Breeding Science 70: 183–192 (2020)
doi: 10.1270/jsbbs.19110

Research Paper

Marker-assisted selection of *qMrdd8* to improve maize resistance to rough dwarf disease

Zhennan Xu[1,2)], Jinge Hua[2)], Feifei Wang[2)], Zixiang Cheng[2)], Qingchang Meng[3)], Yanping Chen[3)], Xiaohua Han[4)], Shuanggui Tie[4)], Changlin Liu[2)], Xinhai Li[2)], Zhenhua Wang*[1)] and Jianfeng Weng*[2)]

[1)] *Northeast Agricultural University*, No. 600 Changjiang Road, XiangFang District, Harbin, Heilongjiang 150030 China
[2)] *Institute of Crop Science, Chinese Academy of Agricultural Science*, No. 12 Zhongguancun South Street, Haidian District, Beijing 100081 China
[3)] *Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences*, No. 50 Zhongling Street, Xuanwu District, Nanjing 210014, China
[4)] *The Cereal Crops Institute, Henan Academy of Agricultural Sciences*, No. 116 Garden Road, Jinshui District, Zhengzhou 450002, China

Maize rough dwarf disease (MRDD) is caused by viruses in the *Fijivirus* genus in the family *Reoviridae*. MRDD resistance can be improved by a combination of marker-assisted selection (MAS) and conventional breeding strategies. In our previous study, we fine-mapped a major QTL *qMrdd8* and developed the functional Indel marker IDP25K. In the present study, *qMrdd8* from the donor parent X178 was introgressed into elite inbred lines derived from the three corn heterotic groups using multi-generation backcrossing and MAS. Recipient lines included Huangzao4, Chang7-2, Ye478, Zheng58, Zhonghuang68, B73, and Ji846. Markers used for foreground selection included IDRQ4, IDRQ47, IDP25K, and IDP27K. Background selection was carried out in the BC_3 or BC_4 using 107 SSR markers to select lines with the highest rate of recovery of the particular recurrent parent genome. Plants from BC_4F_2 and BC_3F_2 that carried the shortest *qMrdd8* interval from X178 and those with the highest rate of recovery of the recurrent parent genome were then selected to create converted homozygous inbred lines. In 2017, seven converted inbred lines and five hybrids exhibited enhanced resistance to MRDD, while other agronomic traits were not affected under nonpathogenic stress conditions. Thus, the MRDD resistance allele at the *qMrdd8* locus, or IDP25K, should be valuable for maize breeding programs in China.

Key Words: maize, rough dwarf disease, resistance gene, *qmrdd8*, marker-assisted selection (MAS).

Introduction

Maize rough dwarf disease (MRDD), which is caused by viruses in the genus *Fijivirus* in the *Reoviridae* family (Zhang *et al.* 2001), seriously threatens maize production worldwide (Bai *et al.* 2002, Dovas *et al.* 2004, Lenardon *et al.* 1998). These viruses are known in Europe as maize rough dwarf virus (MRDV) (Dovas *et al.* 2004), in South America as *Mal de Río Cuarto virus* (MRCV) (Arneodo *et al.* 2005), and in Asia as rice black streaked virus (RBSDV) or southern rice black streaked virus (SRBSDV) (Bai *et al.* 2002, Fang *et al.* 2001). In China, RBSDV is persistently transmitted by its insect vector *Laodelphax striatellus* Fallen among maize, rice, and wheat hosts, and the virus particles appear mainly in the phloem of affected plants (Zhou *et al.* 2015). MRDD symptoms include dwarf stature, stunted growth, dark green leaves, waxy wrinkles on the adaxial surfaces of leaves and sheaths, deformed tassels and uppermost leaves, suppressed flowering, and an absence of ears (Zhang *et al.* 2001). Since the 1990s, the disease has been highly prevalent in the Yellow and Huai River valley maize-growing areas due to the use of susceptible cultivars and extensive maize cultivation in those areas (Wang *et al.* 2006). Yield losses due to MRDD have generally been over 30% and can even be up to 100% in regions with severe epiphytotics. At present, several ways of controlling MRDD have been proposed including applying pesticides to reduce insect vector populations and delaying maize sowing dates to avoid the peak migration periods of the vector. However, the costs of pesticide inputs and their risks to the environment are considerable. Therefore, using resistant cultivars is a more economical and

Communicated by Toyoaki Anai
Received July 17, 2019. Accepted October 7, 2019.
First Published Online in J-STAGE on February 26, 2020.
*Corresponding authors (e-mail: jianfengweng@126.com and zhenhuawang_2006@163.com)

environmentally friendly way to control MRDD.

Screening for resistance to MRDD in maize germplasm and cloning of genes responsible for MRDD resistance are essential for development of resistant cultivars (Ali and Yan 2012). Because MRDD infections can be so severe in China, maize inbred lines used for resistance breeding can be screened under natural infection conditions (Xue *et al.* 2012, Yang *et al.* 2010b) Only afew highly MRDD-resistant lines have been identified, such as Qi319, Jinhuang59, X178, P138, P12, P20, and BS110, which was derived from the US hybrids (Liu *et al.* 2015). Additionally, resistant germplasm has been found in local cultivars in China, such as inbred line 803-2 and in tropical maize germplasm (Lu *et al.* 2001). Consequently, based on existing germplasm resources, the identification and pyramiding of QTL (quantitative trait loci) or genes for MRDD resistance will make MAS in maize breeding much more efficient.

Previous studies showed resistance to MRDD to be a quantitative trait (Bonamico *et al.* 2012, Liu *et al.* 2014, Luan *et al.* 2012, Shi *et al.* 2012). Many QTL conferring resistance to MRDD have been identified using genome-wide association studies or linkage mapping. For example, Di Renzo *et al.* (2004) identified two QTL for MRCV resistance in bins 1.03 and 8.03/4 in BLS14 using an $F_{2:3}$ QTL-mapping strategy (Di Renzo *et al.* 2004). In an F_2 population derived from 90110 × Ye478, three QTLs were identified on bins 6.02, 7.02, and 8.07 (Luan *et al.* 2012, Wang 2007). A major QTL for MRDD resistance was found in maize inbred line X178 on chromosome bin 8.03 and explained 24.6–37.3 % of the phenotypic variance (Shi *et al.* 2012). Subsequently *qMrdd8* was fine-mapped into a 347-kb interval between markers IDRQ2 and IDRQ20 by testing progeny of the recombinants and localizing InDel25 within candidate gene *ZmGDI* (Liu *et al.* 2016). In an independent experiment, the QTL *qMrdd1* was detected and fine-mapped into a 1.2-Mb region (Tao *et al.* 2013).

