plant materials were obtained from the Institute of Crop Science, Chinese Academy of Agricultural Science in China. A RIL population comprised of 314 F_{11} individuals was developed from a cross between the maize inbred lines Qi319 (MRDD resistant) and Ye478 (MRDD susceptible) by single-seed descent. The MRDD-resistant line Qi319, which belongs to the PB heterotic group was originally developed from the US hybrid 78599, and the MRDD-susceptible line Ye478 was developed from the U8112 × 5003 cross. Using the MRDD-resistant line Qi319 as the donor and the MRDD-susceptible line Ye478 as the recurrent parent, 200 CSSLs were developed using a combination of crossing, selfing, and molecular MAS. Detection of SSR markers was performed as described by Wang et al [48]. The lengths of substituted chromosomal segments were assessed using graphical genotypes [49]. Detailed methods and processes we followed for developing CSSLs were described in a previous study in rice [50]. We characterized the 2 to 4 introgression segments in each CSSL in our study according to the physical positions and genotypes of 201 SSRs. The 201 SSRs were evenly distributed along all 10 maize chromosomes at an average marker interval of 9.94 Mb. Both the parental lines and RILs were evaluated for MRDD resistance in two fields including one in Xinxiang (35.05°N, 113.96°E), Henan Province, China in 2016 and one in Xuzhou (34.79°N, 116.57°E), Jiangsu Province, China in 2015 and 2016. The Xuzhou growing area experiences severe outbreaks maize rough dwarf disease. All of the plant materials including the 314 RILs and two parental lines were arranged in the fields in randomized incomplete blocks with two replications per location. In each block, the parents Qi319 and Ye478 were planted as MRDD-resistant and -susceptible controls, respectively. The plots for each line consisted of approximately 17 plants in individual 4-m rows spaced 0.6 m apart at a planting density of 60,000 plants/ha. Standard agricultural management practices for maize were followed during each growing season at each location.

Evaluation of plants for MRDD symptoms

To evaluate the effects of MRDD, plants were grown in three locations including Xinxiang (35.05°N, 113.96°E) in Henan Province, Xuzhou (34.79°N, 116.57°E) in Jiangsu Province, and Jining in Shandong Province, which allowed MRDD infection of plants to occur under natural conditions. All fields were planted on May 16 each year to coincide with SBPH infestations when RBSDV, which causes MRDD, is transmitted. At the R6 maturity stage, we visually assessed the MRDD resistance of all plants and assigned disease scores on a scale from 0 to 4, in which plants scored 0 are highly resistant and those scored 4 are highly susceptible to MRDD [30]. We defined the DSI [30] as: DSI (%) = S (disease rating score number of plants at each score)/maximum disease rating score total number of plants rated in the line) ´100. The phenotypic data for all plants across different replicates were assessed independently. In order to verify whether plant materials were resistant to virus-free adult planthopper, 100 virus-free adult planthoppers were sealed in boxes with Qi319, Ye478, and F_1 plants at the V3 stage, and the survival rate of virus-free adult planthoppers was assessed daily from the first day to the seventh day (with six plants per box and five replications). Multiple comparisons using Student's t-test were used to determine whether any differences in mean survival rate for virus-free SBPH were significant.

Linkage map construction and QTL detection

The 314 RILs were genotyped using a GBS approach on an Illumina HiSeq2500 platform. A high-density genetic map was constructed from a total of 88,268 high-quality SNPs with 4183 bin markers. The map of the RIL population comprised a total genetic distance of 1545.65 cM covering all 10 maize chromosomes with an average physical distance between adjacent markers of ~0.51 M. This detailed genetic map has been described in a previous study [39]. Phenotypic data for resistance to MRDD were collected from the population of 314 RILs during field experiments conducted from 2015 to 2016 in Xuzhou and Xinxiang. A linkage map was constructed by inclusive composite interval mapping (ICIM) using QTL IciMapping software 4.0 [51] and analyzed together with phenotypic information to identify QTL for resistance to MRDD. The positive and negative signs of the estimates indicated whether resistance effects for QTL with additive effects were inherited from Ye478 or Qi319, respectively. Taking each location as an environment and for each of the datasets (2015, 2016, and BLUP), the significance threshold for identifying a putative QTL was set at a logarithm of odds (LOD) score > 3 with 1000 permutations at $P < 0.05$ [51].

