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ORIGINAL PAPER

Mapping quantitative trait loci conferring resistance to rice black-streaked virus in maize (*Zea mays* L.)

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Abstract Maize rough dwarf disease (MRDD) is one of the most serious virus diseases of maize worldwide, and it causes great reduction of maize production. In China, the pathogen was shown to be rice black-streaked virus (RBSDV). Currently, MRDD has spread broadly and leads to significant loss in China. However, there has been little research devoted to this disease. Our aims were to identify the markers and loci underlying resistance to this virus disease. In this study, segregation populations were constructed from two maize elite lines '90110', which is highly resistant to MRDD and 'Ye478', which is highly susceptible to MRDD. The F_2 and BC_1 populations were used for bulk sergeant analysis (BSA) to identify resistance-related markers. One hundred and twenty $F_{7:9}$ RILs were used for quantitative trait loci (QTL) mapping through the experiment of multiple environments over 3 years. Natural occurrence and artificial inoculation were both used and combined to determine the phenotype of plants. Five QTL, *qMRD2, qMRD6, qMRD7, qMRD8* and *qMRD10* were measured in the experiments. The *qMRD8* on chromosome 8 was proved to be one major QTL conferring resistance to RBSDV disease in almost all traits and environments, which explained 12.0–28.9 % of the phenotypic variance for disease severity in this present study.

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Introduction

Maize (*Zea mays* L.) is one of the most important food, forage and energy crops worldwide. Maize rough dwarf disease (MRDD) is a destructive viral disease that causes great losses in maize production yearly in China. This disease was firstly found in Italy in 1949, and then found in other European countries (Harpaz et al. 1958; Dovas et al. 2004). In 1954, it was discovered in south Xinjiang and western Gansu in China, and was subsequently distributed all over China. Diseased farmland reached 2.33×10^6 hm^2 and farmland with no production reached 4.0×10^4 hm^2 in 1996 (Chen and Zhang 2005). From 2008 to 2011 the diseased farmland was above 3×10^6 hm^2/year. It has seriously reduced the maize production and caused severe economic damage in China in recent years.

The typical diseased maize plant presents stunted, dark green color, enations on abaxial surface of leaves and leaf sheaths, suppressed flowers and no ear or born simply nubbins. MRDD is caused by MRDV (Maize rough dwarf virus) in Europe, MRCV (Mal de Río Cuarto virus) in South America and RBSDV (Rice black-streaked virus) in Asia. All of these viruses belong to genus *Fijivirus* of the family Reoviridae. In China, RBSDV was shown to be the pathogen of MRDD (Bai et al. 2002; Fang et al.2001; Isogai et al. 2001).

MRDD is transmitted by an insect vector in a persistent manner and can not be transmitted by any other way in nature. The planthopper (*Laodelphax sreiatellus* Fallen.) is the major RBSDV viral vector in China (Ruan et al. 1984). There would be explosive epiphytotics of MRDD each year when the migratory flight of planthoppers occurred in bulk during the harvest days of spring wheat in Yellow-Huai plain of China.

Nowadays MRDD is controlled by changing the planting time of maize and spraying insecticides in the fields.

Both of the above methods can reduce the severity of MRDD by controlling the planthopper populations, but they have a high cost and poor efficiency. Genetic resistance may be a cost effective and environmentally friendly way to control the disease in severely affected areas. DNA markers can rapidly and reliably be linked to genes of interest by bulked sergeant analysis (BSA) (Michelmore et al. 1991). BSA has been used in a number of studies to identify markers linked to genes controlling monogenic traits and major genes controlling multigenic traits (Chaguè et al. 1997; Chen et al. 2004; Lehmensiek et al. 2001; Quarrie et al. 1999; William et al. 1997; Xu et al. 1999; Yang et al. 2004). Improving the resistance of germplasm in maize is an important breeding objective in many research programs and there have been several studies on the genetic basis of MRDD. Liu et al. (1996) evaluated the MRDD resistance of 96 inbred lines and 136 hybrids and supposed that the resistance is a quantitative trait based on their field survey data. Bar-Tsur et al. (1998) inspected the resistance of corn genotypes to maize rough dwarf virus. Wang et al. (2000) reported the maize resistance to MRDD was a quantitative characteristic that was controlled by polygene with small effect. In Argentina, a partial resistance line was used to evaluate the genetic resistance to Mal de Río Cuarto (MRCV) and this research suggested that the moderate heritability ranged from 0.44 to 0.56 (Di Renzo et al. 2002). Then two QTL that explained a total of 36.2 % phenotypic variance were detected through a $F_{2:3}$ QTL mapping (Di Renzo et al. 2004). Chen et al. (2008) identified two SSR markers that might be related to resistant genes for MRDD in a resistant maize line 87-1 using group inoculation in a net house.

In this study, F_2, BC_1 and RILs populations derived from the cross of 'Ye478' which is the highly sensitive elite inbred, and '90110' which is highly resistant to RBSDV were analyzed. Molecular markers were used to construct genetic map and decipher the resistance to RBSDV. The BSA method was used to find out markers linked to loci for RBSDV resistance. And the method of conventional QTL mapping was used to validate the results of BSA, determine the genetic distances and calculate the effects of QTL. The objectives of the present study were to: (1) find out makers which might relate to RBSDV resistance genes in parental line '90110' through marker-BSA and QTL mapping; (2) provide consistent and useful marker information to establish the foundation for the fine mapping of the resistant loci and MAS breeding in maize.

