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ORIGINAL PAPER

Resistance to wheat yellow mosaic virus in Madsen wheat is controlled by two major complementary QTLs

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Abstract

Key message **Wheat yellow mosaic virus resistance of Madsen is governed by two complementary QTLs, *Qym1* and *Qym2*, located on chromosome arms 2DL and 3BS.**

Abstract Wheat yellow mosaic, caused by *Wheat yellow mosaic virus* (WYMV), is one of the most serious wheat diseases in East Asia. In this study, recombinant inbred lines (RILs, F_9) from a cross between cultivars Madsen (resistant) and Hokushin (susceptible) grown in a WYMV-infected nursery field were tested for the presence of WYMV in leaves by enzyme-linked immunosorbent assay (ELISA) and genotyped by using genome-wide molecular markers. Two major QTLs were detected: *Qym1* located between *Xgwm539* and *Xgwm349* on chromosome 2DL and *Qym2* located between *Xbarc147* and *Xwmc623* on chromosome 3BS. The resistance alleles for both QTLs originated from Madsen. The third QTL *Qym3* located near *Xwmc457* on chromosome 4D, where the resistant allele for this QTL originated from Hokushin. Although the *Qym3* was rather minor, it was essential to complement *Qym1* and *Qym2* for complete avoidance of WYMV infection. Near-isogenic lines carrying the resistance QTLs were developed by repeated backcrosses using Madsen as the donor parent and Hokushin as the recurrent parent. The lines that were resistant to WYMV (as tested by ELISA) were homozygous for the Madsen alleles at both *Qym1* and *Qym2*. *Qym1* dominance was partial, whereas that of *Qym2* was nearly complete. *Qym1* was closely linked to *Xwmc41*; *Qym2* was closely linked to *Xwmc754*. These markers will be useful in marker-assisted selection in wheat breeding for WYMV resistance; this study will facilitate cloning the WYMV resistance genes.

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Introduction

Wheat yellow mosaic virus (WYMV) and *wheat spindle streak mosaic virus* (WSSMV) are closely related bymoviruses (Namba et al. 1998; Xiaoyun et al. 1998). Both viruses cause soil-borne diseases transmitted by a fungus *Polymyxa graminis*. WYMV has been reported in China and Japan (Han et al. 2000; Inoue 1969), whereas WSSMV is mainly distributed in Europe and America. WYMV was first reported in Japan in the 1920s (Sawada 1927) and appeared in Hokkaido, the northern island and the main wheat production area of Japan, by 1991 (Kusume et al. 1997). WYMV became widespread in Hokkaido with the expanded cultivation of the susceptible leading cultivar Hokushin (Yanagisawa et al. 2000): the number of Hokkaido municipalities with WYMV-infected fields increased from six in 1994 to 57 in 2010 (Horita et al. 2011).

WYMV-infected wheat shows yellow or yellow-striped leaves, anthocyanin accumulation in leaves, stunted spring growth, dwarfism (Takeuchi et al. 2010). In particular, in Hokushin, WYMV infection results in ~50 % yield loss compared with a resistant near-isogenic line (Nishimura et al. 2010).

Chemical treatments to control WYMV are expensive and crop rotation is not efficient because of the long-term survival of dormant *P. graminis* spores in the soil (Ohto et al. 2006). Therefore, it is important to develop resistant cultivars in the wheat breeding program. However, evaluation of WYMV resistance is influenced by variability between nursery fields and uncontrolled environmental conditions. Thus, the development of molecular markers to accurately select WYMV resistance genes is desirable, because it would enable screening of resistant lines without nursery fields.

WYMV resistance genes were recently mapped on the long arms of chromosome 2D of the Chinese cultivar Yangfu 9311 (Liu et al. 2005) and European cultivar Ibis (Nishio et al. 2010). They were also detected on chromosomes 3BS, 5AL, and 7BS in the Chinese cultivar Xifeng Wheat (Zhu et al. 2012). In Madsen, an American winter wheat cultivar, which is highly resistant to WYMV, no symptoms of WYMV infection were observed and no WYMV was detected in leaves by enzyme-linked immunosorbent assay (ELISA; Takeuchi et al. 2010). The WYMV resistance of Madsen was reported to be controlled by one dominant gene (Takeuchi et al. 2010). By using isogenic lines that carried the dominant Madsen resistance gene in the Hokushin genetic background, this gene was mapped on chromosome 2DL (Takeuchi et al. 2010). The objective of the present study was to detect additional WYMV resistance genes in Madsen. We used a QTL mapping strategy for comprehensive analysis of WYMV resistance genes in Madsen; this strategy was not used in previous studies.