Validation of *qMrdd2* using the CSSL populations

To validate the effect of *qMrdd2*, eight CSSLs that covered all of chromosome 2 were developed with Ye478 as the recurrent parent and Qi319 as the donor of the randomized MRDD resistance allele tracked using a combination of crossing, backcrossing and molecular MAS (BC_5F_2) with average background recovery rates from 91.45 % to 99.62 %. In 2015 and 2016, the MRDD resistance of parental lines and CSSL haplotypes I through VIII were evaluated in Xuzhou and Xinxiang. Field designs were arranged in randomized incomplete blocks with three replications per location and the parents Qi319 and Ye478 were planted in each block as resistant and susceptible controls, respectively. Approximately 17 plants from each line were grown in single 4-m rows spaced 0.25 m apart. Student's t-test was used to perform multiple comparisons for the MRDD resistance of each genotype.

Genotyping and marker development

Genomic DNA was extracted from leaves of plants at the five-leaf stage using a CTAB procedure following the protocol of Murray and Thompson [52] with modifications. The quality and quantity of DNA samples used for

marker genotyping was assessed by evaluating DNA samples on 1.0% agarose gels and by measuring absorbances using a spectrophotometer (Nanodrop 2000, Thermo Scientific, US). We obtained the SSR primer sequences for our study from the MaizeGDB (http://www.maizegdb.org/). InDel markers were developed from 30×genome sequence data for the resistant parent Qi319 and susceptible parent Ye478 [39]. These InDels were designated with the prefix RD and screened for polymorphisms between Qi319 and Ye478. SSR or InDel primers used for genotyping of plants were synthesized by AuGCT Biotechnology Co. Ltd., China. Each PCR reaction mixture contained 6.8 μL double-distilled water, 1.2 μL 10× Buffer, 0.5 μL dNTPs (2.5 mM), 0.15 μL each primer (0.01 nmol/μL), 0.2 μL *Taq* DNA polymerase (5 U/μL), and 1 μL template DNA in a 10-μL total volume. The touchdown PCR program for amplifying these markers included an initial denaturing step at 94 °C for 4 min, followed by 10 cycles of 30 s at 95 °C, 30 s at 65 °C, and 30 s at 72 °C, with the annealing temperature decreasing by 1 °C per cycle; followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C; and ending by extending for 5 min at 72 °C. The PCR products were then electrophoretically separated on 8% polyacrylamide gels in 1× TBE buffer that were then silver stained for visualization of PCR products.

Fine-mapping of *qMrdd2*

We carried out the recombinant-derived progeny tests to fine-map *qMrdd2* (Fig. 1) [53]. Based on the QTL region mapped using RILs and CSSLs, CSSL-31 (haplotype II) was crossed as a male parent with Ye478 to produce an F_1 in Yunnan in 2015. The 25 F_1 progeny were then self-pollinated to produce the F_2 and crossed with Ye478 to produce the BC_1F_1 in a winter nursery in Hainan in 2015. In the summer of 2016, 850 F_2 and 1000 BC_1F_1 were genotyped, and new recombinants mapped within the region containing the QTL were self-pollinated to produce F_3 and BC_1F_2 progenies at Jining and Xinxiang under natural infection conditions. In the winter of 2016, 6000 F_3 and 6000 BC_1F_2 progeny were planted in the winter nursery and screened for new recombinants. F_3 and BC_1F_2 recombinants with a homozygous Qi319 genotype at the flanking marker on one side and a homozygous Ye478 genotype of on the other side were then selfed. At the same time, we selfed the heterozygous recombinant BC_1F_2 progenies to produce segregating BC_1F_3 populations heterozygous at the flanking marker on one side and homozygous at the flanking marker on the other side. BC_1F_3 recombinants and F_3 recombinants were then classified into different haplotypes by developing markers. These segregating populations and homozygous recombinants were then used to fine map *qMrdd2*.