Disease resistance controlled by quantitative disease resistance alleles is generally broad and stable. Although the effect on resistance of a single disease resistance allele is often moderate, pyramiding resistance alleles of several genes or QTL can confer a high degree of disease resistance (Dingerdissen *et al.* 1996, Mackay *et al.* 2009, Yang *et al.* 2017). In spring and winter wheat, MAS of the QTL *Fhb1* and *Qfhs.ifa-5A* conferred increased resistance to FHB (Schweiger *et al.* 2013). In rice, five genes that impart resistance to the brown plant-hopper have been deployed by MAS. In that study, the efficiency of selection increased by 89.9% and 91.2% using the *bph2*-linked SSR markers RM7102 and RM463, respectively (Sun *et al.* 2006). In maize, Zhao *et al.* (2012) developed 10 susceptible inbred lines carrying the resistance allele of *qHSR1*. The head smut resistance of all 10 of these converted inbred lines and the hybrids derived from them improved substantially, even as the other desirable agronomic traits of these lines and hybrids remained essentially the same (Zhao *et al.* 2012). Interestingly, elite maize *ZmCCT* haplotypes without transposable elements in their promoters showed enhanced resistance to *Gibberella* stalk rot and also improved yield-related traits, with no changes in flowering time (Li *et al.* 2017).

In our previous studies, we mapped MRDD resistance to the quantitative trait locus (QTL) *qMrdd8* and fined-mapped *qMrdd8* into a 347-kb interval of between the IDRQ2 and IDRQ20 markers. Subsequently, a functional marker IDP25K was further developed within the *ZmGDI*. In the present study, our objectives were to (1) to improve resistance to MRDD by introgressing the resistance allele of *qMrdd8* into seven MRDD-susceptible maize inbred lines including B73, Ji846, Chang7-2, Huangzao4, Ye478, Zheng58, and Zhonghuang68 using MAS; (2) evaluate responses of the resulting seven converted inbred lines and five hybrids to MRDD under both natural and artificial MRDD inoculation; and (3) evaluate the agronomic performance of converted inbred lines and hybrids.

Materials and Methods

Plant materials

In our previous studies (Liu *et al.* 2016, Shi *et al.* 2012), a major QTL (*qMrdd8*) that explained 24.6–37.3% of the phenotypic variation in resistance to MRDD was detected on chromosome 8 in elite maize inbred line X178. X178 was derived from the US hybrids 78599 and had better agronomic traits. X178 was the most resistant material and then used as donor for introgression the MRDD resistance allele at *qMrdd8* into other lines, which then showed complete resistance to MRDD under natural infection conditions. The MRDD-susceptible lines from three subgroups (Lancaster, Tangsipingtou, and Reid) that were used as recurrent parents in that experiment included B73, Ji846, Huangzao4, Chang7-2, Ye478, Zheng58, and Zhonghuang68. These seven susceptible materials have high general combining ability, better agronomic traits, and have been widely used in maize breeding. For example, the corn hybrid Zhengdan 958 from the cross between Chang7-2 and Zheng58 has been widely planted in China. In 2012/2013, the above seven susceptible lines and X178, the donor of the *qMrdd8* resistance allele, were planted in the winter nursery at Hainan (18.25°N, 109.5°E). Each of the seven MRDD-susceptible recipient lines was pollinated by X178 to obtain a set of seven hybrids. During 2013 at the Shunyi farm in Beijing (40.13°N, 116.65°E), each F_1 was then backcrossed to its respective MRDD-susceptible recurrent parent to create the BC_1 generation. From generation BC_1 through BC_4, foreground selection was carried out by screening these seven recipient BC families only for individuals harboring the *qMrdd8* donor region. No screening for resistance to MRDD took place during recurrent selection under either natural inoculation or artificial inoculation. The selected BC individuals with phenotypes most closely resembling those of their recurrent parent were then

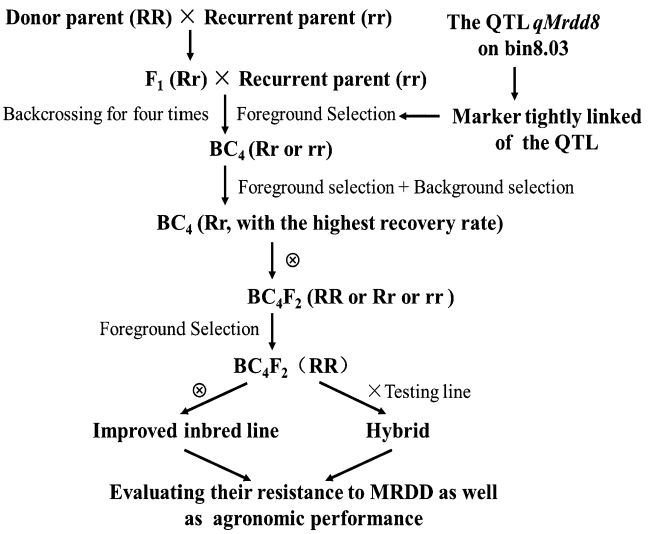

Fig. 1. Flowchart of the procedure for introgression of the *qMrdd8* locus into recurrent parent (rr) genetic backgrounds through marker-assisted selection. Recurrent parents include B73, Ji846, Huangzao4, Chang7-2, Ye478, Zheng58, and Zhonghuang68. The genotypse of the donor parent, the recurrent parent, and the heterozygote at the *qMrdd8* locus are indicated by RR, rr, and Rr, respectively.

backcrossed to their recurrent parental lines to generate the BC_2 through BC_4 families. During 2015 at Yuanjiang in Yunnan Province (23.59°N, 102°E), individuals carrying the shortest *qMrdd8* donor genome (X178) intervals and the greatest proportions of recurrent parental genomes were identified by carrying out both foreground and background selection, respectively, in the BC_4 families. Individuals from BC_4 were then selfed to develop lines carrying the most minimal homozygous donor genotypes at *qMrdd8*. At the winter nursery in Hainan in 2015/2016, BC_4F_2 individuals homozygous at the *qMrdd8* locus were screened and either selfed to produce converted inbred lines or crossed to obtain eight F_1 hybrids. The MRDD resistance of all of these converted inbreds and F_1 hybrids was evaluated in four environments in China, including Jining in Shandong Province (35.38°N, 116.59°E), Xuzhou in Jiangsu Province (34.79°N, 116.57°E), Nanjing in Jiangsu Province (31.14°N, 119.14°E), and Xinxiang in Henan Province (35.05°N, 113.96°E) under natural inoculation, and also under artificial inoculation at Nanjing in Jiangsu in 2017. These lines were simultaneously grown in Beijing to evaluate their agronomic performance (**Fig. 1**).

Genotyping using molecular markers

At the five-leaf stage, samples were taken from plants and were ground into a fine powder in liquid nitrogen. At least 300 individual plants were selected from each BC_1F_1 through BC_4F_1 generation. Extraction of genomic DNA was then carried out following the protocol of Murray and Thompson (1980) with modifications. SSR or InDel primers used for genotyping of plants by PCR (**Supplemental Table 1**) were synthesized by the AuGCT Biotechnology Co. Ltd., China.

Each PCR reaction mixture contained 6.8 μL of double-distilled water, 1.2 μL 10× Buffer, 0.5 μL dNTPs (2.5 mM), 0.15 μL of each primer (0.01 nmol/μL), 0.2 μL of *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 denaturation step at 94°C for 4 min followed by 10 cycles of 30 s at 95°C, 30 s at 65°C, 30 s at 72°C and decreasing the annealing temperature 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 with extending for 5 min at 72°C. PCR products were then separated by electrophoresis on 8% polyacrylamide gels in 1× TBE buffer that were silver stained for visualization of PCR products.