Materials and methods

Plant materials

The WYMV-resistant cultivar Madsen (VPM1/Moisson 951//2*Hill 81) was introduced to Japan from the United States. Because Madsen expresses moderately high resistance to straw-breaker foot rot inherited from VPM1, which derives resistance from *Triticum ventricosum* (Allan et al. 1989). The WYMV-susceptible cultivar Hokushin was developed at Hokkaido Agricultural Experimental Station in 1994 (Yanagisawa et al. 2000). For the analysis of WYMV resistance genes, we established a population of 151 recombinant inbred lines (RILs, F_9) from a cross between Madsen and Hokushin.

Four BC_5F_4 lines, named Takikeimugi 1–4 (TK1–TK4), were derived by repeated backcrossing of Madsen as the single donor parent for resistance genes and Hokushin as the recurrent parent. Field selection (2000–2008) was based on visual inspection of WYMV symptoms, confirmed by ELISA (Takeuchi et al. 2010). These lines were advanced to the BC_5F_6 generation in 2011. In addition, two resistant BC_3F_9 lines, 05I11 and 05I21, were established by repeated backcrossing.

ELISA detection of WYMV

A bulk of leaf samples from five individuals for each RIL was assessed by plate-trapped antigen ELISA (Clark 1981) using polyclonal WYMV-specific antibodies (Ueda et al. 1998). Samples were collected in 2009 (April 25, May 7, and May 26), in 2010 (April 30, May 11, and May 26), and in 2011 (April 26, May 9, and 24). Different plants were sampled on each day (i.e., 15 plants per line per year). The results were scored as 0 = the absence of WYMV in any bulk samples collected during the same year; 1 = detection of WYMV at least once a year.

Visual evaluation of disease severity

Wheat yellow mosaic symptoms were rated visually on a 0–4 scale as described by Takeuchi et al. (2010), where 0 = no visible symptoms, 1 = some leaves show mild symptoms (streak mosaic, yellow), 2 = some leaves show streak mosaic and yellow, but plants are not stunted, 3 = all leaves show streak mosaic and yellow with stunting, 4 = all leaves show distinct mosaic and yellow with obvious stunting. The disease severity was assessed in 2009 (April 15, April 25, May 7, and May 26), in 2010 (April 17, April 30, May 11, and May 26), in 2011 (April 17, April 26, May 9, and May 24), in 2012 (April 26, May 7, and May 21), and in 2013 (April 22, May 8, and May 21). Scores of May 7 in 2009 and May 11 in 2010 were used for QTL analysis because disease symptoms were most clear on these days.

Sources of molecular markers

The 210 SSR markers recommended by Nitta and Nasuda (2012) for hexaploid wheat (http://wheatssr.lab.nig.ac.jp/markerdb/) were screened for polymorphism between Madsen and Hokushin. A total of 160 polymorphic markers distributed genome-wide—at least seven markers per chromosome except for 1D (six markers) and 7B (five markers)—were used for genotyping RILs.

Additional SSR markers on chromosomes 2DL and 3BS were tested for polymorphism between Madsen and Hokushin according to the "wheat génoplante SSR mapping data release: a new set of markers and comprehensive

genetic and physical mapping data" web page (http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/). We also tested the insertion site-based polymorphism markers (Paux et al. 2008) for chromosome arm 3BS.

DNA techniques and map construction

PCR was performed in a 10-μL reaction mixture containing DNA template (1 μL, ~50 ng), 2.5 U Taq Gold DNA polymerase (Applied Biosystems, USA), 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.02 μM of each forward primer, 0.18 μM 6-FAM/VIC/NED/PET-labeled M13 primer (5′-ACGACGTTGTAAAACGAC-3′, Applied Biosystems), and 0.2 μM reverse primer. All forward primers listed on the NBRP-wheat web site were modified to contain a 19-nucleotide 5′ M13 tail (5′-CACGACGTTGT AAAACGAC-3′; Schuelke 2000). PCR amplification was conducted as follows: an initial denaturation at 94 °C for 7 min; 35 cycles of 95 °C for 1 min, 48 °C (for the markers with $T_m < 51$ °C) or 56 °C (for the markers with $T_m > 55$ °C) for 1 min, and 72 °C for 1 min; and a final extension at 72 °C for 5 min. PCR products were separated and detected on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems) using GeneMapper software and GeneScan-600 LIZ as a size standard. We constructed linkage maps with MAPMAKER/Exp v3.0b (Lander et al. 1987). Kosambi's mapping function was used to convert recombination frequencies into map distances (Kosambi 1943).

QTL analysis

For QTL analysis of the categorical data (0 or 1 for WYMV detection by ELISA and 0–4 for visual assessment of disease severity), we used the interval mapping method for categorical traits developed by Hayashi and Awata (2006). This method has been successfully used for QTL analyses of several categorical traits in plants (Khan et al. 2008; Kamei et al. 2010; Pu et al. 2012). In this method, genomic regions that significantly influenced the probabilities of phenotypic scores of individuals or lines being classified into categories can be searched using a logistic regression model. We wrote a Fortran program implementing this method for QTL analysis of the WYMV scores obtained by ELISA and visual assessment in RILs.