In the summer of 2017, we detected differences in DSI under natural infection in Jining, Xinxiang, and Xuzhou between the homozygous families derived from recombinants and Ye478 using Student's *t*-test in SAS version 9.2. DSIs differing significantly ($P < 0.05$) between homozygous recombinant-derived families and Ye478 indicated that *qMrdd2* was located within a homozygous Qi319/Qi319 segment, whereas DSIs differing insignificantly ($P \geq 0.05$) indicated that *qMrdd2* was located within a homozygous Ye478/Ye478 segment. Each homozygous recombinant and Ye478 were grown in plots of approximately 17 plants in 4-m rows spaced 0.6 m apart with three replications per location. At the same time, 150-300 kernels randomly selected from each plot representing the progeny of diverse types of BC_1F_2 recombinants were planted to evaluate for MRDD resistance under natural inoculation conditions in Jining, Xinxiang, and Xuzhou. Each BC_1F_2 recombinant could be categorized as carrying one of two possible segments, heterozygous Qi319/Ye478 or homozygous Qi319/Qi319, flanking the recombination breakpoint. Individuals from the selfed BC_1F_2 recombinant progeny were categorized into one of three possible genotypes in the *qMrdd2* region: homozygous Qi319/Qi319, homozygous Ye478/Ye478, or heterozygous Qi319/Ye478. One-way ANOVA was used to compare the DSIs of these three

genotypic classes in SAS version 9.2 (SAS Inc., Cary, NC, US, 2009). The DSIs of the three genotypic classes differing significantly ($P < 0.05$) indicated that the MRDD resistance gene was located within a heterozygous region, whereas the DSIs of three genotypic classes differing insignificantly ($P \geq 0.05$) indicated that the MRDD resistance gene was located within a homozygous segment.

Analysis of phenotypic data

As described above, the disease response phenotypes of all recombinant-derived progenies were assessed in terms of DSI (See above for calculation of DSI). All of the genotypic and phenotypic datasets were calculated using Microsoft Excel 2010 software. We estimated the broad-sense heritability (H^2) of MRDD resistance across three environments according to Knapp et al. [54]. We calculated heritability as: $H^2 = \delta^2_g / (\delta^2_g + \delta^2_{ge}/e + \delta^2/er)$, where δ^2_g is the genetic variance, δ^2_{ge} is the genotype × environment interaction, δ^2 is error variance, e is the number of environments, and r is the number of replications per environment. The estimates for δ^2_g, δ^2_{ge}, and δ^2 were calculated with analysis of variance (ANOVA) using PROC MLM, the Mixed Linear Model procedure, in Statistical Analysis System (SAS) software version 9.2 [SAS Inc., Cary, NC, US, 2009].

Abbreviations

DSI: Disease severity index

Mb: Megabase pairs

MRDD: Maize rough dwarf disease

PCR: Polymerase chain reaction

QTL: Quantitative trait loci

RIL: Recombinant inbred line

SSR: Simple sequence repeats

InDel: insertion-deletion

CSSL: Chromosome segment substitution line

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All of datasets supporting the conclusions of this article are included within the article. The maize reference genome datasets used during the current study are obtained from the maizeGDB database (https://www.maizegdb.org/).

Authors' contributions

ZNX wrote the draft manuscript, carried out molecular genetic studies, performed statistical analyses and participated in phenotypic evaluations. ZQZ, CLL, ZFH, MSL, DGZ, and JNH helped with the development and genotyping of the RIL population. FFW, QCM, YPC, SGT, and XHH performed the maize field cultivation and pollination, as well as phenotypic evaluations. XHL, ZHW, and JFW supervised the research, designed the experiments, and were involved in data analysis. All authors have read and approved the final manuscript.