Materials and methods

Plant materials

Maize inbred line 'Ye478', an elite inbred line in China, is highly susceptible to MRDD, and was provided by Prof Deng-hai Li (Agricultural Academy of Laizhou in China). Another inbred line '90110' is highly resistant to MRDD, and was provided by Prof. Cheng-he Zhang (Agricultural Academy of Hebei Province in China). The two parental lines, F_2, BC_1 and a RIL population of 120 $F_{7:9}$ were used in this study (Fig. 1a). The F_2, BC_1 and two parental lines were grown at two plots in Jinan and Laizhou of China during summers of 2006 and 2007. The RIL population and parental lines were also grown at two plots in Jinan and Laizhou of China during summers of 2008, 2009 and 2010. From 2008 to 2010, RBSDV disease explosions were serious in the summers of Jinan and Laizhou. The disease was most severe at Jinan in 2009 and 2010.

Field test

Each line of the populations and their parents was sown in a one-row plot in the experiment fields with two replications in a completely randomized block design. The rows were 3 m in length with a 0.5 m alley way at the end of plots and 20 individual plants with a distance of 0.15 m between plants in each row. The inter-row space was

Fig. 1 **a** The phenotypes of inbred lines in the experimental field at Jinan in 2010 including the susceptible parental line 'Ye478', the resistant parental line '90110', the susceptible RIL F00233111 and the resistant RIL F039233211 at the 80th day after the transplantation in Jinan. **b** The net house for artificial inoculation of RIL population and the parental lines

0.66 m. The plants were grown in early May in Jinan and late May in Laizhou during the explosion of MRDD. For the whole life cycle, the plants were cultured with normal field management without any insecticide used.

The virus acquisition and detection of planthoppers

The planthoppers were fed the leaves from RBSDV-infected maize for 3 days to allow them to acquire the virus. Then the planthoppers were transferred to wheat in plastic buckets for 14 days to pass the circulative period of virus (Wang et al. 2006). Then five planthoppers of each bucket were mixed to be one sample for the detection of RBSDV. A Tube-Capture RT-PCR procedure (Delano 1999) was used to extract the RBSDV virion. A pair of primer which is RBSDV specific was used based on the S6 segment of RBSDV. The sequence of forward primer was named P1 (5′-TCAGCAAAAGGTAAAGGAAGG-3′) and the sequence of reverse primer was named RP (5′-GCTCC TACTGAGTTGCCTGTC-3′) (Wang et al. 2006).

Combined test

To enhance the incidence rate of MRDD and accurately evaluate the disease severity index (DSI), a combined test was designed in 2010. The combined test consists of two steps: the inoculation step and the natural infection step. At the inoculation step, the RILs and their parents were germinated at 28 °C in plastic plates with two replications on May 1st in green house and then were transported to the net house at the 1-leaf period according to the method of "net shed" with modification (Wang et al. 1998). The net house was made of 100-mesh nylon net and consisted of two rooms (Fig. 1b). The outer room was 6 m long, 4 m wide and 2 m high; while the inner room was 3 m long, 2 m wide and 1 m high. After the confirmation of RBSDV detection, the planthoppers were dispersed into the inner room. The average density of planthoppers was about 500/m^2. After a 5-day inoculation, insecticides were used to kill all of the planthoppers, and the seedlings were maintained to grow continually in the plastic plates. At the natural infection step, the plants at the 3-leaf stage were carefully transplanted to experiment fields on May 10th in Jinan. The culture was implemented as the field test, and the plants were infected again by the planthoppers in the fields at the migratory flight phase of planthoppers. The phenotypes of these RILs were subsequently evaluated at the flowering stage.

The evaluation of MRDD

For the populations of F_2, BC_1, RILs and two parents 'Ye478', '90110', the MRDD was evaluated at the flowering stage. There were more than 20 plants evaluated in each RIL. The following typical traits and rating scale of RBSDV disease were used: (1) shortened superior internode (denoted Internode: 0 = normal, 1 = shortened), superior internodes of RILs were evaluated for the trait; (2) enation (denoted Enation: 0 = no enation, 1 = mild enation, 2 = enlarged vein, 3 = galls), enations of abaxial surface on upper leaves of RILs were evaluated for the trait; (3) tassel type (denoted Tassel: 0 = normal, 1 = reduced flower number but fertile flowers, 2 = reduced flower number and sterile flowers, 3 = no flowers and no tassel); (4) disease severity of MRDD (grade scale from 0 to 3) according to the disease grades established by Ornaghi et al. (1993). Then the DSI was used to evaluate the severity of MRDD, which is calculated from disease severity grade for the RILs lines according to Grau et al. (1982):

$$\mathrm{DSI} = \sum (\text{grade} \times \text{number of plants in grade}) \times 100/(3 \times \text{total number of plants}).$$

The preparation of genomic DNA and genotyping

Genomic DNA was extracted from F_2 and BC_1 population after plants were phenotyped, and then the DNA was mixed based on the resistance of plants to produce resistance pools and sensitive pools. Both of pools contained equal amounts DNA of 15 typical plants. Genomic DNA of each RILs was isolated from the leaves at the 3-leaf stage and samples were stored in liquid nitrogen. All of genomic DNA was extracted by the CTAB method (Maguire et al. 1994).