Foreground selection

A total of 12 linked InDel markers were developed when the *qMrdd8* QTL was fine-mapped (Liu *et al.* 2016). These markers were distributed within a 715-kb region containing *qMrdd8* in the B73 RefGen_v3 map (http://www.maizegdb.org/). Polymorphisms in these 12 markers were identified between X178, the donor parent, and each of seven recurrent parents (**Supplemental Table 1**). These InDel markers delimited either side of *qMrdd8* in the region from 103.53 to 104.03 Mb. Markers for which distinct polymorphic bands could be amplified were then used to select individuals carrying the MRDD resistance allele at the *qMrdd8* locus during foreground selection of the backcrossed and selfed generations. At least two markers at each end of the region containing *qMrdd8* were used to screen each individual plant. After backcrossing to their recurrent parents, plants that were heterozygous at these markers were defined as carrying the intact *qMrdd8* region from X178 and were then included in the next cycle of selection of recombinants. Among these markers, the functional marker IDP25K was localized within the candidate gene *ZmGDI* (Liu *et al.* 2016).

Background selection

A survey of parental polymorphisms between the donor parent X178 and each of seven recurrent parents was performed by genotyping 107 highly polymorphic SSR markers distributed throughout the maize genome. The primer sequences for these SSR markers were retrieved from the MaizeGDB (http://www.maizegdb.org/) and were synthesized by the AuGCT Biotechnology Co. Ltd., China. Background selection was then carried out using evenly spaced polymorphic markers. Between eight and 20 individuals carrying the MRDD resistance allele at the *qMrdd8* region, according to foreground marker genotypes, were identified in the BC_4 family of each converted inbred line from background selection. After genotyping all of the polymorphic SSR markers, we calculated the rate of recovery of the recurrent parent genome (RPG) for the selected individuals as: % RPG = (R + 1/2H) × 100/2P, where R is the number of

homozygous markers for the recurrent parent allele, H is the number of markers that remained heterozygous, and P is the total number of polymorphic SSR markers used during background selection. Then the individual from each BC_4 family with the highest RPG recovery rate was selected and self-pollinated twice to generate converted inbred lines carrying the MRDD resistance allele at *qMrdd8*.

Evaluation of MRDD resistance of the converted inbred lines and their hybrids

To evaluate the effect of the MRDD resistance allele at *qMrdd8* on MRDD resistance in converted lines, we compared the response to inoculation with the MRDD pathogen among the converted inbred lines, the recurrent parents, and their hybrids using a disease severity index (DSI). The converted hybrids include Yandan14-R (Mo17 × Huangzao4-R), Yedan13-R (Dan340 × Ye478-R), Zhengdan958-R (Zheng58-R × Chang72), Jidan159-R (Ji846-R × Dan340), and B73-R × Mo17. In 2017, the MRDD resistance of the converted inbred lines, the recurrent parents, and their hybrids was evaluated under conditions of natural infection at Jining in Shandong, Xuzhou in Jiangsu, Xinxiang in Henan, and also under artificial inoculation at Nanjing in Jiangsu. The method of artificial inoculation we used involved combining inoculation and transplanting the cage group as described in Liu *et al.* (2016). All experimental material was planted in four rows 0.6 m wide and 4.0 m long in a randomized complete block design in duplicate. At maturation, all plants were assessed visually and MRDD resistance was scored on a scale from 0 to 4, in which plants scored 0 are highly resistant and those scored 4 are highly susceptible to MRDD. The DSI was defined as follows (Liu *et al.* 2016). DSI (%) = Σ(disease rating score × number of plants with each score)/maximum disease rating score × total number of plants rated in the line) × 100. One-way analysis of variance (ANOVA) using SAS was performed to analyze these data.

Evaluation of agronomic traits of the converted inbred lines and their hybrids

To investigate agronomic traits without MRDD stress in 2017, we planted the converted inbred lines, the recurrent parents, and their hybrids in the experimental field at Changping in Beijing (40.21°N, 116.19°E) in four-row plots with 17 plants per 4.0-m row spaced 0.6 m apart in a randomized complete block design with two replications. At the appropriate growth stages, plants or ears with no MRDD symptoms were chosen otherwise randomly from each plot for evaluation of maize agronomic traits. Data were recorded for 40 plants or ears per plot and replicate to evaluate agronomic performance for three ear traits (ear length, ear diameter, and cob color), four kernel traits (100-kernel weight, 10-kernel length,10-kernel width, and 10-kernel thickness), one yield trait (grain yield per plot in kg to calculate yield in kg per hectare), two plant architecture traits (plant height and ear height), and three reproductive development traits (tasseling, pollen shed, and silking). One-way ANOVA was used to analyze all data.

Results

Identification of polymorphic markers and recombinant selections

We found that the molecular markers IDRQ4, IDP25K, IDP27K, and IDRQ47 on either side of the *qMrdd8* locus and the 12 molecular markers were polymorphic between donor X178 and the seven recurrent parental inbred lines. The donor parent X178 and the seven recurrent parents were crossed (F_1) and continuously backcrossed to X178 to obtain BC_1, BC_2, BC_3, and BC_4 individuals. Seedlings were genotyped using the markers IDRQ4, IDP25K, IDP27K, and IDRQ47 for foreground selection to identify heterozygous individuals in the population carrying the disease resistance allele at *qMrdd8* (**Supplemental Fig. 1**). Markers IDRQ4 and IDRQ47 were used for foreground selection in the BC_1 and BC_2 segregating populations and IDRQ4 and IDP27K were used in the BC_3 segregating populations. IDP25K was used for foreground selection in the BC_4 and BC_4F_2 segregating populations. The markers IDRQ4, IDP25K, IDP27K, and IDRQ47 are tightly linked, with IDRQ4, IDP27K, and IDRQ47 located outside the gene and IDP25K located within the gene. The resulting selected plants were found to be heterozygous at all linked markers and were screened to identify plants that were phenotypically most similar to their recurrent parents for morphological, grain, and yield-related traits. The foreground selection markers used for screening *qMrdd8* alleles in the BC_4 segregating population included IDRQ4, IDP25K, and IDP27K. IDP25K is a functional marker based on the large InDel within the *ZmGDI* gene. Genotyping results showed that these three markers co-segregated in the BC_4 segregating population. Therefore, only the internal marker IDP25K for *qMrdd8* was used to screen for single individuals in the BC_4F_2 population carrying the homozygous *qMrdd8* allele for MRDD resistance that could be selfed to create inbreds or crossed to create hybrids. These genotyping steps identified 71 BC_4 plants and 12 BC_3 plants carrying the smallest X178 donor genome fragments at *qMrdd8* that were then selected to generate BC_4F_2 families and BC_3F_2 families. The selected 71 BC_4 plants and 12 BC_3 plants consisted of 16, 12, 12, 10, 9, 12, and 12 plants from B73, Huangzao4, Chang7-2, Ye478, Zheng58, Zhonghuang68 BC_4 families, and Ji846 BC_3 families, respectively.