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Tables

Table 1 Phenotypes of the parental lines and RILs across three environments

Year	Qi319 Mean (%)	Ye478 Mean (%)	RILs Mean ±SD[a] (%)	Range (%)	Skewness	Kurtosis	CV[b] (%)	*Heritability* (%)
E1	11.47 ±2.68	52.08 ± 3.26	56.36 ± 5.36	20 - 86	0.0891	0.7762	10.26	76.98
E2	15.62 ±3.12	80.31 ± 4.15	45.98 ± 6.35	12 - 79	-0.0741	0.8736	8.95	80.12
E3	13.67 ±2.49	86.67 ± 5.67	61.35 ± 6.78	13 - 90	0.0871	0.9531	9.16	81.67
Combined	13.75 ±2.27	71.35 ± 4.98	55.36 ± 6.12	15 - 88	0.0764	0.8561	9.75	80.64

[a] SD, standard deviation;

[b] CV, coefficient of variation;

E1: 2015 in Xuzhou, Jiangsu Province

E2: 2016 in Xuzhou, Jiangsu Province

E3: 2016 in Xinxiang, Henan Province.

Table 2 Mapping of the QTL for MRDD resistance, *qMrdd2*, using RILs from a cross between Qi319 and Ye478

Field trial	QTL [a]	Bin [b]	Flanking marker [c]	Marker interval [d] (Mb)	LOD [e]	PVE [f] (%)	Add. [g]	SRA
E1 [h]	*qMrdd2*	2.02	MK806-MK811	11.35-12.0	5.83	8.17	-4.53	Qi319
E2 [h]	*qMrdd2*	2.02	MK807-MK808	11.45-11.6	5.96	8.64	-4.65	Qi319
E3 [h]	*qMrdd2*	2.02	MK807-MK808	11.45-11.6	7.93	11.02	-4.50	Qi319
BLUP	*qMrdd2*	2.02	MK807-MK811	11.45-12.0	7.36	10.68	-4.58	Qi319

[a] The name of each QTL is a composite of the influenced trait, maize rough dwarf disease

[b] Chromosome bin location of QTL

[c] Markers to the left and right of the QTL

[d] Interval between the two markers on the B73 reference genome RefGen_v3sequence.

Table 3 Primers developed for fine-mapping *qMrdd2*

maker	Position (bp)	Forward primer (5'-3')	Reverse primer (5'-3')	Type
umc1824a	7.411.594	ATCGTGCTTAAGCGGTTATAGGAAT	TGCACATGCTTTGTATAAGATGCC	SSR
RD-33	8.283.417	AAAGGCAGCGATGGGTGAT	CTGGTGGAGTGTTGTTATGG	InDel
RD-28	9.418.302	GCGACTGAAAGGAACCACTG	CTGCTCTGAACATTCCGATG	InDel
RD-42	10.478.148	GTGACTGACAGGACGAGGCT	GGATTCAGGCAACGACCAA	InDel
RD-97	10.661.704	ACGTCACATGAAGGTGGTGGA	CAATCGCTAGGCTGCTGGTAG	InDel
RD-3	11.318.029	TGAAGTGACACCCCTAAACC	GGAGGAGCACCAGAACAAAT	InDel
RD-52	11.943771	ATCACTTTATTTTGCTCTTCAG	GCAGTTCCAGCCTCATCCTAC	InDel
RD-56	12.143699	TCAGACCTAACGACCTGTTGT	TACGAGCTTGAATCATCATTG	InDel
RD-81	12.260.380	ATTGCTTGCTCGCTCGCTTTG	GCTAGGGGCGGAGGACCAGAT	InDel
RD-26	12.354.844	CCAGGAGGCGGTAACGCTA	AGAGCGAGGACTCACTGTCCG	InDel
RD-87	12.575.608	GAGCCAACAGTTTGTCATCTT	CCTGTCTTACTATACTGCCTGA	InDel
RD-114	12.685.280	ATCGCATCCATCCAAATCACA	TCCTGCACCTCAATCGCCTA	InDel
RD-93	12.805.802	CACAAGACGACGGATGATACAA	TAGCGGAAGTGAGTACAAGGT	InDel
RD-20	13.303.791	AGGATTCCAACACGGAAAA	TAACGCATACAGCGCACAT	InDel
bnlg125	13.602.840	GGGACAAAAGAAGAAGCAGAG	GAAATGGGACAGAGACAGACAAT	SSR

Primer positions are according to B73 RefGen_v3.