Five hundred and twelve SSR markers from the Maize Genetics and Genomics Database (MaizeGDB at http://www.maizegdb.org) were used to screen polymorphism between the two parents, and then the markers that had polymorphism, clear and stable bands were used to screen the mixed bulk groups and the RILs populations.

For the SSR reaction, a 10-μl PCR mixture was used. It contained 50 ng templates of genomic DNA, 1× buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP and 0.6 U *Taq* polymerase. A touchdown PCR profile was applied for amplification. The mixture was incubated at 95 °C for 5 min, then ten cycles of 1 min of denaturing at 95 °C, 1 min of annealing at 65 °C with a decrease of 1 °C in each subsequent cycle, and 1 min of extension at 72 °C. Then PCR was continued through an additional 29 cycles of 45 s at 95°C, 1 min at 55 °C, and 1 min at 72 °C with a final extension at 72 °C for 7 min. The PCR products were separated in a 6 % denaturing polyacrylamide gel electrophoresis. The bands were detected and read by silver staining method (Bassam et al. 1991) and valuated finally.

SSR-BSA analysis

For bulk sergeant analysis, resistant F_2 plants were pooled to form the F_2 resistant bulk group and resistant BC_1 plants formed the BC_1 resistant bulk group. The diseased F_2 plants formed the F_2 diseased bulk group; while the diseased BC_1 plants formed the BC_1diseased bulk group. In screening, the markers which exhibited polymorphism between the F_2 pools were further used to test the BC_1 pools. The markers were identified as the potential markers linked to the resistant loci, when the polymorphism was exhibited between the two BC_1 pools in accordance with those between the two F_2 pools.

QTL mapping and analysis

One hundred and two lines of RILs populations were used for QTL mapping for resistance to RBSDV disease through the experiment from 2008 to 2010 in Jinan and Laizhou, finally. MAPMAKER 3.0b was used for linkage analysis (Lincoln et al. 1992) with Kosambi function (Kosambi 1944). QTL analysis was performed with QTL IciMapping v3.0 (http://www.isbreeding.net) with the methods of ICIM-ADD for additive effect (Li et al. 2007) and ICIM-EPI for epistatic effect (Li et al. 2008). The logarithm of odds (LOD) value of 3.0 and 5.0 was claimed as the threshold through a permutation of 1,000 times for mapping with additive effect and epistatic effect, respectively ($\alpha < 0.05$).

Statistical analysis

Both of the genotypic and phenotypic data were gathered and calculated with the Microsoft Excel 2003 Software. Then the broad sense heritability (h^2), trait distribution and the Pearson correlation coefficients were calculated by SAS 9.13 Software (SAS Institute Inc, Raleigh, USA). The correlations were calculated using the 'PROC CORR' option among different traits. Analyses of variance (ANOVA) were carried out using the general linear model procedure (GLM) to estimate the effects of genotype, environment and interaction between the genotypic and environmental factors. The broad sense heritability was calculated on the basis of each RIL family mean in each environment and overall environment. The broad sense heritability that is based on the ratio of genetic variance to phenotypic variance was estimated using the following formulae (Hallauer and Miranda 1988):

Single environment by:

$$h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r)$$

Overall environment by:

$$h^2 = \sigma_g^2/\left(\sigma_g^2 + \sigma_{ge}^2/l + \sigma_e^2/lr\right)$$

σ_g^2 is the variance component of genotype, σ_e^2 is the variance component of environment and σ_{ge}^2 is the interaction between genotype and environment. The l is the number of environments, and r is the number of replications in each environment.

Results

SSR-BSA analysis

To map the resistant loci to RBSDV disease, SSR-BSA analysis was performed by using F_2 DNA pools and BC_1 DNA pools. Four hundred and fifty-three pairs of SSR markers were used to determine the genotypes of both parental lines and F_2 DNA pools. The markers that were linked to a resistant locus were further tested with the BC_1 DNA pools. Four SSR markers umc1656 (bin 6.02), umc1401 (bin 7.02), bnlg1823 (bin 8.07) and umc1268 (bin 8.07) may be linked to the MRDD resistant loci were identified.

The tube-capture RT-PCR result of infected planthoppers

Fourteen samples that came from 14 different plastic buckets were used for the tube-capture RT-PCR test. Ten samples of RBSDV-infected planthoppers in the plastic buckets were positive, and RBSDV was not detected in the other four samples (Fig. 2). The planthoppers from the RBSDV-detected buckets were subsequently dispersed in the net house for group inoculation.