Based on the B73 RefGen_V3 map published at the Maize Genome Database (http://www.maizegdb.org/), a total of 910 polymorphic SSR markers was detected genome-wide between donor and recurrent parents and 107 polymorphic SSR markers were identified between donor X178 and the seven recurrent parental inbred lines, ranging from six to 13 markers evenly distributed across the 10 maize chromosomes (**Supplemental Fig. 2**, **Supplemental**

Table 2). During foreground selection, we selected 71 BC_4 plants and 12 BC_3 plants including 16, 12, 12, 10, 9, 12, and 12 plants from B73, Huangzao4, Chang7-2, Ye478, Zheng58, Zhonghuang68 BC_4 families, and Ji846 BC_3 families, respectively. After genotyping for background selection, the rate of recovery of the genetic backgrounds of the recurrent parents in selected progenies ranged from 90.34% to 98.02% (**Supplemental Table 3**). Among BC_3F_1 plants from Ji846, the average rate of recovery of the genetic background of the recurrent parent was 95.47% and the highest background recovery rate was 97.80%. For BC_4F_1 plants from B73, Huangzao4, Chang7-2, Ye478, Zheng58, and Zhonghuang68, the highest rates of recovery of the recurrent parent genome were 93.96%, 98.02%, 97.52%, 96.74%, 95.70%, and 96.85%, respectively. For reference, the theoretical average background recovery rates for the BC_3 and BC_4 populations are 93.75% and 96.88% (**Supplemental Table 3**). Therefore, we chose the plants with the highest rates of recovery of the recurrent parent genomes and selfed all of the selected plants to generate six BC_4F_2 families for B73, Huangzao4, Chang7-2, Ye478, Zheng58, and Zhonghuang68, and one BC_3F_2 family for Ji846. A total of 1260 BC_4F_2 plants and 110 BC_3F_2 plants were then subjected to foreground selection. Eighty BC_4F_2 plants and 19 BC_3F_2 plants were homozygous at the linked marker IDP25K. The BC_4F_2 progeny that were homozygous for the MRDD resistance allele at the *qMrdd8* locus were considered as converted inbred lines and were designated B73-R, Huangzao4-R, Chang7-2-R, Ye478-R, Zheng58-R, Zhonghuang68-R, and Ji846-R, respectively.

Evaluation of converted lines and their hybrids for resistance to MRDD

Under artificial infection, the DSIs of converted lines B73-R, Huangzao4-R, Zheng58-R, Chang7-2-R, Ye478-R, Ji846-R, and Zhonghuang68-R were 58.47%, 30.15%, 33.75%, 30.86%, 36.23%, 43.17%, and 64.2% lower, respectively, than those of their recurrent parents (**Fig. 2**). The resistance to MRDD of seven converted lines was then further investigated under natural infection conditions. Consistently, these converted lines showed enhanced MRDD resistance compared to their recurrent parents ranging from 15.6 to 47.32% at Yuanyang in Henan, Jining in Shandong, and Xuzhou in Jiangsu (**Fig. 2**, **3**).

These converted inbred lines and their corresponding recurrent parents were then used to produce hybrids. We found that the DSIs of Yandan14-R (Mo17 × Huangzao4-R), Yedan13-R (Dan340 × Ye478-R), Zhengdan958-R (Zheng58-R × Chang7-2), Jidan159-R (Ji846-R × Dan340), and B73-R × Mo17 were significantly lower, by 27.2%, 49.5%, 33.1%, 36.5% and 37.2%, respectively, than those of their original hybrids without the MRDD resistance allele at *qMrdd8* under artificial inoculation in Nanjing in 2017 (**Fig. 4a**). We also found that the MRDD resistance of these hybrids at Yuanyang, Jining, and Xuzhou in 2017 was enhanced from 13.9% to 48.3% compared to their original hybrids without the MRDD resistance allele at *qMrdd8* (**Fig. 4b–4d**). We further investigated the MRDD resistance of four hybrids including Zhengdan958-R2 (Zheng58-R × Chang7-2-R), Zhengdan958-R1 (Zheng58 × Chang7-2-R), Zhengdan958-R (Zheng58-R × Chang7-2),

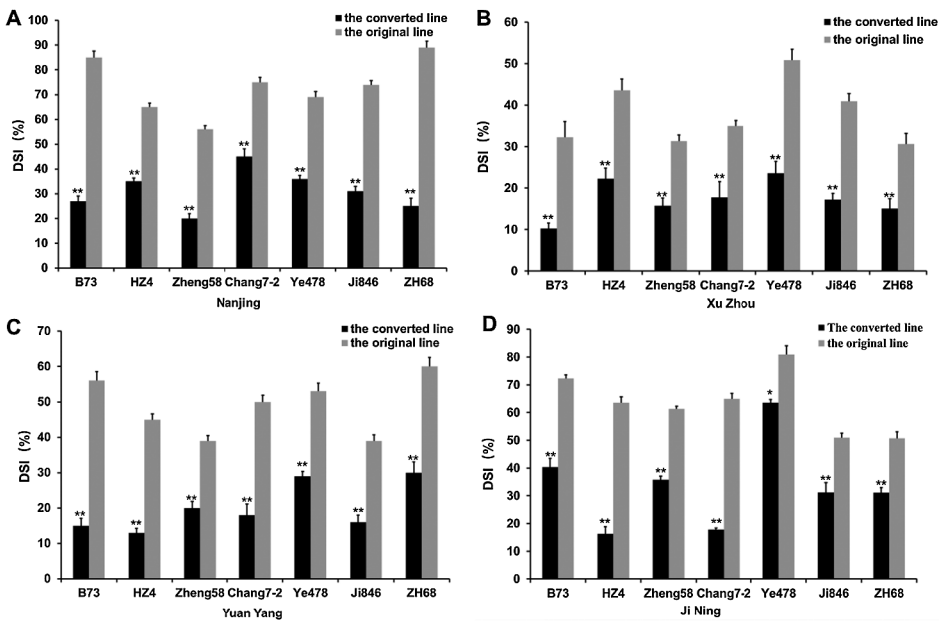

Fig. 2. Pairwise comparisons of DSIs (disease severity indices) of converted and original inbred parental lines. (A) The DSIs of all pairs of converted and original inbred lines were estimated using artificial inoculation at Nanjing in Jiangsu Province in 2017. (B, C, D) The DSIs of pairs of converted and original inbred lines were estimated under natural conditions of inoculation at Yuanyang in Henan Province, Jining in Shandong, and Xuzhou in Jiangsu in 2017, respectively. ** Differences between the converted and original lines are significant at $P \leq 0.01$. * Significant difference between the converted and original lines at $P = 0.05$.

Fig. 3. MRDD disease symptoms in the converted inbred lines (named B73-R, Chang7-2-R, Zhonghuang68-R, Ye478-R, Ji846-R and Zheng58-R) and their corresponding original inbred lines (CK represents each original inbred line, named B73, Chang7-2, Zhonghuang68, Ye478, Ji846, and Zheng58) growing under conditions of natural infection at Jining in Shandong Province in 2017.