Table 4 Comparison of the MRDD resistance of CSSL haplotypes to the control line Ye478

CSSL	Target region	Background recovery rate (%)	DSI (%)			Bin	Linked marker
			2015-Xuzhou	2016-Xuzhou	2016-Jining		
Haplotype I	▯/▯	99.21	55.00	68.41	68.48	Bin2.01	bnlg1338
Haplotype II	▯/▯	98.75	20.01*	56.73*	51.71*	Bin2.02	umc1824-bnlg125
Haplotype III	▯/▯	99.25	54.41	69.64 *	61.80	Bin2.06	umc2178
Haplotype IV	▯/▯	99.51	75.83	76.43	59.63*	Bin2.06-2.07	umc2178-umc1637
Haplotype V	▯/▯	99.62	60.92	87.50	81.92	Bin2.07	umc1637-bnlg1267
Haplotype VI	▯/▯	91.61	49.76	75.00	89.65	Bin2.07	bnlg1267-bnlg2077
Haplotype VII	▯/▯	91.45	50.00	81.25	87.06	Bin2.08	umc2202
Haplotype VIII	▯/▯	99.45	48.68	82.60	87.30	Bin2.09	bnlg1520
Ye478	▯/▯		52.08	80.31	86.67		

"▯/▯": The target region is a homozygous allele of Qi319 (R); "▯/▯": The target region is a homozygous allele of Ye478 (S). Recurrent parent Ye478 (S) was used as the control line for resistance evaluation. "*" Significant at $P < 0.05$.