Variation of traits associated with MRDD

The ratings of parental line '90110' were zero for all traits and overall mean traits, and the ratings of the other parental line 'Ye478' were in the most seriously diseased group. It

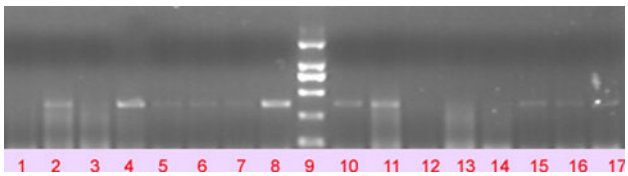

Fig. 2 The result of tube-capture RT-PCR. *Lane 1* is the negative control with five mixed uninfected planthoppers. *Lane 8* is the positive control which is the cDNA of RBSDV extracted and reverse transcribed from the typical leaves of maize infected with MRDD. *Lane 9* is the marker DL2000. *Lanes 2–7*, *Lanes 10–17* are samples, and each of them was mixed with five candidate planthoppers

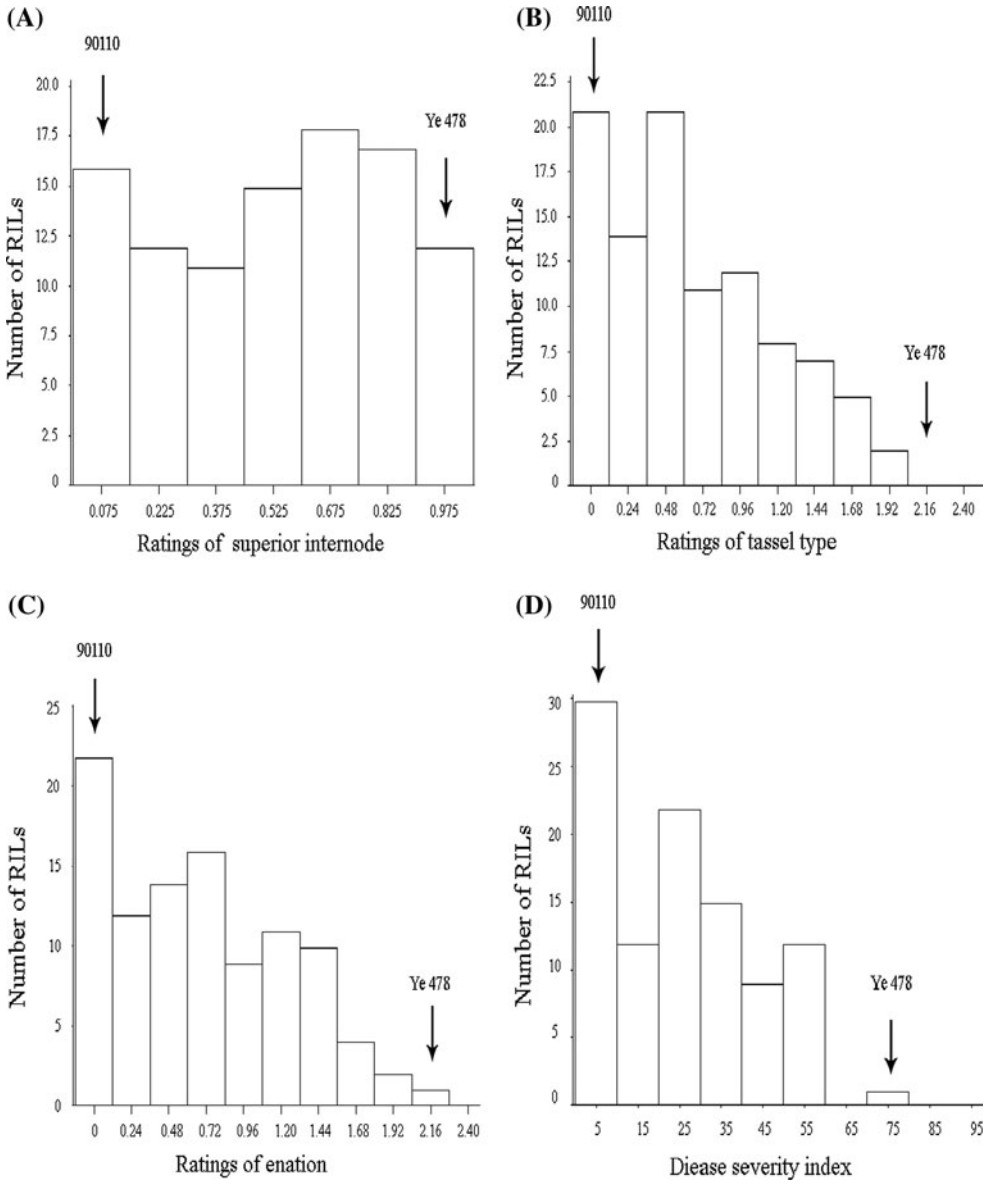

Fig. 3 Distribution among 102 lines for the RBSDV related mean traits in the RILs population of 'Ye478' × '90110'. Values on the *horizontal axis* are midpoint range values. **a** The frequency distribution of mean ratings of superior internode (Internode overall). **b** The frequency distribution of mean ratings of enation (Enation overall). **c** The frequency distribution of mean ratings of tassel type (Tassel overall). **d** The frequency distribution of mean disease severity index (DSI overall)

suggested that the resistance QTL might all come from the resistant line '90110'. The trait of superior internode showed nearly normal distribution; while other traits in RILs population showed skewed distributions toward the resistant parent (Fig. 3). It suggested there might be loci with large effects which controlled RBSDV resistance in the RILs population. All traits measured according to our tests were highly heritable in the RILs populations, though the interaction existed through our calculation between the genetic and environmental factors (Table 1). The heritability of overall shortened superior was 0.95, the overall enation was 0.82, the overall tassel type was 0.73 and the overall disease severity was 0.95 (Table 2). This suggested the resistance to RBSDV disease mainly depended on an inheritance factor. The Pearson correlation coefficients showed all traits that we measured were positive from moderate to high among these tests in all four environments ($P < 0.0001$) (Table 3).