Assuming a categorical trait with $m + 1$ categories (C_0, C_1,…, and C_m for n RILs), we consider the probability of the phenotypic score of the ith RIL being classified into C_j, which is denoted by p_{ij} ($i = 1, 2,\ldots, n$; $j = 0, 1,\ldots, m$). We adopted a polychotomous logistic function $z_{ij} = \log(p_{ij}/p_{i0})$ for $j = 1, 2,\ldots, m$ considering C_0 as a reference category, and applied the following linear model for z_{ij};

$$z_{ij} = \mu_j + u_i a_j, \quad (1)$$

where μ_j is an intercept of the model, u_i is an indicator variable for the genotype of the ith RIL for a putative QTL located in the tested position, taking values of 1 and 0 corresponding to QTL genotypes QQ and qq with Q and q denoting two alleles of the QTL derived from Madsen and Hokushin, respectively, and a_j is the effect of the QTL. The genotype of a QTL can be predicted from the genotypes of the flanking markers in the interval mapping framework (Lander and Botstein 1989).

The detection of QTLs is performed with the log of odds ratio (LOD), $\log_{10} L_1/L_0$, where L_1 denotes the likelihood maximized under model (1) and L_0 is the likelihood maximized in the absence of the QTL, i.e., under the condition of $a_j = 0$ for $j = 1, 2,\ldots, m$ in model (1). The threshold values for LOD are determined by permutation tests (Churchill and Doerge 1994). Here, we carried out 1000 repetitions of the permutation test. Further computational details are described in Hayashi and Awata (2006).

Each probability, p_{ij} ($i = 1, 2,\ldots, n$; $j = 0, 1, 2,\ldots, m$), is predicted using model (1) from the QTL effect and QTL genotype, and is expressed in terms of z_{ij} as.

$$p_{i0} = 1 \Big/ \left\{ 1 + \sum_{(j=1)}^{m} \exp\,(z_{ij}) \right\}$$

and

$$p_{ij} = \exp\,(z_{ij}) \Big/ \left\{ 1 + \sum_{(j=1)}^{m} \exp\,(z_{ij}) \right\} \quad \text{for } j = 1,\ 2,\ \ldots,\ m.$$

The influence of the detected QTL on the categorical trait is evaluated on the basis of the difference of probabilities of categories between the two QTL genotypes, QQ and qq. For example, letting C_0 denote the absence of WYMV in ELISA detection, when p_{i0} for QQ is greater than that for qq, Q is regarded as a resistance allele (q is a susceptible allele) for WYMV.

We determined that a significant QTL was detected when the LOD score exceeded the threshold value of the genome-wide 1 % significance level obtained by 1000 repetitions of the permutation test. The peak position of LOD score was regarded as the position of the detected QTL.

Results

RIL characterization

The RILs were homozygous based on the 160 markers recommended by NBRP. Plants of each RIL were homogeneous for agronomic traits (heading date, plant height, and awn character). These results indicated that the RIL plants (F_9) were homozygous and therefore suitable for our QTL analysis.

