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ORIGINAL PAPER

Genetic analysis of resistance to six virus diseases in a multiple virus-resistant maize inbred line

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Abstract
Key message **Novel and previously known resistance loci for six phylogenetically diverse viruses were tightly clustered on chromosomes 2, 3, 6 and 10 in the multiply virus-resistant maize inbred line, Oh1VI.**

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Abstract Virus diseases in maize can cause severe yield reductions that threaten crop production and food supplies in some regions of the world. Genetic resistance to different viruses has been characterized in maize populations in diverse environments using different screening techniques, and resistance loci have been mapped to all maize chromosomes. The maize inbred line, Oh1VI, is resistant to at least ten viruses, including viruses in five different families. To determine the genes and inheritance mechanisms responsible for the multiple virus resistance in this line, F_1 hybrids, F_2 progeny and a recombinant inbred line (RIL) population derived from a cross of Oh1VI and the virus-susceptible inbred line Oh28 were evaluated. Progeny were screened for their responses to *Maize dwarf mosaic virus*, *Sugarcane mosaic virus*, *Wheat streak mosaic virus*, *Maize chlorotic dwarf virus*, *Maize fine streak virus,* and *Maize mosaic virus*. Depending on the virus, dominant, recessive, or additive gene effects were responsible for the resistance observed in F_1 plants. One to three gene models explained the observed segregation of resistance in the F_2 generation for all six viruses. Composite interval mapping in the RIL population identified 17 resistance QTLs associated with the six viruses. Of these, 15 were clustered in specific regions of chr. 2, 3, 6, and 10. It is unknown whether these QTL clusters contain single or multiple virus resistance genes, but the coupling phase linkage of genes conferring

resistance to multiple virus diseases in this population could facilitate breeding efforts to develop multi-virus resistant crops.

Abbreviations

RIL	Recombinant inbred line
F_1	Filial 1
F_2	Filial 2
CIM	Composite interval mapping
QTL	Quantitative trait loci
REML	Restricted maximum likelihood
AUDPC	Area under disease progress curve
LOD	Logarithm of the odds

Introduction

Maize is a natural host for more than 50 viruses and an experimental host for about 30 more (Lapierre and Signoret 2004), but only some cause diseases that seriously affect yield (Ali and Yan 2012; Redinbaugh and Pratt 2009). Among the most damaging are members of the *Potyviridae* and *Maize chlorotic mottle virus* (MCMV), which form the devastating complex known as maize lethal necrosis (Uyemoto et al. 1980; Wangai et al. 2012). Plants have evolved passive and active defense mechanisms that suppress virus multiplication and spread. Such mechanisms require interaction of plant and viral factors to confer plant resistance or susceptibility (Gomez et al. 2009; Kang et al. 2005). Identifying the loci conferring resistance to virus diseases offers an approach to develop genetically resistant lines that reduce yield losses caused by existing and emerging viral diseases.

Viruses in at least eight different families cause significant agronomic losses in maize (Ali and Yan 2012; Redinbaugh and Pratt 2009). Distributed worldwide, potyviruses are the most common and most studied viruses of maize (Ali and Yan 2012; Lapierre and Signoret 2004). Potyviruses are single stranded, positive sense, monopartite RNA viruses with a single open reading frame encoding a polyprotein that is post-translationally processed into at least 10 mature proteins. Maize-infecting members of the family *Potyviridae* include *Maize dwarf mosaic virus* (MDMV), *Sugarcane mosaic virus* (SCMV) and *Wheat streak mosaic virus* (WSMV) (Ali and Yan 2012; Lapierre and Signoret 2004). In nature, MDMV and SCMV are nonpersistently transmitted by aphids (Nault and Knoke 1981) and WSMV is semi-persistently transmitted by mites (Slykhuis 1955).

Rhabdoviruses are single stranded, negative sense RNA viruses with a monopartite genome and five structural proteins. *Maize mosaic virus* (MMV) causes an important disease of maize in regions of Africa, South America, Hawaii and Australia (Ming et al. 1997; Redinbaugh and Pratt 2009). *Maize fine streak virus* (MFSV) is a phylogenetically distinct rhabdovirus that was isolated from maize collected near Bainbridge, GA (Redinbaugh et al. 2002). MMV and MFSV are persistently and propagatively transmitted by the maize planthopper *Peregrinus maidis* (Ashmead) and the black-faced leafhopper *Graminella nigrifrons* (Forbes), respectively (Nault and Knoke 1981; Redinbaugh et al. 2002).