QTL analysis

Five QTL in different region were detected through QTL mapping, and they were located on chromosome 2(bin 2.02), 6(bin 6.02), 7(bin 7.02), 8(bin 8.07) and 10(bin

Table 1 General statistics for DSI of the 'Ye478 × 90110' RIL population in four environments and overall environments

Parameters	Environment					*F* test			
	J2009	J2010	L2008	L2009	Overall environments[a]	Source	MS	*F*	*P*
Mean ± SEM	23.63 ± 1.17	24.78 ± 1.23	24.84 ± 1.34	24.99 ± 1.17	24.56 ± 0.96	Genotype	1,321.89	29.48	<0.0001
Variance components						Environment	40.17	0.9	0.4427
σ_g^2	331.54*	347.83*	307.33*	339.13*	157.58*	Genotype × environment	61.27	1.36	0.0004
σ_e^2	90.94	120.36	243.85	82.89	44.84	Error	44.84		
σ_{ge}^2					8.22				

* $P < 0.01$

[a] Overall analysis of DSI was calculated with the averaged phenotype data of four environments

10.05), which were named *qMRD2, qMRD6, qMRD7, qMRD8* and *qMRD10*, respectively. The *qMRD8* that was located between SSR markers bnlg1823 and umc1069 was consistently significant in all experiments. It is a major QTL contributing to lower the disease levels in this study. And other four QTL were relatively minor QTL. The *qMRD2* was flanked by the markers umc1823 and umc1845, and *qMRD6* was flanked by umc1656 and bnlg2191. The *qMRD7* was located between the markers umc1695 and umc1666, and the *qMRD10* was flanked by the markers umc1677 and umc1648 (Fig. 4; Table 4). However, we did not detect any epistatic effects among these QTL for all traits evaluated in all environments through the epistatic mapping. All of the alleles above contributing to low score (disease level) came from the parental line '90110' (Table 4).

Table 2 Heritability of each trait, per year and overall environments

Trait[a]	Heritability (%)
Internode J2009	92
Internode J2010	98
Internode L2008	86
Internode L2009	74
Enation J2009	73
Enation J2010	80
Tassel J2009	74
Tassel J2010	76
DSI J2009	88
DSI J2010	85
DSI L2008	71
DSI L2009	89
Internode overall	95
Enation overall	82
Tassel overall	73
DSI overall	95

[a] The traits related to MRDD. The traits were assessed in four environments: in the field in Laizhou (denoted L), during the summer 2008 and 2009. In the field at Jinan (denoted J) during the summer 2009 and in combined field and net house at Jinan during the summer of 2010. The trait of shortened superior internode was evaluated from 2008 to 2010 at Jinan (Internode J2009 and Internode J2010), Laizhou (Internode L2008 and Internode L2009). The trait of enations was only evaluated from 2009 to 2010 at Jinan (Enation J2009 and Enation J2010). The trait of tassel type was also evaluated from 2009 to 2010 at Jinan (Tassel J2009 and Tassel J2010), only. The DSI was evaluated from 2008 to 2010 at Jinan (DSI J2009 and DSI J2010), and Laizhou (DSI L2008 and DSI L2009). The Internode overall, Enation overall, Tassel overall and Disease overall were mean scores of trait across all environments above

For the trait of shortened superior internode, there were 4 loci (bin 2.02, bin 6.02, bin 7.02 and bin 8.07) detected in both field test and combined test through a 3-year experiment. The QTL *qMRD8* had a major locus effect, which explained a variation of phenotype ranging from 11.9 to 34.8 %. The QTL *qMRD6* explained a variation of phenotype ranging from 13.2 to 18.7 %, which was detected in the field test at Laizhou during 2008 and Jinan during 2009. The QTL *qMRD2* and *qMRD7* were detected in the field test at Laizhou during 2009 and the combined test at Jinan during 2010 explained 11.4 and 13.8 % of phenotypic variation, respectively. Through overall environments, the *qMRD6* and *qMRD8* were detected related to the trait of superior internode explaining 12.0 and 32.1 % of phenotypic variation, respectively (Fig. 4; Table 4).

For the trait of the enation, four loci (bin 2.02, bin 6.02, bin 8.07 and bin 10.05) were detected. The QTL *qMRD8* explained about 26.3 % of phenotypic variation detected in the field test at Jinan in summer 2009. There were three QTL detected in the combined test at Jinan in summer 2010. The QTL *qMRD2, qMRD6* and *qMRD10* explained 13.7, 9.0 and 9.0 % of phenotypic variation, respectively. The QTL *qMRD2* and *qMRD8* were also detected across all environments explaining ~30 % of phenotypic variance, together (Fig. 4; Table 4).

For the trait of tassel type, there was only one locus detected on bin 7.02 in the combined test at Jinan during 2010 and it explained 27.3 % of phenotypic variation.