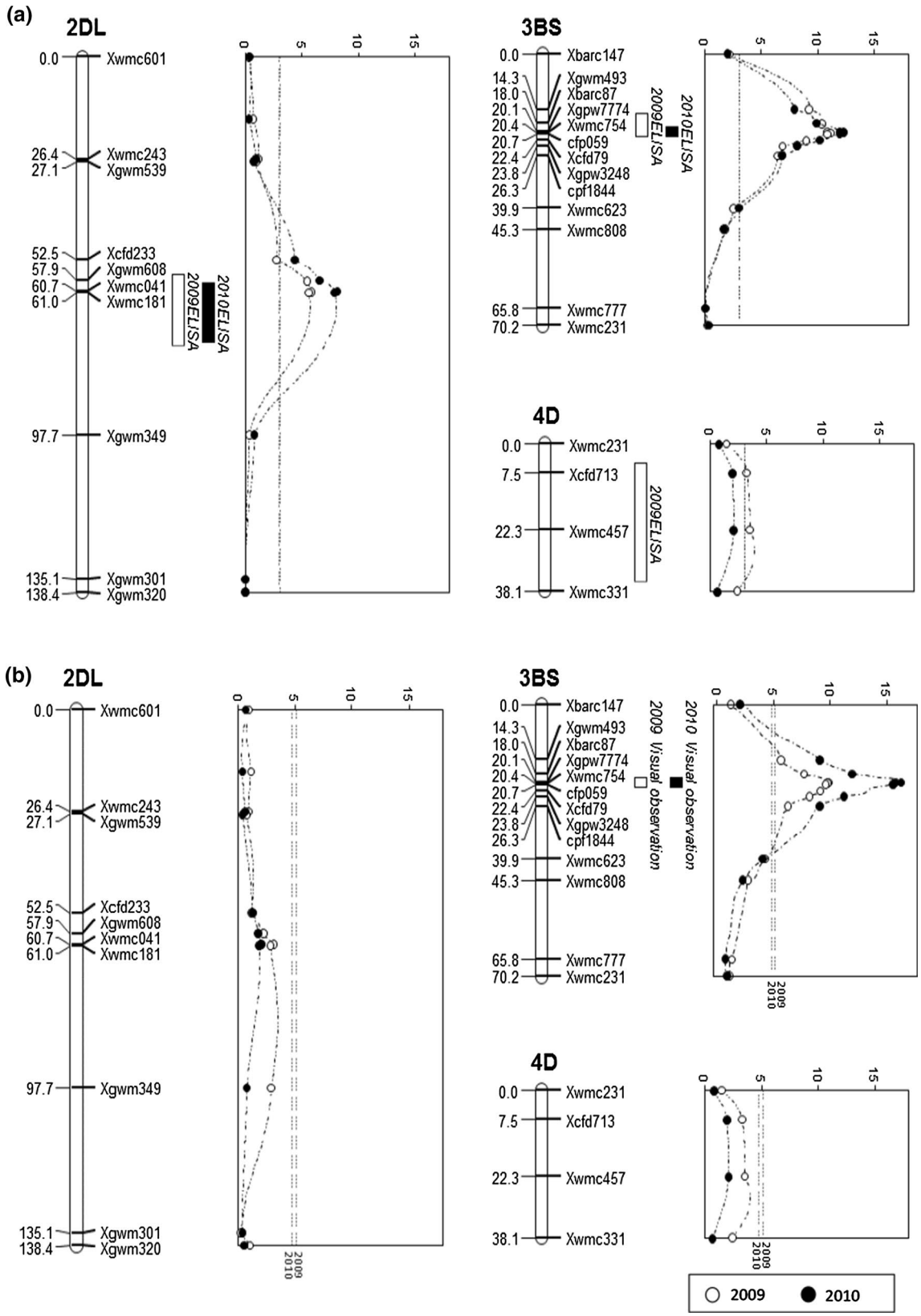

Fig. 1 LOD score plots for QTLs detected in a population of recombinant inbred lines derived from a cross between Madsen and Hokushin. **a** QTLs for the presence of WYMV as detected by ELISA, **b** QTLs for visually evaluated disease severity. *Bars* on the left of the graphs indicate 1-LOD intervals for QTL regions. *Straight lines* in the graphs indicate threshold values; **a** 2009: 3.04, 2010: 3.02, **b** 2009: 5.12, 2010: 4.78

Table 1 QTLs for wheat yellow mosaic virus resistance detected in 151 recombinant inbred lines (RILs) produced from a cross between Madsen and Hokushin

QTL	Year	Resistance allele	Evalation method	Chromosome	Position (cM)	Proximal marker	LOD score	Probabilities of category 0[a]	
Qym1	2009	Madsen	ELISA	2DL	60.7	*Xwmc41*	5.8 (3.04)[b]	QQ: 0.402	qq: 0.063
	2010	Madsen	ELISA	2DL	60.7	*Xwmc41*	8.2 (3.02)	QQ: 0.486	qq: 0.063
Qym2	2009	Madsen	ELISA	3BS	20.4	*Xwmc754*	11.1 (3.04)	QQ: 0.458	qq: 0.013
	2009	Madsen	Visual observation	3BS	20.1	*Xgpw7774*	9.9 (5.12)	QQ: 0.548	qq: 0.128
	2010	Madsen	ELISA	3BS	20.4	*Xwmc754*	12.2 (3.02)	QQ: 0.528	qq: 0.025
	2010	Madsen	Visual observation	3BS	20.1	*Xgpw7774*	16.4 (4.78)	QQ: 0.630	qq: 0.064
Qym3	2009	Hokushin	ELISA	4D	27.3	*Xwmc457*	4.0 (3.04)	QQ: 0.072	qq: 0.402

QTL genotypes, *Q* allele from Madsen, *q* allele from Hokushin

[a] Predicted probabilities of RILs being classified into category 0 (no WYMV detected by ELISA and no WYMV disease symptoms detected by visual observation)

[b] Numbers in parentheses indicate LOD score threshold values at the genome-wide 1 % significance level, which were obtained by a permutation test with 1000 replications

QTL detection on the basis of ELISA data

In both years (2009 and 2010), two major QTLs involved in WYMV resistance scored by ELISA analysis were detected. One QTL (*Qym1*) was detected just at the position of *Xwmc41* on chromosome 2DL (Fig. 1a); its LOD score was 5.8 in 2009 and 8.2 in 2010 (Table 1). For this QTL, the predicted probabilities of the absence of WYMV (category 0) were 0.402 (2009) and 0.486 (2010) for the Madsen-type homozygote, whereas they were 0.063 (both years) for the Hokushin-type homozygote.