Figures
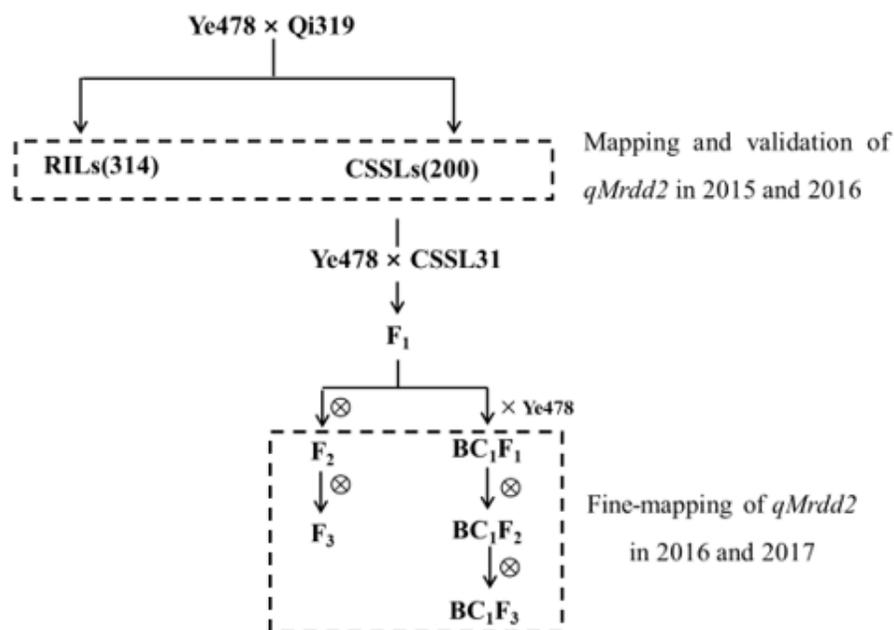
Figure 1

Experimental flow chart for identification, validation, and fine-mapping of qMrdd2, a QTL for MRDD resistance
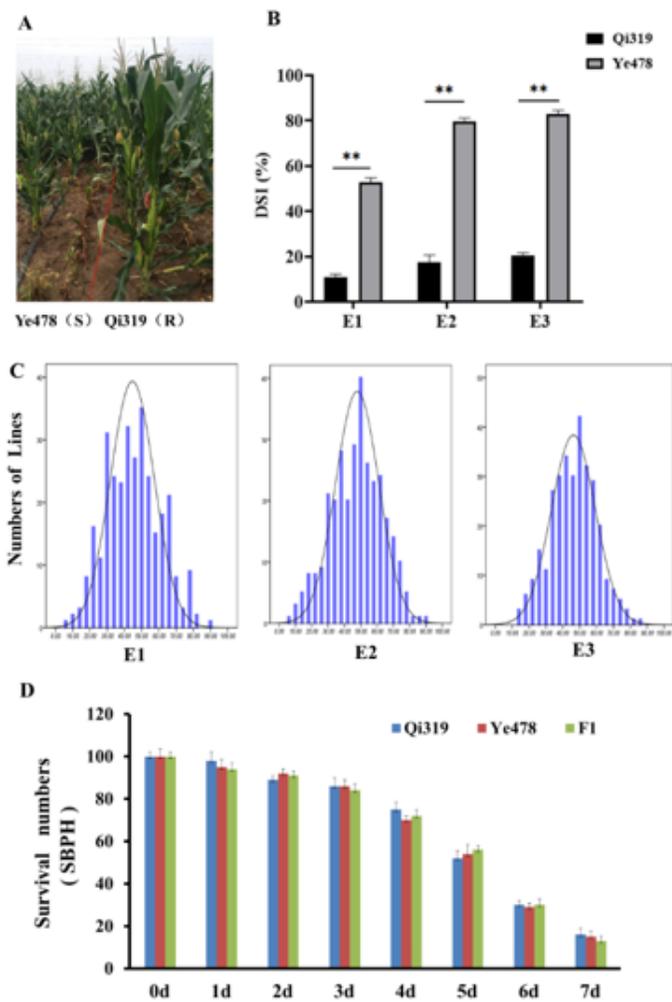
Figure 2

Phenotypic variants for MRDD resistance in the parental lines and distribution of DSIs in the RILs A Parental phenotypes for MRDD. "R" and "S" represent the resistant and susceptible parents, Qi319 andYe478, respectively. B Comparison of parental phenotypes for MRDD resistance. E1: 2015 in Xuzhou, Jiangsu Province, E2: 2016 in Xuzhou, Jiangsu Province, E3: 2016 in Xinxiang, Henan Province. "**" significant at $P < 0.01$. C DSI distributions in the RILs of the Qi319 × Ye478 population in each environment. D Assessment of the survival of virus-free small brown planthoppers (SBPH, Laodelphax striatellus) on maize inbreds Qi319, Ye478, and their F1.

A

B

Source	Chr 1	Chr 2 bin2.01	bin2.02	bin2.06	bin2.06-2.07	bin2.07	bin2.07	bin2.08	bin2.09	Chr 3-10	DSI% 2015-Xuzhou	2016-Xuzhou	2016-Xinxiang
Ye478											52.08	80.31	86.67
Qi319											3.33	17.32	13.33
Type1											55.00	68.41	68.48
Type2											20.01*	56.73*	51.71*
Type3											54.41	69.64	61.80
Type4											75.83	76.43	59.63
Type5											60.92	87.50	81.92
Type6											49.76	75.00	89.65
Type7											50.00	81.25	87.06
Type8											48.68	82.60	87.30