Table 3 The Pearson correlation coefficients among measured traits related to RBSDV disease in four environments

Trait	Internode J2010	Internode L2008	Internode L2009	Enation J2009	Enation J2010	Tassel J2009	Tassel J2010	DSI J2009	DSI J2010	DSI L2008	DSI L2009
Internode J2009	0.77	0.76	0.85	0.81	0.68	0.58	0.59	0.85	0.76	0.76	0.82
Internode J2010		0.94	0.88	0.63	0.78	0.39	0.68	0.70	0.90	0.86	0.80
Internode L2008			0.90	0.65	0.76	0.40	0.66	0.69	0.85	0.91	0.82
Internode L2009				0.71	0.73	0.48	0.70	0.77	0.84	0.86	0.89
Enation J2009					0.73	0.56	0.53	0.83	0.69	0.74	0.80
Enation J2010						0.32	0.69	0.63	0.84	0.78	0.71
Tassel J2009							0.52	0.60	0.35	0.43	0.49
Tassel J2010								0.51	0.73	0.63	0.61
DSI J2009									0.74	0.77	0.90
DSI J2010										0.89	0.85
DSI L2008											0.87

The *qMRD7* was also detected through the overall environments with a 24.5 % explanation of phenotypic variance (Fig. 4; Table 4).

For DSI of MRDD, four loci (bin 2.02, bin 6.02, bin 7.02 and bin 8.07), which were detected in other traits, were also identified. The *qMRD8* conferred resistance both in the field test and the combined test, and explained 28.9 % of phenotypic variation in Laizhou in 2008. It explained 23.9 % of the phenotypic variation at Laizhou in 2009, 12.0 % at Jinan during 2009 and 16.3 % during 2010. QTL *qMRD2* was detected in the field test at Laizhou 2009 and the combined test at Jinan in 2010, and explained 11.4 and 9.3 % of the disease severity variation, respectively. The QTL *qMRD7* was both detected in the experiment at Laizhou 2008 and Jinan 2010, and explained 10.0 and 8.6 % of the phenotypic variation, respectively. The QTL *qMRD6* was only detected in the test at Jinan during 2009, and explained a 12.8 % phenotypic variation. The QTL *qMRD2* and *qMRD8*, both of which were detected through overall environments, together explained ~38.3 % of phenotypic variance for DSI (Fig. 4; Table 4).

Discussion

The MRDD is a viral disease that requires the insect vectors for transmission. Under normal conditions, the pathogen is only distributed by the planthopper (Boccardo and Milne 1984; Ruan et al. 1984). The disease incidence and severity both are influenced to a great extent by the density of planthopper (*Laodelphax sreiatellus* Fallen.). There were several artificial inoculation methods to aim at virus infection for a single plant including rice, maize and other crops (Louie and Abt 2004; Shikata and Kitagawa 1977; Wang et al. 2006; Zhou et al. 2011). The method named "net shed" was used for group inoculation (Wang et al. 1998). All of the methods were effective for the artificial infection, but had their own disadvantages including unstable rates of inoculation, bad repetition or low throughput of inoculation. The previous segregation population experiments always employed field natural infection which might lead to non-identical results in different places and years. For the accuracy of resistance detection in the present study, we employed a combined method to evaluate the segregation population which combined the "net shed" inoculation method and the natural infection. The plant materials were infected by planthoppers twice with the combined method in the summer of 2010 in Jinan. If the RBSDV-infection rate of "net shed" inoculation is x, the natural infection rate is y, and then the infection rate of combined test is z.

$$z = 1 - (1 - x) \times (1 - y)$$

This may provide a more reliable way for evaluation of MRDD experiments in large populations.

It had been proved that the resistance of parental lines ('90110' and 'Ye 478') to MRDD was not due to the resistance to the insect vector but to RBSDV in China (Lu et al. 2009). The heritability ranged from 0.71 to 0.95 for DSI through our experiments of multiple plots and years, which was higher than the evaluation in Argentina (Di Renzo et al. 2002). The high heritability may be caused by differences in the genetic evaluation of the different parental lines, the method of infection and differences in the pathogen. From the genetic evaluation in our experiment, we concluded that the resistance to RBSDV disease in our population was mainly due to the genetic factor with a relative minor influence from differences of environment and the interaction between genetic factor and environmental factor (Tables 1, 2).