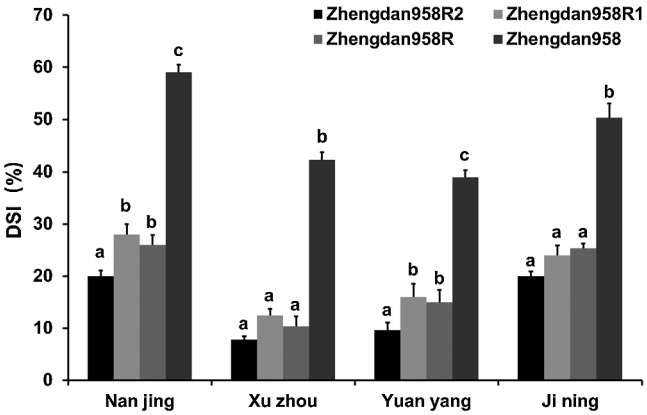

Fig. 5. DSIs (disease severity indices) of the four converted hybrids Zhengdan958R2 (Zheng58R × Chang7-2R), Zhengdan958R1 (Zheng58 × Chang7-2R), Zhengdan958R (Zheng58R × Chang7-2), Zhengdan958 (Zheng58 × Chang7-2) were tested in Yuanyang, Jining, Xuzhou, and Nanjing in 2017 under natural infection and artificial inoculation. The significance level of Duncan's Multiple Range Test was set to 0.05 in one-way analysis of variance. Means shown with different letters are significantly different.

and Zhengdan958 (Zheng58 × Chang7-2) in Yuanyang, Jining, Xuzhou, and Nanjing in 2017 under both natural infection conditions and artificial inoculation. The DSIs of Zhengdan958-R, Zhengdan958-R1, and Zhengdan958-R2 were 40.02%, 31.56%, and 33.17% lower, respectively, than that of Zhengdan958 at Nanjing in 2017 (**Fig. 5**). The DSI of Zhengdan958-R2 was lower than that of the other two resistant hybrids, suggesting that the MRDD resistance allele at the *qMrdd8* has an additive effect but no significant dominant effect on MRDD resistance and that resistance conferred by two alleles was superior to that conferred by one.

Evaluation of the effects of qMrdd8 on agronomic performance traits in the absence of pathogenic stress conditions

Under natural conditions without inoculation at Changping in Beijing in 2017, the agronomic traits of the

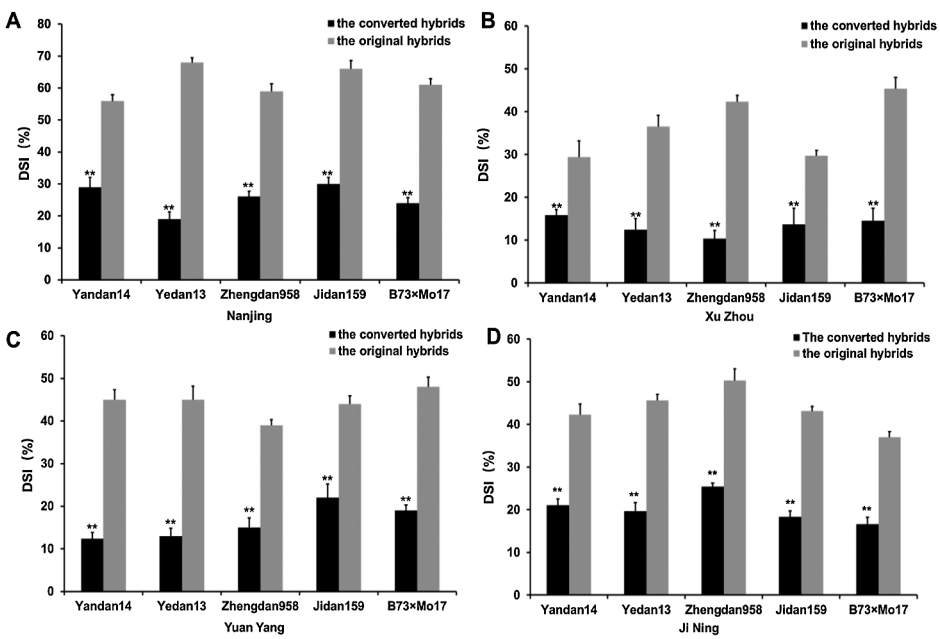

Fig. 4. Pairwise comparisons of DSIs (disease severity indices) between converted hybrids and the original hybrids. (A) The DSIs of pairs of converted hybrids and their original hybrids were estimated using artificial inoculation at Nanjing in 2017. (B, C, D) The DSIs (disease severity indices) of converted hybrids and their original hybrids were estimated under conditions of natural inoculation at Yuanyang in Henan Province, Jining in Shandong, and Xuzhou in Jiangsu in 2017, respectively. ** Differences between the converted and original lines are significant at $P = 0.01$.

Table 1. Comparison of agronomic performance of converted and original lines

Converted lines (R) and original lines	Plant height (m)	Ear height (m)	10-kernel length (mm)	10-kernel width (mm)	10-kernel thickness (mm)	Ear length (mm)	Ear diameter (mm)	Tasseling (d)	Pollen shed (d)	Silking (d)	100-kernel weight (g)
B73-R	2.28*	1.24	100.84	74.63	43.75	142.32	24.35	59.00*	62.50*	65.50*	24.25
B73	2.49	1.29	101.28	74.75	43.49	144.09	24.38	61.50	65.00	67.00	24.65
Huangzao4-R	2.41	1.18	97.89	90.54	41.65	108.07	26.54	60.50	64.00	66.50	24.95
Huangzao4	2.40	1.14	97.42	90.78	41.36	109.30	26.45	60.50	64.00	66.00	25.19
Zheng58-R	1.52*	0.50	108.18	86.57	52.93	151.91	21.49	60.50	66.00	68.00	35.14
Zheng58	1.74	0.51	108.59	87.48	52.57	151.71	21.30	60.00	66.00	68.00	35.23
Chang7-2-R	2.18	1.11	108.56	77.40	40.87	115.85	24.59	64.50	67.00	70.00	29.46
Chang7-2	2.16	1.13	108.55	77.65	40.79	115.77	24.95	64.50	67.00	70.00	29.44
Ye478-R	1.72	0.79	107.61	86.11	45.10	152.49	23.51	64.50	68.00	71.50	27.11
Ye478	1.79	0.73	107.44	86.35	45.52	153.56	22.54	64.50	68.00	71.00	27.10
Zhonghuang68-R	2.03	0.91	116.19	90.40	44.73	139.45	32.76	60.00	64.00	68.00	36.26
Zhonghuang68	2.08	0.93	116.33	90.37	44.48	139.08	32.75	60.00	64.50	68.50	36.32
Ji846-R	2.06	0.70	82.45	74.67	47.84	170.50	24.04	63.00	68.00	70.00	28.25
Ji846	2.09	0.71	82.68	74.45	47.23	171.49	23.64	63.00	68.00	70.50	28.41

* Differences between converted and original lines were considered significant at $P = 0.05$.

converted inbred lines and the isogenic inbreds without the MRDD resistance allele at the *qMrdd8* locus were evaluated and no significant differences were found except that B73 and Zheng58 were taller than B73-R and Zheng58-R, respectively. Similarly, we found that tasseling, pollen shed, and silking occurred 2–3 days earlier in B73-R for than in B73 (**Table 1**). We also investigated the agronomic traits of the original hybrids without the MRDD resistance allele at *qMrdd8* and the converted hybrids carrying the MRDD resistance allele at *qMrdd8* at Changping, Beijing in 2017. Under natural conditions without inoculation, Yandan14-R, Yedan13-R, Zhengdan958-R, Jidan159-R, and B73-R × Mo17 showed no significant differences in agronomic traits (**Supplemental Table 4**). Due to the symptoms and number of strains of MRDD in Changping, under artificial inoculation and natural inoculation, we compared the yield of converted inbred lines and hybrids carrying the MRDD resistance allele from the recurrent parents at *qMrdd8* to that of their recurrent parents and hybrids without the MRDD resistance allele at *qMrdd8* (data not shown here). Therefore, the improvement of MRDD resistance by introducing the X178 MRDD resistance allele into other backgrounds did not change the agronomic traits, especially yield, of inbred lines or hybrids under nonpathogenic conditions.