C
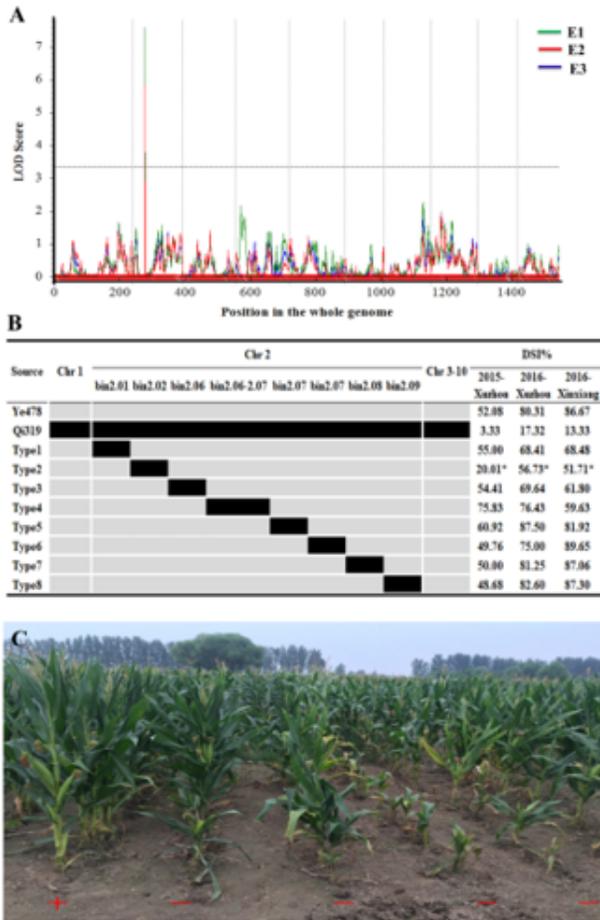
Figure 3

Mapping and validation of qMrdd2. A Diagram of QTL for MRDD resistance on the whole maize genome in 314 RILs. The LOD score profile, relative positions, and relevant markers are shown using QTL Cartographer version 3.5. B and C Validation of the effect of qMrdd2 in a CSSL population from the cross between Qi319 and Ye478. Black and gray rectangles correspond to homozygous Qi319 alleles and homozygous Ye478 alleles, respectively. "▨" represents the CSSL carrying the donor fragment including qMrdd2, whereas "▨" represents the CSSL carrying donor fragments that do not include qMrdd2. E1: 2015 in Xuzhou, Jiangsu Province; E2: 2016 in Xuzhou, Jiangsu Province; E3: 2016 in Xinxiang, Henan Province. "*" significant at P < 0.05.

	DP	Xinxiang NoP	DSI (%) Qi319	H	Ye478	P-value	Xuzhou NoP	DSI (%) Qi319	H	Ye478	P-value	Jining NoP	DSI (%) Qi319	H	Ye478	P-value
I	S	200	35.89	36.82	56.95	0.0363	198	15.63	19.56	35.61	0.0077	186	45.32	89.62	86.21	0.0002
II	NS	187	36.12	39.45	38.56	0.9398	180	35.69	36.98	36.52	0.9905	175	65.31	69.35	68.12	0.9385
III	NS	156	48.96	50.12	51.23	0.9541	105	23.62	26.65	29.36	0.7075	162	56.96	62.31	62.45	0.8187
IV	NS	160	23.36	25.63	25.55	0.9465	116	29.56	32.36	33.74	0.8701	175	78.98	77.65	78.31	0.9871
V	NS	200	37.56	38.69	38.12	0.9911	169	32.23	36.95	30.56	0.7516	189	69.87	69.65	69.75	0.9989
VI	NS	201	45.12	45.62	48.96	0.9371	210	30.03	30.25	36.12	0.6873	192	72.56	74.65	75.68	0.9474
VII	NS	46	56.92	Na	54.54	0.4466	47	33.54	Na	33.81	0.2938	45	82.23	Na	89.12	0.1895
VIII	NS	35	54.74	Na	54.54	0.0653	45	33.02	Na	33.81	0.2845	42	82.24	Na	89.02	0.1898
IX	NS	35	54.81	Na	54.54	0.0646	29	33.57	Na	33.81	0.2956	31	81.36	Na	89.12	0.1786
X	NS	37	64.06	Na	54.54	-0.0017	31	28.59	Na	33.81	0.0491	35	82.01	Na	89.12	0.1832
XI	NS	44	54.69	Na	54.54	0.0716	47	33.51	Na	33.81	0.2924	46	82.04	Na	89.12	0.1835
XII	NS	40	44.76	Na	54.54	<0.05	47	21.21	Na	33.81	<0.05	45	69.02	Na	89.12	<0.05
XIII	NS	43	42.33	Na	54.54	<0.05	39	21.89	Na	33.81	<0.05	41	68.14	Na	89.12	<0.05
XIV	NS	47	44.63	Na	54.54	<0.05	48	7.62	Na	33.81	<0.05	49	69.31	Na	89.12	<0.05
XV	NS	41	38.45	Na	54.54	<0.05	32	17.18	Na	33.81	<0.05	49	65.02	Na	89.12	<0.05
XVI	NS	37	33.28	Na	54.54	<0.05	45	19.91	Na	33.81	<0.05	38	67.11	Na	89.12	<0.05