The waikavirus *Maize chlorotic dwarf virus* (MCDV) is present in the southern and southeastern regions of the United States where it has caused significant problems (Lapierre and Signoret 2004). MCDV virions contain a monopartite single-stranded positive sense RNA genome that encodes a large post-translationally processed polyprotein (Hull 2002). MCDV is semi-persistently transmitted by *G. nigrifrons* (Nault and Knoke 1981).

Loci conferring resistance to various virus diseases have been reported on all maize chromosomes (Bonamico et al. 2012; Redinbaugh and Pratt 2009; Wisser et al. 2006). These studies involved different viruses, maize populations, environments and screening techniques. Resistance loci to the potyviruses MDMV, SCMV, *Sorghum mosaic virus* (SrMV) and *Johnsongrass mosaic virus* (JGMV) and to WSMV cluster in specific regions of chromosomes (chr.) 3, 6 and 10 (Dussle et al. 2000; Jones et al. 2007; McMullen and Louie 1991; McMullen et al. 1994; De Souza et al. 2008; Redinbaugh and Pratt 2009; Stewart et al. 2013; Wu et al. 2007; Xia et al. 1999; Zhang et al. 2003).

Our goal was to determine the inheritance and location of genes conferring resistance to a diverse set of viruses in the multiply virus resistant maize inbred line Oh1VI (Louie et al. 2002), and to further test whether resistance loci to these taxonomically diverse viruses cluster in the aforementioned chromosomal regions. To accomplish this, F_1 hybrids, F_2 progeny and a recombinant inbred line (RIL) population derived from a cross between Oh1VI and the virus-susceptible line Oh28 were tested for their responses to inoculation with six viruses from three different families (Table 1). RIL plants were genotyped, a genetic map was built, and the positions of resistance loci were determined.

Materials and methods

Plant material

The inbred line Oh1VI (PI 614734) is a flint corn type derived from a Virgin Islands population developed by the USDA-ARS and OARDC (Louie et al. 2002). Oh28 {(CI.1 12-1 × Oh920) × (I11.A × I11.B)} is a yellow dent corn released in 1943 (R. Pratt, personal communication) that is susceptible to all six viruses used in this study (Jones et al. 2007; Louie 1995; McMullen and Louie 1989; McMullen

Table 1 Summary of experimental parameters

Acron[a]	Virus	Family[b]	Genus	Inoculation[c]	Location[d]	Exp. design[e]	Plants/rep[f]
MDMV	*Maize dwarf mosaic virus*	*Potyviridae*	*Potyvirus*	Airbrush	Field	2 years/2 rep	17
SCMV	*Sugarcane mosaic virus*	*Potyviridae*	*Potyvirus*	Airbrush	Field	2 years/2 rep	17
WSMV	*Wheat streak mosaic virus*	*Potyviridae*	*Tritimovirus*	Leaf rub	GH	1 year/1 rep	12
MCDV	*Maize chlorotic dwarf virus*	*Secoviridae*	*Waikavirus*	Vector (LH)	GH	4 rep	1
MMV	*Maize mosaic virus*	*Rhabdoviridae*	*Nucleorhabdovirus*	Vector (PH)	GH	3 rep	1
MFSV	*Maize fine streak virus*	*Rhabdoviridae*	*Nucleorhabdovirus*	Vector (LH)	GH	3 rep	1

[a] Acron., Virus acronym

[b] The family and genus for the virus

[c] The method used for virus inoculation. The leafhopper (LH) *Graminella nigrifrons* or the planthopper (PH) *Perigrinus maidis* was used for vector transmission

[d] Evaluations for virus resistance were carried out in either the field or greenhouse

[e] The experimental design used for resistance evaluation. *Rep* the number of replications

[f] The number of plants of each recombinant inbred line used per replication

et al. 1994; Redinbaugh et al. 2002). F_1 hybrids, F_2 progeny and RIL families were generated from a cross between Oh1VI and Oh28. F_1 seed was produced in the summers of 1996 and 2003. Through 2006, 511 F_2 ears were generated. Seeds of F_2 plants were planted ear to row and self-pollinated. Successive generations were similarly planted and pollinated every year. By 2010, seed for 260 RILs, that had been self-pollinated between seven and nine times without selection, was available. All lines were maintained at the OARDC in Wooster, OH.

Viruses and vectors

The MDMV, SCMV, WSMV, and MCDV-severe isolates were collected in Ohio (Hunt et al. 1988; Louie 1986). The MFSV and MMV isolates were collected in Georgia and Hawaii, respectively (Ming et al. 1997; Redinbaugh et al. 2002). Virus identity was verified by enzyme-linked immunosorbent assay (ELISA) and bioassay (MDMV, SCMV and WSMV) as previously described (Jones et al. 2007). The MCDV, MMV and MFSV isolates were maintained by serial transmission using vascular puncture inoculation (Louie 1995) or insect transmission (Louie and Anderson 1993; Todd et al. 2010). MDMV, SCMV, and WSMV were maintained by serial rub-inoculation on susceptible maize (Oh28).