The other major QTL (*Qym2*) was located just at the position of *Xwmc754* on chromosome 3BS (Fig. 1a); its LOD score was 11.1 in 2009 and 12.2 in 2010 (Table 1). For this QTL, the predicted probabilities of the absence of WYMV were 0.458 (2009) and 0.528 (2010) for the Madsen-type homozygote, whereas they were 0.013 (2009) and 0.025 (2010) for the Hokushin-type homozygote.

An additional QTL (*Qym3*) was detected near *Xwmc457* on chromosome 4D in 2009 (Fig. 1a). The predicted probabilities of the absence of WYMV were 0.072 for the Hokushin-type homozygote and 0.402 for the Madsen-type homozygote. Thus, the susceptible parent Hokushin carried the resistance allele (Table 1). The LOD score (4.0) was smaller than those of the QTLs on chromosomes 2DL and 3BS. Therefore, *Qym3* was considered to be relatively minor.

QTL detection on the basis of visual evaluation

QTLs for WYMV disease severity, evaluated by means of visual observation, were identified in the same regions (Fig. 1b). On chromosome 3BS, a QTL was identified in both 2009 and 2010 at the position of *Xgpw7774* (only 0.3 cM from *Xwmc754*, the position of *Qym2* detected using the ELISA data). Madsen carried the resistance allele of this QTL. The predicted probabilities of score 0 (no visible symptoms) were 0.548 (2009) and 0.630 (2010) for the Madsen-type homozygote, whereas they were 0.128 (2009) and 0.064 (2010) for the Hokushin-type homozygote (Table 1). In 2009, the following probabilities of higher scores were predicted: 0.164 (1), 0.123 (2), 0.096 (3), and 0.068 (4) for the Madsen-type homozygote, and 0.115 (1), 0.179 (2), 0.141 (3), and 0.436 (4) for the Hokushin-type homozygote. In 2010, these probabilities were 0.164 (1), 0.041 (2), 0.082 (3), and 0.082 (4) for the Madsen-type homozygote, and 0.115 (1), 0.154 (2), 0.128 (3), and 0.538 (4) for the Hokushin-type homozygote. No significant QTLs were identified on other chromosomes, including chromosome 2DL, where *Qym1* was detected on the basis of ELISA data.

Interactions between QTLs

We tested the interaction between the two major QTLs detected with the ELISA data (Table 2). Segregation analysis of the RILs showed the absence of WYMV in 34 lines in 2009 and 41 lines in 2010 and its presence in 117 lines in 2009 and 110 lines in 2010. Chi-square tests supported the hypothesis that two complementary genes were involved in resistance to WYMV infection.

To better understand the effects of QTL genotype combination on WYMV resistance, RILs homozygous for the flanking markers were analyzed in detail. For genotyping accuracy, RILs recombinant for the flanking markers was excluded from the analysis. Only one combination, with Madsen alleles for both *Qym1* and *Qym2*, showed resistance to WYMV (Table 3), whereas all other combinations

Table 2 Segregation of recombinant inbred lines produced from a cross between Madsen and Hokushin for resistance to WYMV infection

Test year	Uninfected	Infected	χ^2 (1:3)	*P*
2009	34	117	0.50	0.48
2010	41	110	0.37	0.54
Expected[a]	37.75	113.25		

Lines were scored as 'infected' when WYMV was detected by ELISA in at least one plant out of nine individuals tested

[a] Theoretical segregation of two genes (*Qym1* and *Qym2*)

were susceptible. The only exceptions were three lines in 2009 and one line in 2010. Although these lines carried the Madsen alleles for both *Qym1* and *Qym2*, they showed disease symptoms and were positive for WYMV by ELISA. We found that all three lines carried the homozygous Madsen allele of *Qym3* near *Xwmc457* on chromosome 4D. This result indicated that the Hokushin allele at the *Qym3* locus is effective, in addition to Madsen alleles at *Qym1* and *Qym2,* for complete avoidance of WYMV infection, and that *Qym1* and *Qym2,* and probably *Qym3*, are complementary for WYMV resistance.

Both major alleles are essential for resistance to WYMV

More than 90 % of the genomes of 05I11, 05I21, TK2, and TK3 including *Qym3*, is derived from Hokushin (Fig. 2). At the *Qym1* region, these lines were homozygous for Madsen. At the *Qym2* region, TK2 and TK3 were also homozygous for Madsen, whereas TK1 was homozygous for Hokushin and TK4 was segregating. No WYMV was detected by ELISA in TK2 and TK3, whereas WYMV was detected in TK1 and TK4 in 2011 (Table 4). These results indicate that Madsen alleles at both *Qym1* and *Qym2* are essential for resistance to WYMV and that selection at WYMV-infected nursery fields was not sufficiently stringent to select the genotype resistant to WYMV.

