qMrdd2, a novel quantitative resistance locus for maize rough dwarf disease

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Abstract
Background: Maize rough dwarf disease (MRDD), a widespread disease caused by four pathogenic viruses, severely reduces maize yield and grain quality. Resistance against MRDD is a complex trait that controlled by many quantitative trait loci (QTL) and easily influenced by environmental conditions. So far, many studies have reported numbers of resistant QTL, however, only one QTL have been cloned, so it is especially important to map and clone more genes that confer resistance to MRDD.

Results: In the study, a major quantitative trait locus (QTL) qMrdd2, which confers resistance to MRDD, was identified and fine mapped. qMrdd2, located on chromosome 2, was consistently identified in a 15-Mb interval between the simple sequence repeat (SSR) markers D184 and D1600 by using a recombinant inbred line (RIL) population derived from a cross between resistant (“80007”) and susceptible (“80044”) inbred lines. Using a recombinant-derived progeny test strategy, qMrdd2 was delineated to an interval of 577 kb flanked by markers N31 and N42. We further demonstrated that qMrdd2 is an incompletely dominant resistance locus for MRDD that reduced the disease severity index by 20.4%.

Conclusions: A major resistance QTL (qMrdd2) have been identified and successfully refined into 577 kb region. This locus will be valuable for improving maize variety resistance to MRDD via marker-assisted selection (MAS).

Keywords: Maize, MRDD, QTL, Fine-mapping, MAS

Background
Maize rough dwarf disease (MRDD) is a serious viral disease worldwide. MRDD is caused by four pathogenic viruses [1], namely, Maize rough dwarf virus (MRDV), Mal de Rio Cuarto virus (MRCV), Rice black-streaked dwarf virus (RBSDV) and Southern rice black-streaked dwarf virus (SRBSDV), which are prevalent in Europe [2], South America [3], Asia [4], and Northern China [5], respectively. These pathogenic viruses are naturally transmitted by the small brown planthopper Laodelphax striatellus in a persistent manner [6] and are not spread by seed or mechanical inoculation. Once infected, L. striatellus can transmit these viruses for life, though transmission to eggs does not occur [7].

MRDD causes substantial yield losses in maize production globally [2]. Indeed, between 2008 and 2011, MRDD was responsible for yield losses among more than 3,000,000 ha of crops each year in North China with yield losses reaching 100% in some seriously affected areas [8]. The disease leads to severe symptoms, such as plant dwarfing, internode shortening, waxy enation, malformed tassels and dark-green leaves [9]. Current methods for controlling MRDD are agricultural measures (i.e., delayed sowing) and chemical control. However, delayed sowing leads to the underutilization of photothermal resources and renders the seedlings vulnerable to waterlogging and late ripening.
as occurred in the Yellow-Huai-Hai River plain [10]. Small-scale chemical control is also not ideal and leads to environmental pollution. Accordingly, cloning natural resistance genes and breeding resistant maize varieties would be the most environmentally responsible and cost-effective means of controlling MRDD. Hence, collecting and identifying various resistant germplasms will facilitate the development of resistant maize cultivars, and it is especially important to map and clone resistant genes to MRDD.