G. nigrifrons, the vector of MFSV and MCDV, was collected from fields near Wooster, OH. *P. maidis*, the vector of MMV, was a gift from Dr. William Belote (Dupont, Stine-Haskell Research Center, Newark, DE). Colonies of *G. nigrifrons* and *P. maidis* were maintained on oat 'Armor' and sweet corn 'Early Sunglow' (Schlessman Seed Co. Milan, OH) seedlings, respectively, in growth chambers at 25 °C with a 15 h light period (800 μmol m^{-2} s^{-1}) and 9 h dark period.

Viruliferous *G. nigrifrons* and *P. maidis* were obtained by allowing virus-naive nymphs to feed on MFSV- or MMV-infected plants for 26 days before being used for inoculation (Todd et al. 2010). *G. nigrifrons* viruliferous for MCDV was obtained by feeding virus-naive adults on infected MCDV plants for 2 days before being used for inoculation (Louie and Anderson 1993).

Inheritance of resistance

F_1 and F_2 seedlings derived from crosses between Oh1VI and Oh28 were independently inoculated with the six viruses to determine the inherence of the resistance. Experiments using MFSV, MMV, and MCDV were conducted in growth chambers and greenhouses, and those for MDMV and SCMV were conducted at the OARDC Snyder Farm (Wooster, OH) during the summers of 2006 and 2010 (see Table 1 for a summary of experimental conditions). Screening with WSMV was conducted in the field during the summer of 2010. The environments selected for these experiments (growth chambers, greenhouses, or field) were determined, in part, by the conditions of USDA, Animal and Plant Health Inspection Service permits for working with the viruses.

For the evaluation of MFSV, MCDV, and MMV, 100 F_2 and 60 F_1 6-day-old seedlings were randomized and divided between two Dacron covered cages each containing 500 viruliferous vector insects as previously described (Zambrano et al. 2013). Cages also contained ten seedlings each of the resistant and the susceptible parents as controls. Cages were then moved to a growth chamber (12 h light/dark periods at 600 μmol m^{-2} s^{-1} and 25 °C) for a 7-day inoculation access period (IAP). For MCDV, the multiple inoculation method described by Louie and Anderson (1993) was used. After the IAP, plants were fumigated and

transferred to a greenhouse with 25 °C/18 °C day/night temperatures for symptom development. Natural light was supplemented with 400 W high-pressure sodium lamps (P. L. Light System, Beamsville, ON, Canada) between October and April to obtain a 12 h light period. These experiments were conducted at different times during 2011 and 2012. Disease incidence and severity were recorded at 21 days post inoculation, with the end of the IAP defined as 0 dpi. Incidence was determined as the ratio of symptomatic plants/total number of plants, and severity was evaluated on the uppermost expanded leaf of individual plants using a 3-point scale, where 1 = no symptoms, 3 = intermediate symptoms, and 5 = severe disease symptoms (Fig. 1).

For field evaluations, plots consisted of 15 to 18 rows of F_2 plants bordered by two rows each of Oh1VI and F_1 plants, and five rows of Oh28. Rows (3.5 m) planted with 17 seeds were spaced at 0.76 m. Plots were inoculated four times with MDMV, SCMV or WSMV at 2-day intervals beginning at the V2 stage (two leaves present with a visible collar) using a Model H3 airbrush (Paasche Airbrush Co., Chicago, IL) as described (Louie et al. 1983). Disease incidence was scored between 14 and 21 dpi as the number of symptomatic plants/total number of plants (Jones et al. 2011).

Phenotypic data for F_1 and F_2 plants were analyzed relative to parents to assess the degree of dominance associated with resistance (Wu et al. 2007). A Chi-square test was conducted to assess goodness of fit for single gene (3:1), two gene including variants (15:1, 9:7, 11:5, 13:3), and three gene (63:1) models for disease incidence.

Phenotypic analyses

The responses of RILs to inoculation with MFSV, MMV, MCDV and WSMV were evaluated in a greenhouse at the OARDC. For MFSV, MMV, and MCDV, a single seed of each RIL was planted into a "cone-tainer" (16.4 × 2.5 cm; Stuewe and Sons Inc., Tangent, OR) containing autoclaved soil, and randomly distributed in three racks (30.5 × 30.5 cm; Stuewe and Sons Inc.) at 6 days after planting (dap). Ten seedlings of the susceptible parent (Oh28) were placed in each cage as controls, and inoculations were conducted using viruliferous insects as described above. For MMV and MFSV, the experiment consisted of three independent replications, and for MCDV four replications were used. Disease incidence and severity were evaluated as outlined above at 7, 14, and 21 dpi. The area under disease progress curve (AUDPC) was calculated from the severity data at the three rating dates.