Dominance of two major QTLs

F_2 plants from the crosses between Hokushin and TK3 segregated 214 uninfected individuals and 39 infected individuals at the nursery field in 2012. The segregation ratio deviated from the 9:7 ratio expected for two complementary dominant genes and suggested quantitative expression of resistance. No WYMV was detected in plants that were homozygous for the Madsen allele at *Qym1* and were not homozygous for the Hokushin allele at *Qym2* (Table 5). On the other hand, WYMV was detected in 7.1 % of plants that were heterozygous at *Qym1* and homozygous for the Madsen allele at *Qym2* (Table 5). Dominance of *Qym1* was 0.39 (in the presence of the Madsen allele at *Qym2*) and 0.13 (in the presence of the Hokushin allele at *Qym2*), indicating partial dominance, whereas *Qym2* dominance was 1.00 (either Madsen or Hokushin alleles at *Qym1*).

We selected the F_3 plants homozygous for either Madsen or Hokushin allele at either *Qym1* or *Qym2* with random segregation for the other QTL from the cross between Hokushin and TK3.These plants were evaluated for the presence of WYMV in 2013 (Table 5). *Qym1* dominance was calculated as 0.31 (Madsen allele at *Qym2*) or 0.10 (Hokushin allele at *Qym2*). *Qym2* dominance was calculated as 0.79 (Madsen allele at *Qym1*) or 0.71 (Hokushin allele at *Qym1*).

Discussion

Our study showed that the WYMV resistance of Madsen was governed by two complementary QTLs, *Qym1* and *Qym2*, located on chromosome arms 2DL and 3BS. In previous studies, resistance genes *YmYF* of cultivar Yangfu 9311 (Liu et al. 2005) and *YmIB* of cultivar Ibis (Nishio et al. 2010) were mapped on chromosome 2DL. It is unknown whether *YmYF*, *YmIB*, and *Qym1* are alleles of the same locus. The resistance gene(s) on chromosome 2DL might be multiallelic, because the three resistant

Table 3 Effect of the combination of the QTLs *Qym1* and *Qym2* on WYMV resistance

Allele type		Year 2009						Year 2010					
		The number of ELISA-positive RILs		Average disease index				The number of ELISA-positive RILs		Average disease index			
Qym1[a]	*Qym2*[b]	Uninfected	Infected	15 Apr	23 Apr	7 May	26 May	Uninfected	Infected	17 Apr	30 Apr	11 May	26 May
Madsen	Madsen	7	3	0.20	0.00	0.01	0.01	9	1	0.10	0.10	0.20	0.10
Madsen	Hokushin	0	9	1.67	2.33	2.78	2.17	0	9	1.56	2.67	3.22	3.11
Hokushin	Madsen	0	4	2.75	2.88	3.00	2.50	0	4	1.00	1.75	2.75	2.50
Hokushin	Hokushin	0	11	2.73	2.45	2.64	2.59	0	11	2.64	2.27	2.27	2.36