Most research indicates that maize resistance to MRDD is controlled by many genes, each with different effects [2, 11, 12]. Maize germplasms shows different levels of resistance to MRDD under natural conditions, with the most resistant germplasm originating from US hybrid P78599 [13–16]. By using F3 families of Mo17 and BSL14, Di Renzo et al. [17] found that in Argentina, the heritability of MRCC resistance in maize ranged from 0.44 to 0.56, and based on an F2:3 QTL-mapping strategy, 2 quantitative trait loci (QTLs) were localized to maize chromosome bins 1.03 and 8.03/4 [18]. Luan et al. [19] screened four linked molecular markers and mapped three QTLs for resistance to MRDD within chromosome bins 6.02, 7.02 and 8.07 by using an F2 population derived from the resistant inbred line 90,110 and the susceptible inbred line Ye478. Through association analysis with 163 inbred lines, Shi detected one associated single nucleotide polymorphism (SNP) in the ZmElf4E promoter, which accounting for 3.33 and 9.04% of the phenotypic variation, respectively in two environments, [20]. Using 152 maize germplasm isolates and 89 recombinant inbred lines (RILs) derived from resistant line X178 and susceptible line B73, Shi et al. [21] detected three QTLs for MRDD resistance in bins 2.07, 5.04 and 8.03, and the QTL on bin 8.03 explained 24.6–37.3% of the phenotypic variance. This major QTL (qMrdd48) on chromosome bin 8.03 was then fine-mapped to a 347-kb region by Shi’s laboratory [22]. With 527 inbred lines and 556,000 SNPs, 15 candidate genes associated with MRDD resistance have been identified [23]. Conventional linkage and high-throughput sequencing analysis have been employed to locate the resistance-related genomic region, and an associated region was identified within the genomic interval 68,396,487–69,523,478 bp on chromosome 6 [24]. A major QTL (qMrdd1) that reduces the DSI by 24.2–39.3% was fine-mapped to a 1.2-Mb region by applying recombinant-derived progeny testing to self-pollinated backcrossed families [25]. Although many QTLs have been identified, few have been fine-mapped or even cloned. Liu et al. (2020) reported that the Rab GDP dissociation inhibitor (ZmGDIα) is a candidate gene for qMrdd1 which confers recessive resistance to MRDD and resolves the underlying molecular mechanisms controlling viral diseases. qMrdd1 is also the only resistance gene cloned to date [26].

Considering the damage caused by MRDD and the complexity of disease resistance, it is critical to identify new resistance QTLs that can be used to genetically control MRDD and to deepen our understanding of resistance mechanisms.

**Results**

**Initial QTL analysis of MRDD**

For each field trial, the RIL population showed continuous variation in DSI (range 0–100%), with averages of 54.2 and 55.5% in two field trials (2013-A and 2013-B) respectively (Fig. 1a). Spearman’s rank correlation coefficient ($r_s$) among two sets of phenotypic data was calculated. The data among two replications were significantly correlated ($r_s = 0.74E^{-15}$) with $r_s$ was 0.49. The broad-sense heritability ($H^2$) of disease resistance was estimated to be 0.66 (Table S1), and the variance was mainly derived from differences among RILs (Table 1). These results suggested that the genetic factor plays a major role in maize resistance to MRDD within the RIL population.

Two maize lines, “80007” and “80044”, were evaluated for resistance to MRDD under artificial inoculation 3 times: in 2017 and 2019 in Taian and in 2018 in Hainan. For each field trial, line “80007” showed effective resistance to MRDD, whereas line “80044” was highly susceptible; the average DSIs of “80007” and “80044” were 34.6 and 82.03%, respectively (Fig. 1b, c).

**Initial QTL mapping of MRDD resistance**

Based on 199 RILs and 804 informative SNPs, we constructed a genetic linkage map that covered 1291.05 cM, with an average of 1.61 cM between adjacent markers (Fig. S1, Fig. S2, Table S2 and Table S3). The DSI for the 199 RILs was used as phenotypic data for QTL mapping of MRDD resistance. Two QTLs were consistently detected in all three field trials (2013-A, 2013-B and the average DSI phenotype of each RILs in 2013-A and 2013-B) (Fig. S3, Fig. S4 and Table 2). One of the QTLs, qMrdd2, is located on chromosome 2 within a 15-Mb region and explained 12.88–15.67% of the total phenotypic variation. Another QTL, located on chromosome 5, explained ~6% of the total phenotypic variation (Table 2).

**Fine mapping of qMrdd2**

According to the initial QTL analysis, two flanking markers (D184 and D1600) and one marker residing within the qMrdd2 region (D829-0) were used to screen the RIL population. Ultimately, six recombinant RILs that had crossover breakpoints within the qMrdd2 region were detected and further genotyped with seven newly isolated
markers (D387-2, D550, D664, D829-0, D936-2, D122-6 and D1347) (Table S4). All six F5 recombinants were self-pollinated to produce corresponding F6 progeny. In the summer of 2017, the F6 progeny (1010 plants) were planted in Taian to test for MRDD resistance in the field. Three genotypes within each F6 progeny derived from individual heterozygous recombinant plants were categorized: $q_{Mrdd2}$ (80007) homozygote and heterozygote and non$q_{Mrdd2}$ (80044) homozygote. The DSI value for each genotype was calculated. MRDD resistance among the three genotypes of recombinant types a-I, a-III, a-IV, a-V and a-VI was not significantly different ($P<0.05$), indicating that $q_{Mrdd2}$ is located in the homozygous region of the parental region. For the remaining recombinant type a-II, a statistically significant difference ($P<0.05$) in MRDD resistance among the three genotypes was observed, suggesting the presence of $q_{Mrdd2}$ in the heterozygous region of the parental recombinant. This analysis shows that the $q_{Mrdd2}$ locus was localized to between