The RIL responses to WSMV were evaluated in a greenhouse as previously described (Jones et al. 2007). Briefly, rows of twelve seeds of each RIL were planted into plastic trays containing autoclaved soil, and then inoculated using leaf-rub four times at 2-day intervals, beginning at 14 dap. Plants were scored for the presence of symptoms at 21 dpi. Disease incidence was estimated as percentage of the number of plants with disease symptoms.

The responses of RILs to MDMV and to SCMV were evaluated as previously described (Jones et al. 2011). Plots were set up as outlined above, with two replicate blocks containing one row of each RIL in a complete randomized block design. Virus inoculation was carried out using an airbrush as described above. Disease incidence was evaluated as the proportion of symptomatic plants in each row at 21 dpi.

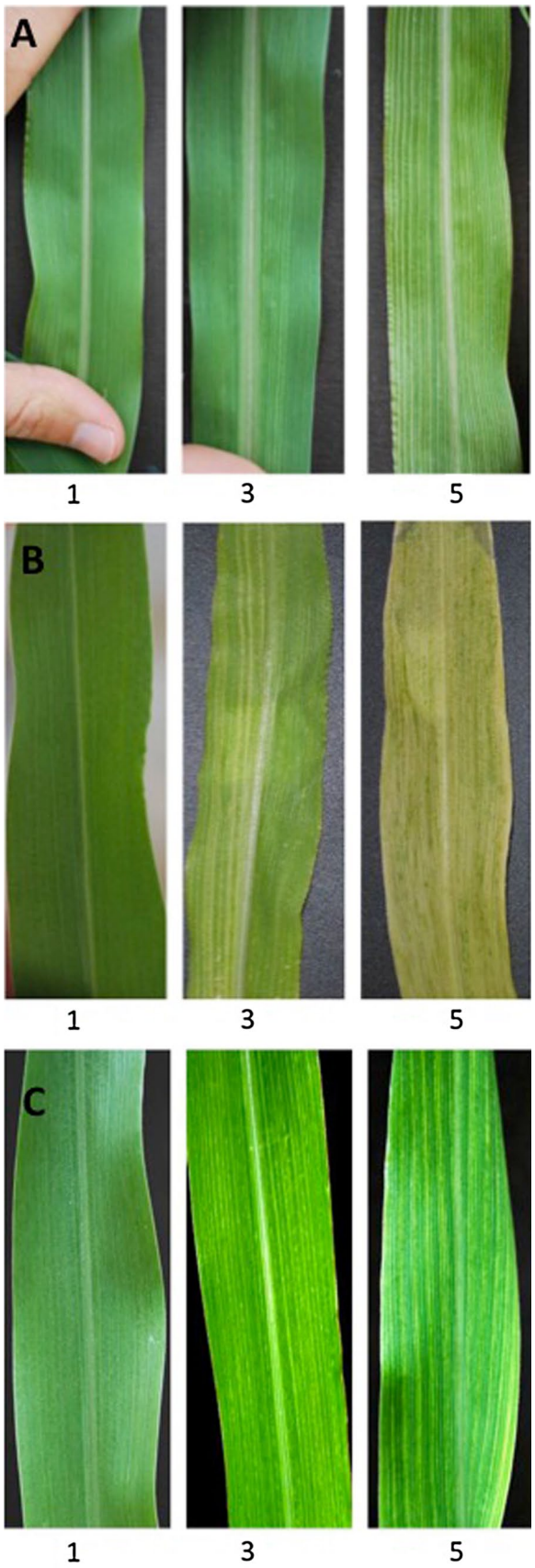
Best linear unbiased predictors or BLUPs (Balzarini and Milligan 2003) for incidence and AUDPC were estimated for each RIL using the PROC MIXED procedure in SAS (version 9.2, SAS Institute Inc., Cary, NC). The model applied was: $y_{ij} = \mu + \text{gen}_i + \text{rep}_j + e_{ij}$; where y_{ij} was the phenotypic response value to virus inoculation for the ith genotype in the jth replication, gen_i represented the individual effect of the ith line, rep_j was the effect of the jth replication, and e_{ij} the residual error term in the model. For MDMV and SCMV, replication was replaced by year in the model. All factors were considered random. Variance components for disease incidence and AUDPC were estimated by restricted maximum likelihood (REML).