Discussion

The genetic effects of the disease resistance QTL qMrdd8 in different genetic backgrounds

The spatial and temporal distribution of MRDD in fields tends to be uneven. Thus, phenotypic screening under natural pathogenesis conditions in the field requires multiple methods to identify resistance and can be inefficient (Han *et al.* 2019). Using molecular markers that are closely linked to QTL or genes conferring resistance to maize diseases to improve the disease resistance of elite inbred lines by MAS has been an effective way to develop resistant varieties (Khan *et al.* 2015, Wijerathna 2015, Zhou *et al.* 2007). At present, most of the inbred lines resistant to MRDD in China have been derived from the American hybrid P78599; therefore, the resources for resistance to MRDD in China are relatively narrow (Liu *et al.* 2015). Discovering QTL for resistance to MRDD in elite maize germplasm can be useful for pyramiding disease resistance genes through MAS into different genetic backgrounds and thereby improving breeding efficiency (He *et al.* 2014, Kim *et al.* 2019). Disease resistance in maize is quantitatively inherited, often via QTL. Using MAS, Li *et al.* (2017) found that an elite *ZmCCT* haplotype without a transposable element in its promoter was more resistant to *Gibberella* stalk rot but had normal flowering time (Li *et al.* 2017). In rice, marker-assisted backcrosses introgressed genes for resistance to blast (*Pi2* and *Pi54*) and bacterial blight (BB) (*xa13* and *Xa21*) into Pusa Basmati1121 (PB1121) and Pusa Basmati 6 genetic backgrounds. The converted rice lines were superior to the parental line in disease resistance, early maturity, and high yield, and SSR or SNP marker estimates both indicated similar rates of RPG recovery (Ellur *et al.* 2016). In the present study, the introduction of an MRDD resistance allele at the *qMrdd8* locus improved the disease resistance of seven different maize inbred lines from different backgrounds and that of their hybrids grown in different locations. Both the converted inbred lines and their hybrids at the *qMrdd8* locus were stably resistant to MRDD. Fine mapping of the *qMrdd8* locus and the linked markers IDRQ4, IDP27K, IDRQ47, and the functional marker IDP25K within the candidate gene *ZmGDI* has allowed accurate tracking of the MRDD resistance allele at the *qMrdd8* locus and reduced

linkage drag (Liu *et al.* 2016). Therefore, the use of the MRDD resistance QTL *qMrdd8* or the functional marker IDP25K in MAS should be very effective for improving the resistance of maize germplasm to MRDD.

Resistance to MRDD in maize is quantitatively inherited. Tao *et al.* (2013) found no difference in DSI between genotypes that were heterozygous or homozygous for the NT409 marker at the *qMrdd1* locus, which shows that the MRDD resistance conferred by *qMrdd1* is recessive. Phenotyping results indicated the chilling tolerance of plants with the heterozygous genotype HAN1$^{Teqing/02428}$ was close to the intermediate value, suggesting that HAN1 had an additive effect and no significant dominant effect on chilling tolerance (Mao *et al.* 2019). Similarly, stalk rot resistant plants carrying homozygous *qRfg1* alleles were slightly more resistant to *Gibberella* stalk rot than were those carrying a single *qRfg1* allele, suggesting that *qRfg1* acted in a partially dominant manner (Yang *et al.* 2010a). In the present study, the DSIs of Zhengdan958-R, Zhengdan958-R1, and Zhengdan958-R2 were 40.02%, 31.56%, and 33.17% lower, respectively, than that of Zhengdan958 at Nanjing in 2017 (**Fig. 5**). The DSI of Zhengdan958-R2 was lower than that of the other two resistant hybrids. Thus, when disease resistance is controlled by a dominant allele, only one parent of the hybrid needs to be improved. However, when the resistance is conferred by a recessive or partially dominant allele, both parents of the hybrid need to be improved.

Genetic markers and genetic background selection to develop converted inbred lines

MAS is the most commonly used method for modern molecular breeding in maize. Compared with traditional breeding, MAS can overcome the uncertainty of phenotypic selection and can significantly improve selection efficiency. However, MAS requires accurate information about the location of known genes and can be very effective for select for a trait using functional markers or tightly linked markers (Nudin *et al.* 2017). At the same time, linkage drag and pleiotropy can reduce the efficiency of MAS. Linkage drag refers to possible interfering genetic effects caused by the physical proximity of closely linked genes that control different agronomic traits (Klindworth *et al.* 2013). Pleiotropy refers to the effect of a single gene on the phenotypes of multiple traits (Chen and Lübberstedt 2010). Linkage drag can limit the efficiency of breeding and its effects on target genes in different genetic backgrounds can vary (Klindworth *et al.* 2013). Linkage drag is also a primary factor in the size of donor fragments introduced during MAS, thus, the appropriate selection of recombinants can effectively control linkage drag. Therefore, detecting the effects of introduced alleles in early generations is typically necessary for the success of MAS. In the present study, stable resistance to MRDD was identified in several different maize populations. Detection of the MRDD resistance effect after its introduction into different inbred lines also required post-selfing detection of the allele by genotyping progeny at the IDP25K functional marker. Due to the recessive nature of MRDD resistance, we could not detect the disease resistance effect of *qMrdd8* early during MAS, so identification of disease resistance was carried out after obtaining lines homozygous for the MRDD resistance allele at *qMrdd8*. Subsequent characterization of phenotypes in the field indicated that there was no effect of linkage drag on selection for MRDD resistance in the present study.

Combining molecular MAS with conventional breeding during backcross breeding can quickly restore the desired genetic background of the recipient parent. If conditions permit, background selection for genomes in early generations can more effectively improve the rate of recovery of the recipient genetic background (Frisch and Melchinger 2001, 2005). Herzog and Frisch (2012) showed through simulation experiments that genetic background selection based on 2 or 3 markers per chromosome results in a 90% probability of obtaining a plant with 96% recovery of the recurrent genetic background in the BC_2 generation. Thus, field selection of plants with the agronomic phenotypes of the recurrent parents from among the offspring of each backcross will help quickly restore the genetic background of the recipient inbred lines. In our study, BC_3F_1 or BC_4F_1 plants from different receptor inbred lines were genotyped at 107 SSR markers to obtain a single plant with a high rate of recovery of the recurrent parent genetic background. Conversion of the genetic background of the Ji846 backcross converted population was completed in the BC_3 generation. The average rate of recovery of the recurrent parent genetic background in the 12 Ji846 BC_3F_1 plants was 1.72% higher than the theoretical value. However, the average genetic background recovery rate of the backcross progeny plants of the other six recipient inbred lines was lower than the theoretical value, ranging from 0.30% to 4.12%. Among these, the highest rate of recovery rate of the recurrent genetic background was found in the backcross plants of Ye478 and Zheng58, which were 0.12% to 1.18% lower than the theoretical values. These values were likely observed because fewer individual plants could be used for analysis of selection for genetic background. Compared with high-throughput sequencing technology, genetic background selection based on SSR markers is easier and less expensive (Nudin *et al.* 2017). However, high-density markers across the entire maize genome can be used to simultaneously analyze the target gene, donor fragment size, and genetic background recovery rate, all of which can shorten breeding cycles and improve the efficiency of molecular breeding.