Table 1  Analysis of variance of data in the RIL population

| Sources      | df | SS  | MS    | $F$-value | $P$-value | Variance |
|--------------|----|-----|-------|-----------|-----------|----------|
| RILs         | 199| 65.53 | 0.29  | 8.67      | 3.66E-76 *** | 1.35E-03 |
| Environments | 2  | 2.17 | 1.13  | 76.0      | 8.23E-26 *** | 5.16E-03 |
| Residuals    | 398| 17.3 | 3.16E-03 |          | 2.15E-03   |          |

Sources Variation sources, df Degrees of freedom, SS Sum of squares, MS Mean squares, $P$-value Significant difference among sources

*** $P<0.001$
markers D829-0 and D122-6 with a physical distance of ~3.9 Mb based on the B73 physical map (AGPv4).

In the winter of 2017 in Hainan, nine F7 progeny (603 plants) were planted in Hainan, and five InDel markers (A1, A10, C2, C3 and C6) were designed to genotype new recombinants. Ten types of recombinants were identified and self-pollinated to produce 10 F8 families (1159 plants) that were planted in Taian in 2018. Ten markers (D550, D664, D829-0, A1, A10, C2, C3, C6, D122-6 and D1347) were used to genotype the 10 types of recombinants (I–X; Fig. 2b). Based on the statistically significant difference (P<0.05) in DSI value between “80007” and “80044” homozygotes, recombinants b-I, b-III, and b-V were considered to be resistant to MRDD (Fig. 2b), suggesting that qMrdd2 is located in the heterozygous region of these recombinant types. In contrast, recombinant types b-II, b-IV, and b-VI−b-X did not show a statistically significant difference (P>0.05) in DSI between “80007” and “80044” homozygotes (Fig. 2b), indicating that qMrdd2 is in the homozygous region. Thus, qMrdd2 was localized to the 2.81-Mb (AGPv4) (http://www.maizegdb.org) interval between markers A10 and C3 (Fig. 2b).

Seven types of recombinants were identified with markers A10 and C3 from the F8 progeny which were then self-pollinated to generate 7 F9 families (765 plants). All F9 progeny were planted in Hainan Province in the winter of 2018. Three markers (S33, D1116 and C2) in the mapped A10/C3 interval and another seven newly developed markers (D829-0, A1, A10, C3, C6, C13 and C18) were applied to genotype the 7 recombinants (Fig. 2b). The same progeny test strategy was utilized to determine whether there was a statistically significant difference (P<0.05) in DSI value between “80007” and “80044” homozygotes. The results for type c-I, c-IV and c-VI recombinants indicated that qMrdd2 is in the heterozygous region. Analogously, qMrdd2 was localized to the homozygous region of type c-II, c-III, c-V and c-VII recombinants (Fig. 2c). Therefore, qMrdd2 was mapped between markers S33 and D1116 with a physical distance of 1.28-Mb (AGPv4).

We conducted the fourth fine-mapping in the summer of 2019 in Taian. Three InDel markers (N37, N31 and N42) were developed in the newly mapped 1.28-Mb region, and 1421 individuals from 9 recombinants were obtained from F9 progeny. The progeny of types d-I, d-IV, d-VI and d-IX show significantly (P<0.05) different DSI values, and types d-II, d-III, d-V, d-VII and d-VIII did not show significant differences (P>0.05) in DSI. These data further allowed us to localize qMrdd2 to the 577-kb (AGPv4) interval flanked by markers N31 and N42 (Fig. 2d).