Genotypic analysis

A set of 21 simple sequence repeats (SSR) distributed through the maize genome and known to be polymorphic between Oh1VI and Oh28, and 768 single nucleotide polymorphism (SNP) markers (Jones et al. 2009) were used to genotype the 260 RIL. Genotyping with SSR markers was conducted as previously described (Jones et al. 2004), and genotyping with SNP markers was conducted using a marker multiplex assay for the Illumina® BedArray™ platform (Illumina, Inc., San Diego, CA, USA) at the DuPont Pioneer Marker Lab, Johnston, IA.

Linkage and mapping analysis

Genetic distances and linkage groups were obtained using JoinMap, ver. 3 (van Ooijen and Voorrips 2001) with a minimum LOD threshold of 4. Markers with lower LOD values were discarded. The segregation of alleles at each locus was tested for the expected 1:1 ratio using a Chi-square test ($p > 0.05$). The Kosambi mapping function was used to convert recombination values to map distances (Kosambi 1944). Genetic map quality was assessed by comparing the positions of the markers in our map with their positions in the B73 v2 reference genome. The physical position of markers was estimated using BLAST analysis of sequences



◀ **Fig. 1** Symptom severity scale for: **a** *Maize mosaic virus*; **b** *Maize fine streak virus*; and **c** *Maize chlorotic dwarf virus*; where *1* no disease symptoms, *3* mild or incomplete symptoms, and *5* severe symptoms

flanking SNPs (Jones et al. 2009) with the B73 Reference Genome v2 (Schnable et al. 2009). The positions of SSR markers were obtained from the Maize Genetics and Genomics Database (http://www.maizegdb.org). Markers whose positions could not be estimated by BLAST were placed based on minimizing recombination frequency relative to the nearest BLAST-located marker and compared with its location on the intermated B73 × Mo17 (IBM2) map (Jones et al. 2009).

To identify the region(s) of the genome responsible for the virus resistance as QTLs in the RIL population, composite interval mapping (CIM) was conducted (Jansen and Stam 1994) using MapQTL, version 4 (van Ooijen et al. 2002). The significance threshold of the LOD score ($p < 0.01$) was determined by permutation over each linkage group (Churchill and Doerge 1994). CIM mapping results for incidence of MFSV, MMV and MCDV were confirmed by single marker regression and a Kruskal–Wallis test (Clewer and Scarisbrick 2001). Circos software (Krzywinski et al. 2009) was used to display the position of resistance QTLs in the Oh1VI × Oh28 RIL genetic map.

Interactions between each pair of QTLs were determined by two-way ANOVA using the PROC GLM procedure of SAS and Cockerham orthogonal contrast (Kao and Zeng 2002) as previously described (Coaker 2003). To compare the effects on disease incidence of individual markers with their interaction, variance components were estimated by REML.

Results

Inheritance of resistance

The responses of F_1 and F_2 progeny of the cross between Oh1VI and Oh28 to MFSV, MMV, MCDV, MDMV, SCMV, and WSMV indicated that Oh1VI was resistant to all six viruses, with no disease incidence and a severity score of 1 (no disease symptoms). Oh28 was fully susceptible to all six viruses with ≥95 % infection and disease severity scores of 5 (strong disease symptoms), except for WSMV for which disease incidence was 86 % (Table 2). Previous research using serological assays indicated an absence of MDMV, SCMV and WSMV in inoculated plants of Oh1VI (Redinbaugh, unpublished results). Similarly, no virus was detected in Oh1VI inoculated with *Maize rayado fino virus*

Table 2 Inheritance of maize resistance to six viruses in F_1 and F_2 generations derived from a cross of the inbred lines Oh1VI (resistant) and Oh28 (susceptible)

Virus	Trait[a]	Oh1VI	Oh28	F_1	F_2	F_2 ratio[b]	Chi-square *p* value[c]
MFSV	Incidence	0/20	19/19	1/55	20/88	3:1, 13:3	0.623, 0.339
	Severity	1.0 ± 0	5.0 ± 0	3.0 ± 0	4.3 ± 1		
MMV	Incidence	0/15	19/20	7/60	39/95	9:7	0.596
	Severity	1.0 ± 0	5.0 ± 0	3.0 ± 0	4.4 ± 1		
MCDV	Incidence	0/14	16/16	42/48	88/100	13:3	0.084
	Severity	1.0 ± 0	5.0 ± 0	3.2 ± 1	3.5 ± 1		
MDMV	Incidence	0/14	85/85	0/10	21/219		
		0/17	41/42	0/34	18/229	15:1	0.314
SCMV	Incidence	0/23	63/66	0/16	65/235	3:1, 11:5	0.346, 0.235
		0/7	18/19	1/20	50/294	13:3	0.444
WSMV	Incidence	0/34	68/79	0/26	2/202	63:1	0.512