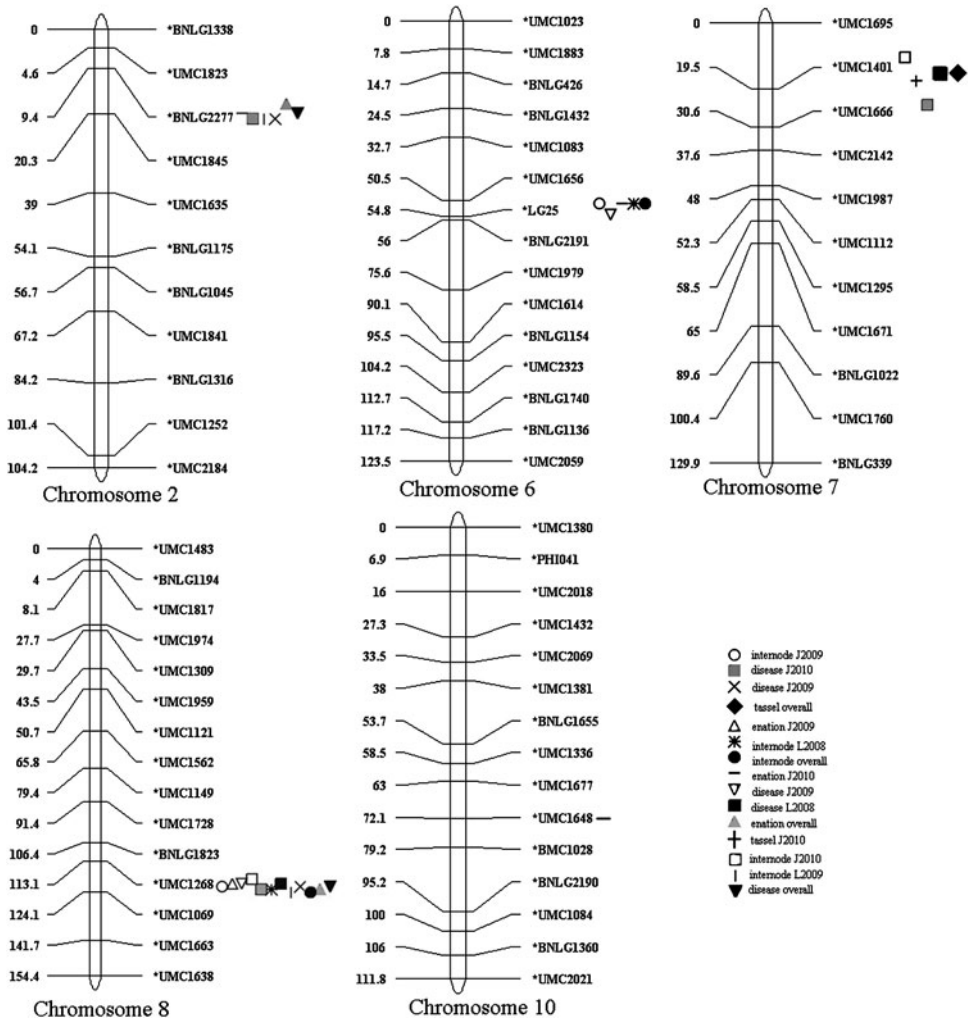

Fig. 4 Linkage group locations of the QTL underling resistance to RBSDV in $F_{7:9}$ RILs population generated from the cross of 'Ye 478' and '90110'

The bulked segregant analysis (BSA) method (Michelmore et al. 1991) had been shown to be a reliable and rapid method for detecting resistance genes or loci in many previous studies (Farinhó et al. 2004; Liu et al. 2007; Tullu et al. 2003). To date, several QTL or loci that are related to resistance have been successfully identified (Xu et al. 1999; Redinbaugh et al. 2004; Dintinger et al. 2005; Lübberstedt et al. 2006). In this study, the SSR markers found through BSA method pointed to the same or nearby regions of QTL across environment, such as umc1656 (bin 6.02), umc1401 (bin 7.02), bnlg1823 (bin 8.07) and umc1268 (bin 8.07). The QTL mapping results indicated that there were five QTL for RBSDV resistance *qMRD2, qMRD6, qMRD7, qMRD8* and *qMRD10* detected through our tests, four of which displayed consistency in different plots and years. It suggested that there might well be several genes or loci related to resistance in these regions which were both detected in BSA experiments and QTL tests.

In previous studies, two QTL with significant effects for MRCV were detected on chromosomes 1 and 8 (Di Renzo et al. 2004), and then there were four QTL detected on chromosomes 1, 4, 8 and 10 for MRCV resistance in a separate study with a $F_{2:3}$ population (Kreff et al. 2006). Subsequently, four loci on chromosomes 1, 4 and 10 were detected in a RILs population for MRCV resistance which showed good consistency with previous studies (Bonamico et al. 2012). Three loci associated with RBSDV resistance were detected on chromosomes 6, 7 and 8 in the resistant maize line '90110' (Wang et al. 2007). In this study, we also detected QTL *qMRD8* which had a major effect and pointed to a nearby region on chromosome 8 in almost all traits and different environments. These results suggested that a major gene or locus conferring resistance to MRDD with the main effect might exist on chromosome 8. Both of *qMRD6* and *qMRD7* were detected in QTL tests, and the SSR markers were also detected associated with them in BSA experiments. The *qMRD6* was mapped to the short arm of chromosome 6 which had been reported to contain a cluster conferring virus resistance (Redinbaugh et al. 2004). The *qMRD7* has explained ~24.5 % of phenotypic variance for the trait of enation across environments (Table 4). The *qMRD10* was detected for enation trait at

Table 4 The QTL (quantitative trait loci) interval detected by ICIM-ADD mapping associated with traits underlying resistance to RBSDV