**Table 2** The QTL related to MRDD in three field trials

| Field trial | Chromosome | Position (Mb) | LOD | Additive effect | SRA | R² |
|-------------|------------|---------------|-----|----------------|-----|----|
| Average     | 2          | 15.10−39.30   | 5.581 | -0.0503 | 80007 | 0.1156 |
|             | 3          | 20.42−27.67   | 3.3725 | -0.0397 | 80007 | 0.0730 |
|             | 5          | 50.90−70.26   | 3.8923 | -0.0446 | 80007 | 0.0625 |
|             | 8          | 200.13−219.86 | 2.8755 | -0.0350 | 80007 | 0.0566 |
|             | 10         | 0.44−16.38    | 3.1656 | -0.0370 | 80007 | 0.0617 |
| R 1         | 2          | 14.80−37.48   | 5.1172 | -0.0675 | 80007 | 0.1588 |
|             | 5          | 54.55−70.26   | 2.9262 | -0.0425 | 80007 | 0.0614 |
| R 2         | 2          | 13.41−37.48   | 4.8036 | -0.0648 | 80007 | 0.1288 |
|             | 3          | 20.42−27.67   | 3.1145 | -0.0391 | 80007 | 0.0728 |
|             | 5          | 50.89−70.26   | 2.8259 | -0.0462 | 80007 | 0.0641 |

LOD: Logarithm of odds, SRA: Source of resistance allele, R²: Explained phenotypic variation; “R1” and “R2” represent the field trial 2013-A and 2013-B and “Average” is the average value of 2013-A and 2013-B.

**Genetic model of qMrdd2 resistance to MRDD**

F₆, F₇ and F₉ families were used to investigate the genetic effect of qMrdd2. Plants with homozygous “80007”/“80007”, heterozygous “80007”/“80044” and homozygous “80044”/“80044” genotypes showed DSIs of 30.94, 43.35 and 51.48% in 2017 Taian, 30.94, 43.35 and 51.48% in 2018 Taian, 40.62, 52.28 and 66.23% in 2018 Hainan, respectively. For each field trial, homozygous “80044”/“80044” plants showed an obvious difference in DSI relative to homozygous “80007”/“80007” plants, with an average difference of 20.4%. The DSI of heterozygous plants was, on average, 12.17% greater than that of homozygous “80007”/“80007” plants (Fig. 3). These results indicated that the qMrdd2 QTL acts in an incompletely dominant manner to confer resistance to MRDD.

**Discussion**

The recombinant-derived progeny test is an efficient method and usually used to detect a QTL in backcrossed population such as qRfg2 and qIfm4 [27–29]. Compared
with backcrossed progeny, the self-pollinated progeny can capture the effect of all three genotypes and create more recombinants for fine-mapping. However, if recombinants are homozygous at both flanking markers, they produced by self-pollination cannot be used for fine-mapping. Crosses with other heterozygous plants in the same family can solve this problem very effective.

An artificial infection process [30] was used to evaluate the MRDD resistance of individual plants in this study, in which adequate viruliferous planthoppers carrying RBSDV provided stable and uniform disease pressure. In 2017 in Hainan, because the seedling age was inconsistent with the planthoppers, the planthoppers were old with low motility when the seed germinates. As a result,
planthopper activity was inadequate to assess most of the recombinant individual phenotypes. In the spring of 2019, disease-resistant phenotypes in the F9 population were not as robust as those in previous experiments, which may have resulted from a severe cold snap during the seedling stage. As the first survey of disease symptoms was conducted at 40 days after inoculation, the most severely affected plants could be analyzed before they died, followed by second and third surveys after pollination at 10-day intervals to ensure consistency of the phenotypic data.

We previously identified a major resistance QTL (qMrdd1 on chr.8) from the highly resistant inbred line NT411 and X178 [25]. Additional resistance QTLs have been identified on chr.1, 2, 5, 6, and 7 [19, 24, 31, 32]. In this study, using a different resistant inbred line (80007), we detected another major QTL (qMrdd2, chr.2, bin 2.01/02) and localized it to an interval of ~ 577 kb. A resistance QTL, previously mapped on chr.2 (bin 2.07/08) [8] is clearly different from qMrdd2 (bin 2.01/02). Also, qMrdd2 could be validated in the context of association mapping, by exploring a panel of accessions which usually contains a great diversity from one or multiple germplasms.