[a] Genotype determined by markers between *Xgwm539* and *Xgwm349*

[b] Genotype determined by markers between *Xbarc147* and *Xgwm623*

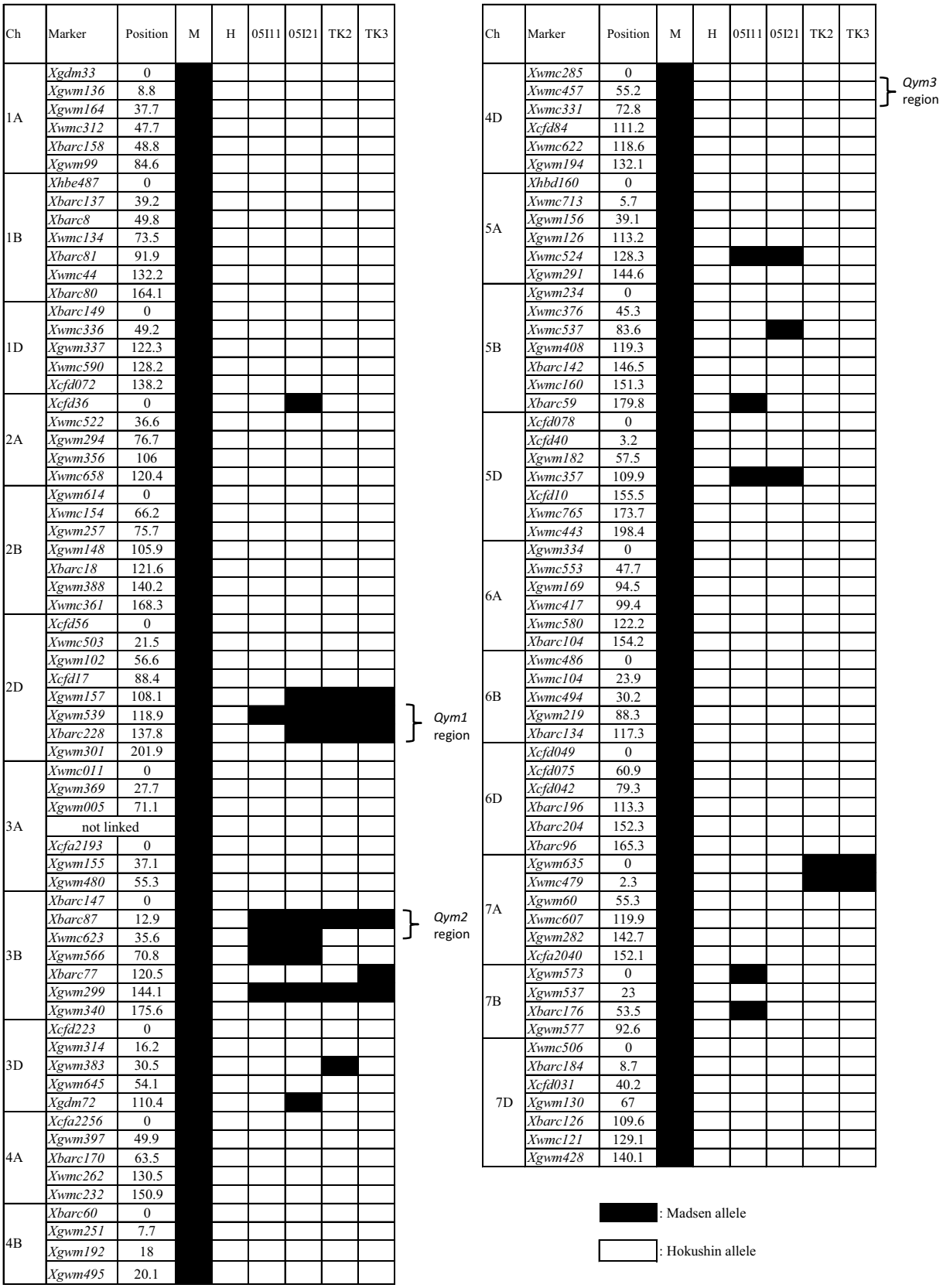

Fig. 2 Graphical genotypes of the *backcross lines*. *Black boxes*, marker alleles for Madsen; *white boxes*, marker alleles for Hokushin. *Ch* chromosome, *H* Hokushin, *M* Madsen

Table 4 Genotypes of near-isogenic lines and their responses to WYMV (2011)

Line #	Plant generation	*Qym1*		*Qym2*			Phenotype	
		Xcfd233	*Xwmc41*	*Xgpw7774*	*Xwmc754*	*Xcfd79*	ELISA	Visual observation
TK1	BC_5F_6	M	M	H	H	H	Detected	+
TK2	BC_5F_6	M	M	M	M	M	Not detected	−
TK3	BC_5F_6	M	M	M	M	M	Not detected	−
TK4	BC_5F_6	M	M	HM	HM	HM	Detected	+
05I11	BC_3F_9	M	M	M	M	M	Not detected	−
05I21	BC_3F_9	M	M	M	M	M	Not detected	−

A total of nine individual plants were tested by ELISA

TK1 to TK4 were derived from a single BC_5F_3 plant

+/− indicate WYMV present/absent

M Madsen allele, *H* Hokushin allele

Table 5 WYMV detection in plants with various combinations of alleles at the QTLs *Qym1* and *Qym2*

Allele type		2012 (F_2 plants[c])		2013 (F_3 plants[c])	
Qym1[a]	*Qym2*[b]	Total plants	WYMV detection (%)	Total plants	WYMV detection (%)
Madsen	Madsen	11	0.0	60	3.3
Madsen	Heterozygote	10	0.0	61	8.2
Madsen	Hokushin	12	16.7	44	59.1
Heterozygote	Madsen	14	7.1	37	24.3
Heterozygote	Heterozygote	21	4.8	–	–
Heterozygote	Hokushin	12	41.7	47	83.0
Hokushin	Madsen	6	33.3	52	69.2
Hokushin	Heterozygote	12	33.3	64	73.4
Hokushin	Hokushin	6	83.3	55	98.2

[a] Genotypes determined by markers between *Xgwm539* and *Xgwm349*

[b] Genotypes determined by markers between *Xbarc147* and *Xgwm623*

[c] These plants were selected from the cross between TK3 and Hokushin

cultivars have diverse origins. Madsen has *T. ventricosum* in its pedigree and possesses the eyespot resistance gene *Pch1* on 7D inherited from *T. ventricosum* via VPM-1. The WYMV resistance gene on 2DL may also be derived from *T. ventricosum.* Takeuchi et al. (2010) detected the WYMV resistance gene *YmMD* in Madsen and mapped it between *Xwmc41* and *Xgwm349* on chromosome 2DL. In the present study, *Qym1* was located near *Xwmc41* in the interval between *Xwmc539* and *Xgwm349*. On the basis of their similar location, we believe that *Qym1* and *YmMD* are likely identical. In our study, the percentage of WYMV-infected plants was higher among *Qym1*-heterozygous plants than among plants homozygous for the Madsen allele (Table 5). Takeuchi et al. (2010) reported the Madsen allele at *YmMD* to be dominant over the susceptible allele. However, we found in this study that the average degree of *Qym1* dominance was 0.23 (range 0.10–0.39).