MFSV, *Maize fine streak virus*; MMV, *Maize mosaic virus*; MCDV, *Maize chlorotic mosaic virus*; MDMV, *Maize dwarf mosaic virus*; SCMV, *Sugarcane mosaic virus*; WSMV, *Wheat streak mosaic virus*

[a] Incidence is given as number of symptomatic plants/total number plants for the two parents (Oh1VI and Oh28), and for F_1 and F_2 progeny. Severity is given as mean ± one standard deviation of severity ratings for symptomatic plants only (1–5 scale), where 1 = no symptoms and 5 = severe symptoms. For MDMV and SCMV, data are for incidence in field trials from 2010 (top line) and 2006 (bottom line)

[b] Possible resistant: susceptible ratios for F_2 progeny determined using a Chi-square test

[c] *P* values for the segregation ratio(s) given

(MRFV) (Zambrano et al. 2013). Therefore, the presence of symptoms was used to indicate virus infection in this study. Few or no seedlings of F_1 progeny developed symptoms when inoculated with MFSV, MMV, MDMV, SCMV, and WSMV. In contrast, 88 % of F_1 seedlings developed symptoms after inoculation with MCDV.

Resistance to each of the six viruses segregated in the Oh1VI × Oh28 F_2 generation (Table 2). Resistant: susceptible segregation ratios for F_2 seedlings were consistent with both 3:1 and 13:3 for MFSV, and 9:7 for MMV. For MCDV, the segregation ratio was consistent with a 13:3 segregation, but the Chi-square *p* value of 0.084 coupled with the large number of tests suggests the significance is marginal. For WSMV, the segregation ratio was consistent a 63:1 ratio. The segregation for MDMV incidence in F_2 plants fit a 15:1 ratio in 2006, but not in 2010 ($p = 0.041$). Segregation of F_2 plants for resistance to SCMV was consistent with both 3:1 and 11:5 ($p \geq 0.235$) in 2010, but fit only a 13:3 ratio ($p = 0.443$) in 2006. These results suggest that one to three major loci could explain resistance for each of the viruses tested.

Most of the susceptible F_2 seedlings showed strong disease symptoms for MFSV and MMV, with a mean severity rate ≥4 (Table 2). From the 20 F_2 MFSV-susceptible seedlings and the 39 F_2 MMV-susceptible seedlings, 14 and 28, respectively, had a severity score of 5. In contrast, 72 out of the 88 MCDV-susceptible F_2 seedlings developed moderate disease symptoms with a mean severity rating of 3.5. These results suggested that resistance to MFSV and MMV had a high dominance component, while MCDV resistance was additive to recessive.

Phenotypic analysis of recombinant inbred lines

Disease incidence in 256 RILs inoculated with MFSV and MMV resembled a binomial distribution, with a large number of RILs scored either as resistant or susceptible (Fig. 2). For example, 105 RILs inoculated with MFSV did not show disease symptoms in any of the three replications, and 98 RILs showed symptoms in all replications. For the remaining 53 lines, inconsistencies were observed among replications suggesting either the presence of disease escapes or incomplete resistance. MDMV incidence in the RILs also resembled a binomial distribution, with 110 RILs developing no symptoms and 105 RILs having 40–100 % disease incidence. Distribution of MCDV incidence in the RILs resembled a right-skewed normal distribution with 132 out of the 256 RILs developing consistent symptoms in all four replications and 17 RILs developing no symptoms across replications. The distribution of SCMV symptoms also resembled a normal distribution with a large number of RILs showing 0 % disease incidence. The frequency distribution for WSMV incidence was highly skewed toward resistance, with 212 out of the 256 RILs developing no disease symptoms. Disease incidence in the virus susceptible control line (Oh28) was ≥98 % and ≥92 % across all the greenhouse and field experiments, respectively.