In this study, the qMrdd2 was narrowed down to an ~ 577 kb region (APV 4). Gene prediction by FGENESH 2.6 (http://linux1.softberry.com/berry.phtml) has revealed 29 putative genes in the qMrdd2 region. Most of the genes have not been annotated yet, only one (Zm00001d002312) is related to disease resistance. The gene Zm00001d002312 was annotated as Leucine-rich repeat transmembrane protein kinase (LRR-TM) in the MazieGDB and the LRR-TM genes are important receptor resistance genes in plants in response to pathogen infection [33]. Both Cf-2 and HcrVf2 were this type of gene, which were related to the resistance to Cladosporium fulvum in tomato and Scab in apple [34, 35], respectively. While Zm00001d002312 is a good candidate gene for qMrdd2, further experiments such as genetic mapping by screening new recombinants within the qMrdd2 locus or gene expression and transgenic testing studies must be performed to restrict the qMrdd2 locus to a single gene.

Because qMrdd2 is an incompletely dominant resistance QTL, the qMrdd2 donor region should be integrated into both parental lines via MAS to guarantee maximally resistant F1 hybrids. However, given that homozygous segments from both parental lines would impair heterosis, it is important to minimize the overlapped qMrdd2 region in the F1 hybrid for successful molecular breeding. We propose that one parental line carry the shortest possible qMrdd2 donor region on the left, while the other parental line carry the shortest possible qMrdd2 donor region on the right via MAS to minimize such overlap. Therefore, developing suitable markers to identify the shortest possible qMrdd2 donor region to minimize such overlap is of great importance. In present study, the genetic distance between marker N31 and N42 was ~ 0.67 cM. Thus, they may be effective for MAS of MRDD resistance because this marker is tightly linked within 5 cM of the QTL [36].

Conclusions
It is critical to identify new resistance QTLs that can be used to genetically control MRDD and to deepen our understanding of resistance mechanisms. With
the availability of a RIL population, we here detected a number of consistent MRDD QTL. Using a recombinant-derived progeny test strategy, a major resistance QTL (qMrdd2) have been identified and successfully refined into 577 kb region. We further demonstrated that qMrdd2 is an incompletely dominant resistance locus for MRDD that reduced the disease severity index by 20.4%. This locus will be valuable for improving maize variety resistance to MRDD via MAS.

Methods

Plant materials

An RIL population consisting of 199 lines developed by single-seed descent from a cross between the inbred lines “80044” and “80007” was used in this study. The “80044” is a susceptible line bred from a Reid heterotic group, and “80007” is a resistant line from the P heterotic group. The two parents as well as the RILs were all developed by Prof. Baoshen Liu of Shandong Agricultural University. For the initial QTL mapping in 2013, we planted 199 RILs as the initial QTL mapping population for conducting two field trials (2013-A and 2013-B) at an experimental station (Jining, Shandong Province). For each RIL, 30 seeds were sown in one row with a spacing of 0.6 m between rows and 0.25 m between plants within a row. For fine mapping, 58 resistant RILs with different chromosomal recombinations in the QTL region were chosen and backcrossed with the susceptible line “80044” to produce F1 populations. Using marker-assisted selection (MAS), the F1 hybrids with the target QTL were self-pollinated four times to produce an F5 population; all mapping populations were developed based on the experimental flow chart presented in Fig. 4. All populations were grown at the experimental station of Shandong Agricultural University (Tai’an, Shandong Province) and experimental base (Sanya, Hainan Province) for fine mapping. For comparison, we planted two parents in each fine mapping, all populations and two parent lines were artificially inoculated with viruliferous planthoppers (see below).

Fig. 4 Experimental flow chart for QTL identification and fine-mapping of QTL qMrdd2, which confers resistance to MRDD. A total of 199 RILs were used for the initial QTL mapping. Recombinants identified within the F4–F9 populations were genotyped and investigated to fine-map the QTL qMrdd2.
Molecular markers within the major QTL region were used to identify recombinants from the F₅ population, and the recombinants were self-pollinated to generate F₆ progeny. This process was repeated during 2017–2019 to develop a series of advanced self-pollinated populations consisting of 1010 F₆, 603 F₇, 1159 F₈, 765 F₉ and 1421 F₁₀ plants for fine mapping of qMrdd2. MAS and progeny testing were conducted across self-pollinated populations until fine mapping of the target QTL was completed.