Disease resistance and agronomic traits of converted inbred lines and their hybrids

When we evaluated MRDD resistance under both natural infection and artificial inoculation conditions, we found that the DSIs of the seven converted inbred lines and the five converted hybrids were significantly lower than those of the controls. Further, when both parents carried the

MRDD resistance allele at *qMrdd8*, the converted hybrids were more resistant than when only a single parent carried the MRDD resistance allele. We found that four markers closely linked to the *qMrdd8* locus can accurately select plants in the backcross population that carry the MRDD resistance allele at the *qMrdd8* locus. Under artificial inoculation, we found that the DSIs of converted lines were 30.15% to 64.2% lower than those of their recurrent parents (**Fig. 2**). When the seven converted lines were further investigated under natural infection conditions, their MRDD resistance was from 15.6% to 47.32% better than that of their recurrent parents at Yuanyang in Henan, Jining in Shandong, and Xuzhou in Jiangsu (**Fig. 2**). Our comparative analysis of agronomic traits of the converted inbred lines and their hybrids found that cob color, plant height, ear height, ear length, ear diameter, 10-kernel length, 10-kernel width, 10-kernel thickness, tasseling, pollen shed, silking, and 100- kernel weight did not significantly differ between the converted inbreds or hybrids and their controls. The only significant difference in performance was that plants of B73 and Zheng58 were both taller than those of B73-R and Zheng58-R. Similarly, tasseling, pollen shed, and silking were 2–3 d earlier in B73R than in B73 (**Table 1**, **Supplemental Table 4**). These differences could have been due to low rates of recovery of the genetic backgrounds of the recurrent parents or linkage drag that allowed remaining X178 donor genome fragments to have an adverse effect on the agronomic traits of the converted lines. Because MRDD resistance is a quantitatively inherited trait, pyramiding of several QTL for MRDD resistance into an elite genetic background will be a powerful tool for developing full and stable resistance to MRDD in the future.

Author Contribution Statement

First, Z.H. Wang and J.F. Weng proposed the original idea and supervised the research project. Second, experimental materials and phenotypic data including disease scoring under artificial inoculation or natural conditions were provided by the lab of J.F. Weng. Screening and evaluation in Yuanyang, Henan Province and in Xuzhou, Jiangsu Province were completed with the assistance of S.G. Tie, X.H. Han, Q.C. Meng, and Y.P. Chen. Third, genotypic data collection for foreground selection and background selection, including DNA extraction, PCR, and sequencing were carried out in the lab of Z.H. Wang. Previous screening work was completed by J.G. Hua, Z.N. Xu, who performed phenotypic evaluation and wrote the article with support from J.G Hua, C.L. Liu, X.H. Li, and J.F. Weng. All authors discussed the results and contributed to the final manuscript.

Acknowledgments

This study was financially supported by funding from the National Natural Science Foundation of China (31771804).

Literature Cited

Ali, F. and J.B. Yan (2012) Disease resistance in maize and the role of molecular breeding in defending against global threat. J. Integr. Plant Biol. 54: 134–151.

Arneodo, J.D., F.A. Guzman, L.R. Conci, I.G. Laguna and G.A. Truol (2005) Transmission features of *Mai de Rio Cuarto virus* in wheat by its planthopper vector *Delphacodes kuscheli*. Ann. Appl. Biol. 141: 195–200.

Bai, F.W., J. Yan, Z.C. Qu, H.W. Zhang, J. Xu, M.M. Ye and D. Shen (2002) Phylogenetic analysis reveals that a dwarfing disease on different cereal crop in China is due to rice black streaked dwarf virus (RBSDV). Virus Genes 25: 201–206.

Bonamico, N.C., M.A. Di Renzo, M.A. Ibañez, M.L. Borghi, D.G. Díaz, J.C. Salerno and M.G. Balzarini (2012) QTL analysis of resistance to *Mal de Río Cuarto* disease in maize using recombinant inbred lines. J. Agric. Sci. 150: 619–629.

Chen, Y. and T. Lübberstedt (2010) Molecular basis of trait correlations. Trends Plant Sci. 15: 454–461.

Di Renzo, M.A., N.C. Bonamico, D.G. Diaz, M.A. Ibanez, M.E. Faricelli, M.G. Balzarini and J.C. Salerno (2004) Microsatellite markers linked to QTL for resistance to *Mal de Rio Cuarto* disease in *Zea mays* L. J. Agric. Sci. 142: 289–295.

Dingerdissen, A.L., H.H. Geiger, M. Lee, A. Schechert and H.G. Welz (1996) Interval mapping of genes for quantitative resistance of maize to *Setosphaeria turcica*, cause of northern leaf blight, in a tropical environment. Mol. Breed. 2: 143–156.

Dovas, C.I., K. Eythymiou and N.I. Katis (2004) First report of maize rough dwarf virus (MRDV) on maize crops in Greece. Plant Pathol. 53: 238–243.

Ellur, R.K., A. Khanna, A. Yadav, S. Pathania, H. Rajashekara, V.K. Singh, S. Gopala Krishnan, P.K. Bhowmick, M. Nagarajan, K.K. Vinod *et al.* (2016) Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. Plant Sci. 242: 330–341.

Fang, S., J. Yu, J. Feng, C. Han, D. Li and Y. Liu (2001) Identification of rice black-streaked dwarf fijivirus in maize with rough dwarf disease in China. Arch. Virol. 146: 167–170.

Frisch, M. and A.E. Melchinger (2001) Marker-assisted backcrossing for introgression of a recessive gene. Crop Sci. 41: 1485–1494.

Frisch, M. and A.E. Melchinger (2005) Selection theory for marker-assisted backcrossing. Genetics 170: 909–917.

Han, X.H., T.M. Chen, R.Q. Yue, S.L. Guo, M.M. Xu, C.X. Lu, Y.P. Fan, N.N. Chen, L. Liu, X.L. Fu *et al.* (2019) A rice black-streaked dwarf virus replication curve model to evaluate maize rough dwarf disease resistance. Plant Dis. 103: 868–873.

He, J.F., X.Q. Zhao, A. Laroche, Z.X. Lu, H.K. Liu and Z.Q. Li (2014) Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front. Plant Sci. 5: 484.

Herzog, E. and M. Frisch (2012) Efficient marker-assisted backcross conversion of seed-parent lines to cytoplasmic male sterility. Plant Breed. 132: 33–41.

Khan, M.H., Z.A. Dar and S.A. Dar (2015) Breeding strategies for improving rice yield—a review. Agric. Sci. 6: 467–478.

Kim, N., W.H. Kang, J. Lee and S.I. Yeom (2019) Development of clustered resistance gene analogs-based markers of resistance to *Phytophthora capsici* in chili pepper. Biomed. Res. Int. 3: 1093186.

Klindworth, D.L., G.A. Hareland, E.M. Elias and S.S. Xu (2013)

Attempted compensation for linkage drag affecting agronomic characteristics of durum wheat 1As/1DL translocation lines. Crop Sci. 53: 422–429.