Takeuchi et al. (2010) proposed a hypothesis that a single dominant gene in Madsen contributed to the WYMV resistance from the test of F_2 population. The hypothesis, however, was not supported well because F_3 progeny derived from each F_2 plant was not tested for the resistance in their study, so that the number and dominance of resistance gene was unclear. We have found in the present study that the dominance of *Qym1* was incomplete and homozygous status of Madsen allele in *Qym1* was necessary for the perfect WYMV resistance (Table 5). Dominance of *Qym2* was nearly complete and heterozygous status of Madsen allele in *Qym2* was sufficient for WYMV resistance. Takeuchi et al. (2010) reported that WYMV resistance of near-isogenic lines (BC_5F_5) to be governed by only *YmMD* (syn. *Qym1*) in chromosome 2D. They disregarded *Qym2* in chromosome 3B based on their molecular marker data that "the gene region was heterozygous" and misled their conclusion.

Zhu et al. (2012) mapped a QTL for WYMV resistance on chromosome 3BS using RILs derived from a cross of Chinese cultivars Xifeng Wheat (highly resistant) and Zhen 9523 (highly susceptible). The QTL, *QYm.njau-3B.1*, was located between *Xwmc754* and *Xwmc623*, close to the location of *Qym2* detected in the present study, suggesting that the two QTLs are alleles of the same locus. *QYm.njau-3B.1* explained only 6.9 % of the phenotypic variance (average of four trials; Zhu et al. 2012). However, Zhu et al. (2012) detected a major QTL on chromosome 5AL in the same mapping population; this QTL explained 45.9 % of the phenotypic variance. The effect of *QYm.njau-3B.1* might have been underestimated in their study, where only homozygous plants were analyzed and the dominance mode of the gene was not determined.

In our study, the rate of infection of F_2 plants carrying genotypes heterozygous and homozygous for the Madsen allele at the *Qym2* locus was almost the same, and their resistance was considerably higher than that of plants carrying the homozygous Hokushin genotype (Table 5). The degree of dominance was regardless of the *Qym1* genotype. The average degree of dominance of *Qym2* was estimated as 1.00 in 2012 and 0.79 or 0.71 in 2013, indicating that *Qym2* dominance is nearly complete. Most genes for barley yellow mosaic virus resistance are recessive, with only one, *Rym17* located on 3HS, showing complete dominance (Kai et al. 2012). The orthology between *Qym2* and *Rym17* would be an interesting hypothesis to be tested in further gene cloning studies.

In 2009, on chromosome 4D, we detected an additional resistance QTL, *Qym3*, with a minor effect (Fig. 1; Table 1). Interestingly, its resistance allele was carried by Hokushin, the susceptible parent. The loss of the Hokushin allele at *Qym3* resulted in susceptibility of plants with the *Qym1* (Madsen) *Qym2* (Madsen) genotype to WYMV (Table 3). Hokushin was a leading winter variety from 1998 to 2008 in Hokkaido and is adapted to harsh winters. Madsen, an American cultivar, had problems with winter adaptation in Hokkaido. *Qym3* may encode a factor that helps plants to withstand the environmental stresses and mediates basic defense against plant diseases, including WYMV. The positions of the known flanking markers (Somers et al. 2004) indicate that *Qym3* is located in the centromeric region of chromosome 4D. As many as six barley yellow mosaic virus resistance genes (*rym1*, *rym8*, *rym9*, *rym11*, *rym12*, and *rym13*), are located in the centromeric region of chromosome 4H (Kai et al. 2012; Yang et al. 2014). However, in barley these loci confer complete resistance or immunity, so it is unlikely that *Qym3* is orthologous to these genes.

Our results with backcrossed lines (Table 5) indicate the necessity of quantitative scoring of resistance phenotypes and the necessity of using segregating F_3 lines for accurate genotyping of each F_2 plant, which is essential for map-based cloning of each locus. TK2 and TK3 carried Hokushin alleles for more than 93 % of the molecular markers tested (Fig. 2). The two lines showed almost the same agronomic and grain quality performance as Hokushin (Nishimura et al. 2010; Takeuchi et al. 2010), indicating that neither *Qym1* nor *Qym2* was closely linked to undesirable genes introgressed from Madsen. The two resistance genes from Madsen and *Qym3* from Hokushin can be transferred to breeding materials by marker-assisted selection. The isogenic lines produced in this study would be also useful for cloning the WYMV resistance genes.

Author contribution statement TS, MM, and SN carried out the marker development, TS and TH carried out the QTL mapping, and YY developed plant lines and populations. TS and TK designed the experiments and wrote the manuscript.

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