Artificial inoculation and scoring of plants for symptoms
Planthoppers carrying RBSDV were provided by Jiangsu Academy of Agricultural Sciences (Nanjing, China) [37]. Before inoculation, maize kernels derived from one recombinant plant were placed in a plastic casing, and a Dot-ELISA [38] was used to detect the infection rate of the planthoppers. Seedlings inoculated at the coleoptile stage with one infected planthopper [39] were cultured at 24 °C in a greenhouse for 72 h; the seedlings were planted in a sample plot with 0.3-m spacing between plants and 0.6-m spacing between rows.

Symptom scoring was conducted at 40 days after inoculation and was repeated twice after pollination at 10-day intervals. For QTL analysis and fine-mapping, a scoring system (0, 0.25, 0.5, 0.75 or 1) based on overall symptoms was adopted to evaluate MRDD resistance (Fig. 5), as follows: 0 = no symptoms; 0.25 = slightly shortened superior internodes, resulting in plants that are ~80% of the height of a healthy plant; 0.5 = dark-green leaves, waxy enations on abaxial surfaces of leaves and sheaths and obviously shortened superior internodes, resulting in plants that are ~66% of the height of a healthy plant; 0.75 = severely shortened internodes and malformed tassels, resulting in plants that are ~50% of the height of a healthy plant; 1 = severe stunting, with suppressed flowering and the absence of ears, resulting in plants that are <50% of the height of a healthy plant. DSI was calculated by the following formula: DSI (%) = \[ \frac{\sum (\text{scale} \times \text{number of plants in scale})}{(1 \times \text{total number of plants})} \times 100\% \], and was used to represent the MRDD severity of families [40].

Analysis of phenotypic data
Statistical analysis was performed using R project [39]. Two sets of phenotypic data (2013-A, 2013-B) were collected for the RIL population and the Spearman’s rank correlation coefficient (\( r_s \)) of them was calculated. We used ANOVA (part of the R project) to test the significance of the genotypic and environmental effects. The model for variance analysis is as follows: \( y_i = \mu + g_i + e_j + \varepsilon \), in which \( y_i \) is the phenotypic value of the \( i \)th RIL line, \( \mu \) is the overall average value, \( g_i \) the genetic effect of \( i \)th RIL, \( e_j \) the environmental effect of \( j \)th trial and \( \varepsilon \) is the residual. The ANOVA result was used to calculate the broad-sense heritability (\( H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} \)), where \( \sigma_g^2 \) is the genetic variance, \( \sigma_e^2 \) is the residual error, and \( e \) is the number of environments [41, 42].

**Fig. 5** Symptom of RBSDV on *Zea mays* L. A Rice black-streaked dwarf virus (RBSDV) is transmitted to its natural host, maize, by the brown planthopper (*Laodelphax striatellus*). Bar = 5 mm. B Waxy enations on abaxial surfaces of leaves present in maize plants infected with RBSDV. Bar = 5 mm. C Maize with MRDD scores of 0–1. Disease scores were primarily based on plant height. Bar = 10 cm
Genotyping

Leaf tissue from each plant in the field was collected at the six- to seven-leaf stage. An SDS procedure [48] was used to extract genomic DNA. Maize insertion or deletion (Indel) and SSR markers were retrieved from MaizeGDB (http://www.maizegdb.org/); the primers were produced by Sangon Biotech (Shanghai, China). Each sample was subjected to genotyping using the maize B73 reference sequence in the QTL region with high-density molecular markers. Two types of markers were developed based on different sequence variations, including SSRs and InDels. To develop SSR markers, the maize B73 reference sequence in the QTL region was downloaded from the website http://plants.ensembl.org/Zea_mays/Info/Index/. The sequences were scanned by the software SSRHunter (Version 1.3) to search for SSRs, and primers flanking the SSRs were designed with the PrimerQuest tool (https://sg.idtdna.com/Primerquest/Home/Index). To develop InDel markers, lines “80007” and “80044” were sequenced by Tsingke Biotech (Beijing, China), and InDels in the 15 Mb interval for the QTL were analyzed. Single-/low-copy sequences were detected by BLAST on MaizeGDB with the B73 genomic sequence database. BLAST results were surveyed for InDels of ≥ 3 bp, which were detected as 3-bp gaps in the alignment of sequences from the two parents. InDels with ≥ 1000 bp of upstream sequence and ≥ 1000 bp of downstream sequence were assessed by PCR. Primers were designed by Primers Input (https://primer3.ut.ee) with the targets (970, 60). All primers met to the following criteria: ~ 20 nucleotides with 40–60% GC content, no consecutive tracts of a single nucleotide and no secondary structure. Ultimately, 21 markers (10 SSRs and 11 InDels) were found to be polymorphic between the parental lines (Table S4).