Lenardon, S.L., G.J. March, S.F. Nome and J.A. Ornaghi (1998) Recent outbreak of "Mal de Rio Cuarto" virus on corn in Argentina. Plant Dis. 82: 448–453.

Li, Y.P., L.X. Tong, L.L. Deng, Q.Y. Liu, Y.X. Xing, C. Wang, B.S. Liu, X.H. Yang and M.L. Xu (2017) Evaluation of *ZmCCT* haplotypes for genetic improvement of maize hybrids. Theor. Appl. Genet. 130: 2587–2600.

Liu, C.L., J.F. Weng, D.G. Zhang, X.C. Zhang, X.Y. Yang, L.Y. Shi, Q.C. Meng, J.H. Yuan, X.P. Guo, Z.F. Hao *et al.* (2014) Genome-wide association study of resistance to rough dwarf disease in maize. Eur. J. Plant Pathol. 139: 205–216.

Liu, C.L., Z.F. Hao, D.G. Zhang, C.X. Xie, M.S. Li, X.C. Zhang, H.J. Yong, S.H. Zhang, J.F. Weng and X.H. Li (2015) Genetic properties of 240 maize inbred lines and identity-by-descent segments revealed by high-density SNP markers. Mol. Breed. 35: 146–151.

Liu, C.L., J.G. Hua, C. Liu, D.G. Zhang, Z.F. Hao, H.J. Yong, C.X. Xie, M.S. Li, S.H. Zhang, J.F. Weng *et al.* (2016) Fine mapping of a quantitative trait locus conferring resistance to maize rough dwarf disease. Theor. Appl. Genet. 129: 2333–2342.

Lu, Y.G., D.P. Di, H.Q. Miao and L.Z. Tian (2001) Identification and analysis of resistance of introduced foreign and domestic maize inbreds to MRDV. J. Hebei Agric. Sci. 5: 22–24.

Luan, J., F. Wang, Y. Li, B. Zhang and J. Zhang (2012) Mapping quantitative trait loci conferring resistance to rice black-streaked virus in maize (*Zea mays* L.). Theor. Appl. Genet. 125: 781–791.

Mackay, T.F., E.A. Stone and J.F. Ayroles (2009) The genetics of quantitative traits: challenges and prospects. Nat. Rev. Genet. 10: 565–577.

Mao, D.H., Y.Y. Xin, Y.J. Tan, X.J. Hu, J.J. Bai, Z.Y. Liu, Y.L. Yu, L.Y. Li, C. Peng, T. Fan *et al.* (2019) Natural variation in the *HAN1* gene confers chilling tolerance in rice and allowed adaptation to a temperate climate. Proc. Natl. Acad. Sci. USA 116: 3494–3501.

Murray, M.G. and W.F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321–4326.

Nudin, N.F.H., A.M. Ali, N. Ngah, N.Z. Mazlan, N. Mat, M.N.A. Ghani, N. Alias, A.J. Zakaria and M.S. Jahan (2017) ISSR marker-assisted genetic diversity analysis of *Dioscorea hispida* and selection of the best variety for sustainable production. C. R. Biol. 340: 359–366.

Schweiger, W., B. Steiner, C. Ametz, G. Siegwart, G. Wiesenberger, F. Berthiller, M. Lemmens, H. Jia, G. Adam, G.J. Muehlbauer *et al.* (2013) Transcriptomic characterization of two major *Fusarium* resistance quantitative trait loci (QTLs), *Fhb1* and *Qfhs.ifa-5A*, identifies novel candidate genes. Mol. Plant Pathol. 14: 772–785.

Shi, L.Y., Z.F. Hao, J.F. Weng, C.X. Xie, C.L. Liu, D.G. Zhang, M.S. Li, L. Bai, X.H. Li and S.H. Zhang (2012) Identification of a major quantitative trait locus for resistance to maize rough dwarf virus in a Chinese maize inbred line X178 using a linkage map based on 514 gene-derived single nucleotide polymorphisms. Mol. Breed. 30: 615–625.

Sun, L.H., C.M. Wang, C.C. Su, Y.Q. Liu, H.Q. Zhai and J.M. Wan (2006) Mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). Acta Genet. Sin. 33: 717–723.

Tao, Y.F., Q.C. Liu, H.H. Wang, Y.J. Zhang, X.Y. Huang, B.B. Wang, J.S. Lai, J.R. Ye, B.S. Liu and M.L. Xu (2013) Identification and fine-mapping of a QTL, *qMrdd1*, that confers recessive resistance to maize rough dwarf disease. BMC Plant Biol. 13: 145.

Wang, F. (2007) Molecular mapping of three loci conferring resistance to maize (*Zea mays* L.) rough dwarf disease, Doctoral Dissertation: Shandong University.

Wang, Z.H., S.G. Fang, Z.Y. Zhang, C.G. Han, D.W. Li and J.L. Yu (2006) Development of an ID-ELISA for the detection of rice black-streaked dwarf virus in plants. J. Virol. Methods 134: 61–65.

Wijerathna, Y.M.A.M. (2015) Marker assisted selection: biotechnology tool for rice molecular breeding. Adv. Crop Sci. Technol. 3: 2329–8863.

Xue, L., D. Zhang, L. Xu, M.M. Jin, C.J. Peng and C.W. Xu (2012) Mining and analyzing genetic diversity for maize rough dwarf disease resistant germplasms and its application in maize breeding. Acta Agron. Sin. 37: 2123–2129.

Yang, Q., G.M. Yin, Y.L. Guo, D.F. Zhang, S.J. Chen and M.L. Xu (2010a) A major QTL for resistance to *Gibberella* stalk rot in maize. Theor. Appl. Genet. 121: 673–687.

Yang, Q., P. Balint-Kurti and M.L. Xu (2017) Quantitative disease resistance: dissection and adoption in maize. Mol. Plant 10: 402–413.

Yang, X.F., G.B. Wen and Y. Yang (2010b) Resistance identification and analysis of different maize germplasm to maize rough dwarf virus. J. Maize Sci. 18: 144–146.

Zhang, H., J. Chen, J. Lei and M.J. Adams (2001) Sequence analysis shows that a dwarfing disease on rice, wheat and maize in China is caused by *Rice black-streaked dwarf virus*. Eur. J. Plant Pathol. 107: 563–567.

Zhao, X.R., G.Q. Tan, Y.X. Xing, L. Wei, Q. Chao, W.L. Zuo, T. Lübberstedt and M.L. Xu (2012) Marker-assisted introgression of *qHSR1* to improve maize resistance to head smut. Mol. Breed. 30: 1077–1088.

Zhou, L., J.K. Wang, Q. Yi, Y.Z. Wang, Y.G. Zhu and Z.H. Zhang (2007) Quantitative trait loci for seedling vigor in rice under field conditions. Field Crops Res. 100: 294–301.

Zhou, Y., J.F. Weng, Y.P. Chen, C.L. Liu, X.H. Han, Z.F. Hao, M.S. Li, H.J. Yong, D.G. Zhang, S.H. Zhang *et al.* (2015) Phylogenetic and recombination analysis of rice black-streaked dwarf virus segment 9 in China. Arch. Virol. 160: 1119–1123.