Trait	QTL	bin[a]	Marker/interval	Peak cM position	LOD[b]	R^2 (%)[c]	a[d]	Total R^2 (%)[e]
Internode L2008	*qMRD6*	6.02	umc1656/LG25	53.5	5.0	13.2	−0.11	50.86
	qMRD8	8.07	umc1268/umc1069	115.0	7.7	23.6	−0.15	
Internode L2009	*qMRD2*	2.02	bnlg2277/umc1845	9.5	4.4	11.4	−0.07	48.11
	qMRD8	8.07	umc1268/umc1069	114.0	8.0	24.0	−0.10	
Internode J2009	*qMRD6*	6.02	umc1656/LG25	53.5	6.5	18.7	−0.14	46.81
	qMRD8	8.07	umc1268/umc1069	113.5	4.4	11.9	−0.11	
Internode J2010	*qMRD7*	7.02	umc1695/umc1401	14.5	3.8	13.8	−0.12	50.10
	qMRD8	8.07	bnlg1823/umc1268	111.5	11.1	34.8	−0.19	
Internode overall	*qMRD6*	6.02	umc1656/LG25	53.5	4.9	12.0	−0.11	60.72
	qMRD8	8.07	umc1268/umc1069	116.0	9.3	32.1	−0.17	
Enation J2009	*qMRD8*	8.07	bnlg1823/umc1268	113.0	6.7	26.3	−0.31	25.38
Enation J2010	*qMRD2*	2.02	umc1823/bnlg2277	9.0	4.6	13.7	−0.22	41.60
	qMRD6	6.02	umc1656/LG25	54.0	3.1	9.0	−0.18	
	qMRD10	10.05	umc1677/umc1648	72.0	3.3	9.0	−0.18	
Enation overall	*qMRD2*	2.02	umc1823/bnlg2277	8.0	4.2	13.8	−0.21	37.82
	qMRD8	8.07	umc1268/umc1069	115.0	4.8	16.8	−0.22	
Tassel J2010	*qMRD7*	7.02	umc1401/umc1666	22.5	5.8	27.3	−0.31	21.57
Tassel overall	*qMRD7*	7.02	umc1401/umc1666	21.5	5.3	24.5	−0.26	19.95
DSI L2008	*qMRD7*	7.02	umc1401/umc1666	21.5	3.4	10.0	−5.97	47.81
	qMRD8	8.07	umc1268/umc1069	112.5	9.5	28.9	−10.13	
DSI L2009	*qMRD2*	2.02	bnlg2277/umc1845	9.5	4.3	11.4	−6.75	47.50
	qMRD8	8.07	umc1268/umc1069	114.0	8.0	23.9	−9.65	
DSI J2009	*qMRD6*	6.02	LG25/bnlg2191	55.0	4.4	12.8	−6.87	41.43
	qMRD8	8.07	bnlg1823/umc1268	113.0	4.4	12.0	−6.81	
DSI J2010	*qMRD2*	2.02	bnlg2277/umc1845	9.5	3.8	9.3	−6.11	50.36
	qMRD7	7.02	umc1401/umc1666	29.0	3.3	8.6	−5.77	
	qMRD8	8.07	umc1268/umc1069	115.5	5.9	16.3	−7.91	
DSI overall	*qMRD2*	2.02	umc1823/bnlg2277	9.5	4.3	10.7	−6.0	50.84
	qMRD8	8.07	umc1268/umc1069	114.5	9.5	27.5	−9.6	

[a] QTL located on chromosome bin of maize genome. The genetic map is divided into 100 segments by core markers, and each of the segments was called a bin. A bin is named following the rule: the chromosome number followed by a decimal with two-digit (e.g. bin 1.01 et al.)

[b] The logarithm of odds (LOD) value. LOD represents the likelihood at the peak of QTL

[c] The R^2 represents coefficients of determination

[d] The additive effects of QTL. The negative number means the allele for decreased score (lower disease level). For disease severity, the DSI is from 0 to 100. For the superior internodes ratings, it is in terms of the 0–1 level. For the tassel type ratings and enation ratings, these are in terms of the 0–3 level

[e] The total R^2 represents the total phenotypic variation explained by all additive QTL

only one site (Table 4), which suggested the region needs to be evaluated in other mapping populations. The region of *qMRD2* which was detected through QTL mapping both in the field tests and combined tests had been previously detected in maize stripe virus (MStV) (Dintinger et al. 2005). This suggested that there might be a region conferring resistance to maize virus diseases near the *qMRD2*. Previous work showed that resistance genes tend to form several clusters within maize genome (Redinbaugh et al. 2004), which suggested the resistance to different viruses might be in the correlated mechanism in maize. A number of virus resistance loci had been detected through QTL mapping; however, there had not been any virus-resistance genes cloned in maize (Redinbaugh and Pratt 2009). Further investigations should be carried out to increase knowledge of virus-resistance mechanisms in maize. The positional cloning and transposon tagging would be good methods for studying the mechanism of resistance.

In this study, we demonstrated that a consistent QTL *qMRD8* that was detected in almost all traits related to RBSDV resistance explained a large part of total resistance for RBSDV disease in RILs populations. At the same time,

we also detected several other QTL which were only detected in several traits related to RBSDV resistance with relative smaller effect, such as *qMRD2*, *qMRD6* and *qMRD7* (Table 4). These results revealed that the resistance to RBSDV disease was a complex trait that was controlled by multiple genes. This is consistent with previous work (Wang et al. 2000). The SSR markers, which were closely linked to the QTL in our study, will be useful for enhancing the resistance to RBSDV disease in maize breeding program by marker-assisted selection (MAS). The minor disease resistance QTL could be also pyramided to improve the virus resistance of maize germplasm (Hu et al. 2008). The secondary population, such as nearly isogenic lines (NILs), single-segment substitution lines (SSSLs) and chromosomal segment substitution lines (CSSLs) will be constructed for fine mapping of the QTL. Then the resistance genes could be cloned for more comprehensive characterization of the detected QTL. Because MRDD is becoming more and more serious in China, it is cost effective and environment friendly to increase the resistance through MAS and QTL pyramiding. The subsequent studies are in progress now.

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