Strategy for fine-mapping of qMrdd2

Appropriate marker density, crossover density and the ability to determine the precise phenotype of each recombinant are essential for fine mapping analysis. Based on the initial QTL mapping, we developed high-density markers within the 15-Mb interval of qMrdd2. Sequential fine mapping of qMrdd2 was carried out by using recombinant-derived progeny [27]. In this study, a robust progeny test strategy was employed to evaluate the MRDD phenotype of all recombinants (Fig. 4). The DSI was used to represent MRDD severity, as previously described in detail (Fig. 5). Recombinants developed from the F1 population were self-pollinated, and the resulting self-pollinated population was grown in the same experimental stations under artificial infection. Each individual was genotyped using markers in the heterozygous region of its parental recombinant and scored for disease severity in the field. A DSI value was estimated for each resulting self-pollinated population. The progeny from each recombinant strain were divided into three genotypes: “80007” homozygote, “80044” homozygote and “80007”/“80044” heterozygote. Statistically significant differences in the DSI value of each genotype were determined using two-tailed Student's t-test. If there was a significant difference (P < 0.05) in DSI value between the two homozygous genotypes, qMrdd2 was considered to be located in the ‘80007’ donor segment, namely, in the heterozygous region of the parental recombinant. If there was no significant difference (P ≥ 0.05) in DSI value between the two homozygous genotypes, qMrdd2 was assumed to be absent in the ‘80007’ donor segment, namely, in the homozygous region of the parental recombinant, or no qMrdd2 was present.

Abbreviations

MRDD: Maize rough dwarf disease; QTL: Quantitative trait loci; SSR: Simple sequence repeat; RIL: Recombinant inbred line; MAS: Marker-assisted selection; MRDV: Maize rough dwarf virus; MRCV: Mal de Rio Cuarto virus; SRBSDV: Southern rice black-streaked dwarf virus; InDel: Insertion or deletion.
Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-021-03107-1.

Additional file 1: Table S1. Spearman's rank correlation coefficient (r) of DSI between the two field trials. Table S2. Marker and genetic distance information for the 10 maize linkage groups. Fig. S1. The ten maize genetic linkage groups. Fig. S2. Linkage disequilibrium (LD) heat map of each linkage group. Table S3. The number of SNP markers accepted as cofactors in the statistical model of QTL mapping. Fig. S3. Detection of QTLs conferring resistance to MRD. Fig. S4. Boxplot graph of DSI values of the initial mapping population. Table S4. Markers developed to map the qMrd2 locus. Table S5. Criteria applied to analysis SNP database.

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Authors' contributions
WXZ performed most of the experiments and wrote the paper. SND and QCL performed the initial QTL mapping, maize cultivation and pollination. YZ performed the artificial inoculation, phenotypic evaluation, and pollination. WX performed the artificial inoculation, polination, and genotyping. YZZ participated in the phenotypic evaluation and pollination. MLX guided the experiments and was involved in the analysis of the results. CMR and MLX performed the artificial inoculation, phenotypic evaluation, and pollination. WXZ performed most of the experiments and wrote the paper. SND and QCL participated in the phenotypic evaluation and pollination. MLX guided the experiments and was also involved in the analysis of the results. CMR and ZBC provided the brown planthopper. BSL designed and supervised the experiments and provided the RIL population. BSL edited and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The sequencing data is available from the corresponding author on reasonable request. Other data produced during this study are included in this article and in its supplementary files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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