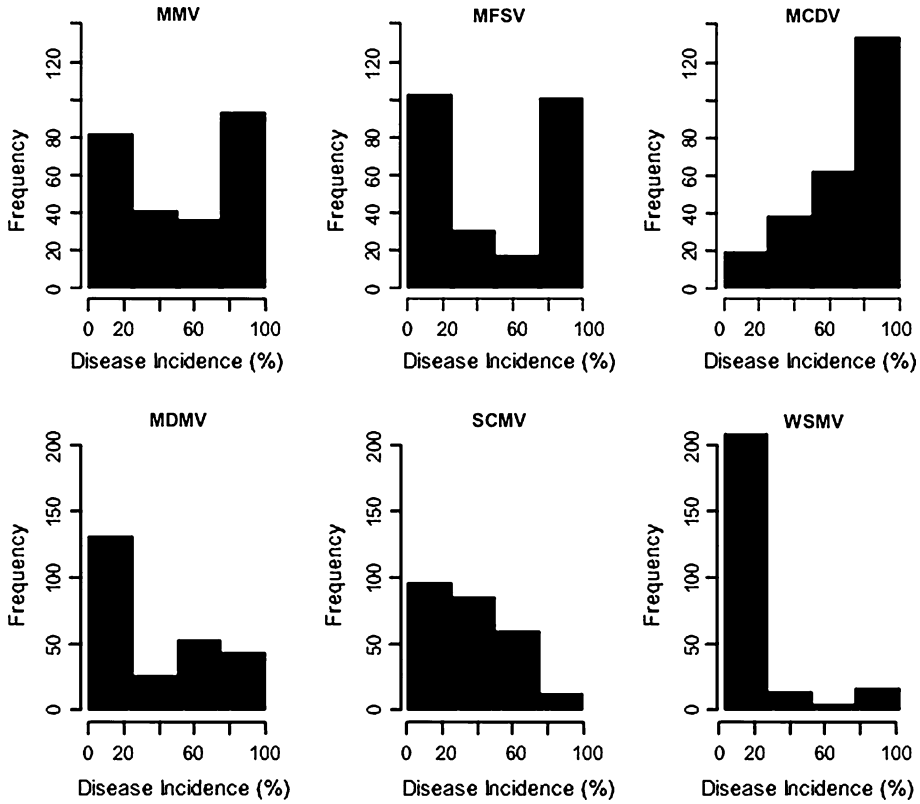


Fig. 2 Mean distribution of symptom incidence among 256 RILs inoculated with *Maize mosaic virus* (MMV), *Maize fine streak virus* (MFSV), *Maize chlorotic dwarf virus* (MCDV) and *Wheat streak mosaic virus* (WSMV) evaluated at 21 days post inoculation in the greenhouse, and *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV) evaluated between 14 and 21 days post inoculation under field conditions

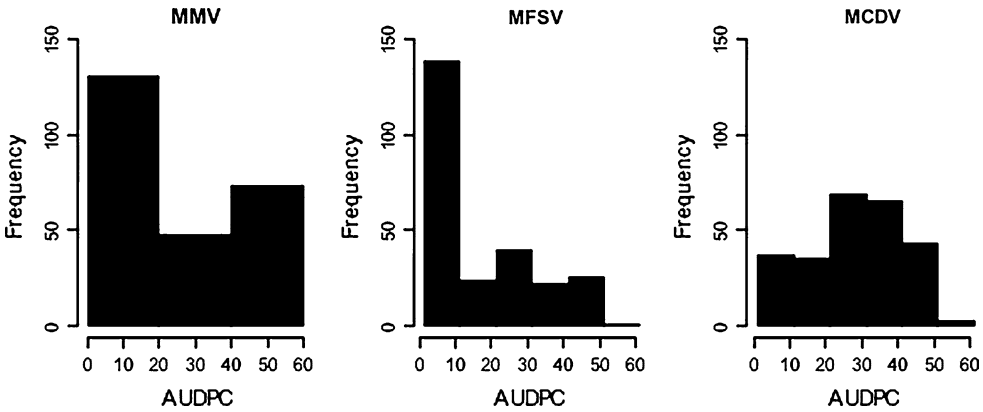


Fig. 3 Mean distribution of area under disease progress curve (AUDPC) scores for 256 RILs inoculated with *Maize mosaic virus* (MMV), *Maize fine streak virus* (MFSV) and *Maize chlorotic dwarf virus* (MCDV) evaluated at 21 days post inoculation in the greenhouse

Table 3 Components of variance for disease incidence and area under disease progress (AUDPC) of a maize recombinant inbred line (RIL) population inoculated with five viruses

Sources	Incidence					AUDPC		
	MFSV	MMV	MCDV	MDMV	SCMV	MFSV	MMV	MCDV
Genotype	16.80	12.88	4.13	1351.50	403.57	196.43	375.97	97.73
Replication	0.47	0.07	1.37	33.40	68.42	32.03	11.38	19.56
Residual	7.96	12.10	12.78	289.86	272.56	106.57	170.82	205.15
Total	25.23	25.05	18.28	1674.76	744.55	335.03	558.17	322.44

MFSV, *Maize fine streak virus*; MMV, *Maize mosaic virus*; MCDV, *Maize chlorotic mosaic virus*; MDMV, *Maize dwarf mosaic virus*; SCMV, *Sugarcane mosaic virus*

Table 4 Location and genetic effects of QTLs associated with virus resistance in maize inbred line Oh1VI