Markers: unc1824 RD-33 RD-28 RD-42 RD-97 RD-3 RD-52 RD-56 RD-81 Indel-26 RD-87 RD-114 RD-93 RD-20 bnlg125

Figure 4

Fine-mapping qMrdd2 Fine-mapping of QTL qMrdd2 was performed using recombinant progeny and homozygous family verification. The genetic composition of each recombinant category is represented by different colors. Black, gray, and white rectangles indicate the homozygous Qi319 genotype, the heterozygous Qi319/Ye478 genotype, and the homozygous Ye478 genotype, respectively. Self-pollinated progeny of these BC1F2 plants were genotyped using markers within the heterozygous region, resulting in three genotypes among progeny. The DSIs of all three genotypes of progeny are listed in the table. Among haplotypes I through VI, significant differences (P < 0.05) among the three genotypes indicated that the MRDD resistance QTL represented by qMrdd2 is located within the heterozygous region and that their parental recombinant(s) were segregating (S). An insignificant difference (P ≥ 0.05) among the three genotypes suggested that qMrdd2 is located within the homozygous region and that their parental recombinant(s) were not segregating (NS). Among haplotypes VII through XVI, a statistically significant difference (P < 0.05) in DSIs between a homozygous Qi319 recombinant-derived family and Ye478 indicates that the MRDD resistance QTL represented by qMrdd2 is located within the homozygous Qi319 region. Finally, qMrdd2 was located between markers RD81 and RD87 within a 315-kb region. DP: deduced phenotype, No. P: number of progenies, Na: not available, Qi319: progeny with a homozygous genotype the same as the MRDD-resistant parental line Qi319, H: progeny with a heterozygous genotype Qi319/Ye478, Ye478: progeny with a homozygous genotype the same as the MRDD-susceptible parental line Ye478.
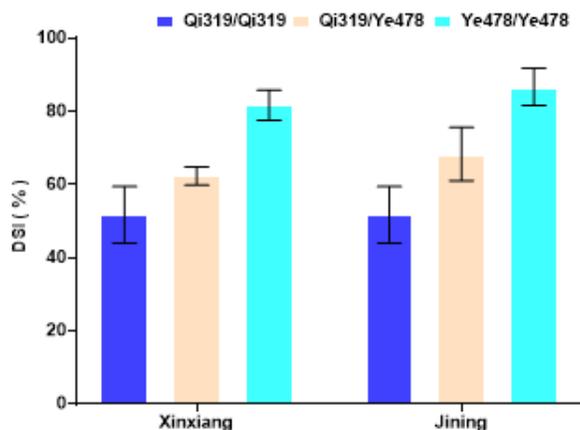

Figure 5

Genetic model of qMrdd2 gene action based on F2 populations in two environments According to genotypes within the qMrdd2 region between marker RD81 and RD87, the F2 populations were divided into three genotypes (Qi319/Qi319, Qi319/Ye478, and Ye478/Ye478) in 2016. The average DSI (%) values are shown. Error bars indicate standard errors of the means.