Chr.[a]	Virus	Trait	QTL Pos. (cM)	LOD[b]	Variance explained (%)	Flanking markers		Physical position of QTL (Mbp)[c]	
1	MMV	Incidence	66.6	4.4	5	PHM2177-85	PHM5098-25	41,104,922	67,646,886
2	MCDV	Incidence	21.9	5.1	7	PHM1511-14	PHM3309-8	2,496,716	5,839,887
		AUDPC	21.9	3.2	4				
	MFSV	Incidence	135.3	40.5	60	PZA02418.2	bnlg1520	217,760,107[d]	224,559,778[d]
		AUDPC	135.3	35.9	52				
	MMV	Incidence	135.3	14.4	20	PZA02964-7	bnlg1520	211,288,307	224,559,778[d]
		AUDPC	135.3	22.9	32				
3	MMV	Incidence	43.3	4.6	6	PZA00627-1	PHM13420-11	57,089,633	158,513,757
		AUDPC	43.3	5.3	6				
	MCDV	Incidence	47.8	12.4	16	PZA00627-1	PHM13420-11	57,089,633	158,513,757
		AUDPC	47.8	13.8	18				
	SCMV	Incidence	47.8	10.4	13	PZA00627-1	PHM13420-11	57,089,633	158,513,757
	WSMV	Incidence	47.8	7.0	10	PZA00627-1	PHM13420-11	57,089,633	158,513,757
	MDMV	Incidence	52.5	3.4	1	PZA02589-1	PHM9914-11	57,656,733	161,257,978
6	MDMV	Incidence	1.1	93.5	79	PHM15961-13	PZA03047-12	9,498,343	31,412,155
	SCMV	Incidence	1.1	13.6	18	PHM15961-13	PZA03047-12	9,498,343	31,412,155
	WSMV	Incidence	1.1	8.3	12	PHM15961-13	PZA03047-12	9,498,343	31,412,155
	MCDV	Incidence	2.1	9.5	12	PHM15961-13	PZA00540-3	9,498,343	39,892,439
		AUDPC	2.1	10.2	13				
	MFSV	Incidence	3.5	3.2	3	PZA03047-12	PZA00540-3	31,412,155	39,892,439
		AUDPC	3.5	4.4	5				
	MMV	Incidence	3.5	8.2	10	PZA00503-5	PZA00540-3	22,666,849	39,892,439
		AUDPC	3.5	6.6	8				
10	WSMV	Incidence	39.4	5.4	7	PHM1812-32	PHM13687-14	38,534,477	117,991,588
	MDMV	Incidence	43.3	3.3	1	PZA00337-3	PHM15868-56	86,424,631	137,500,030

MFSV, *Maize fine streak virus*; MMV, *Maize mosaic virus*; MCDV, *Maize chlorotic dwarf virus*; MDMV, *Maize dwarf mosaic virus*; SCMV, *Sugarcane mosaic virus*; WSMV, *Wheat streak mosaic virus*

[a] Maize chromosome

[b] LOD $p < 0.01$ based on permutation

[c] Physical position of the QTL based on the positions of the markers in the B73 v.2 reference genome (Schnable et al. 2009)

[d] Position is based on nearest physically mapped markers that are on the IBM2 2008 Neighbors map

AUDPC scores for the responses to MFSV, MMV, and MCDV correlated with the disease incidence ($p < 0.0001$), although the AUDPC frequency distributions did not resemble the disease incidence distribution for MFSV and MCDV (Fig. 3). The percentage of variance explained for MFSV incidence and AUDPC due to genetic effects was 67 and 59 %, respectively (Table 3). The variance due to genetic effects for MMV incidence and AUDPC was 51 and 67 %, respectively. For MDMV and SCMV only incidence was measured, and the variance attributed to genetic effects was 81 and 54 %, respectively. Disease incidence and AUDPC for MCDV had the lowest percentages of genetic variance with 23 and 30 %, respectively. Variance due to replication or year effects for all the traits and virus diseases was ≤10 % (Table 3).

Linkage map

Of the 768 SNP markers evaluated, 275 were polymorphic between Oh1VI and Oh28. Thirty-six polymorphic SNP markers were eliminated from the analysis because of low LOD scores (≤4) for association with their predicted linkage group based on the physical location of markers in the B73 reference genome. In addition, four RIL genotypes were excluded from the analysis because data were missing for more than 10 % of the markers. With the remaining markers and 256 genotypes, a linkage map spanning 1226 cM was constructed. The map included 10 linkage groups and 260 markers, with an average density of one marker every 4.9 cM (Online Resources 2 and 3). Almost half of the markers (110) deviated from the expected 1:1

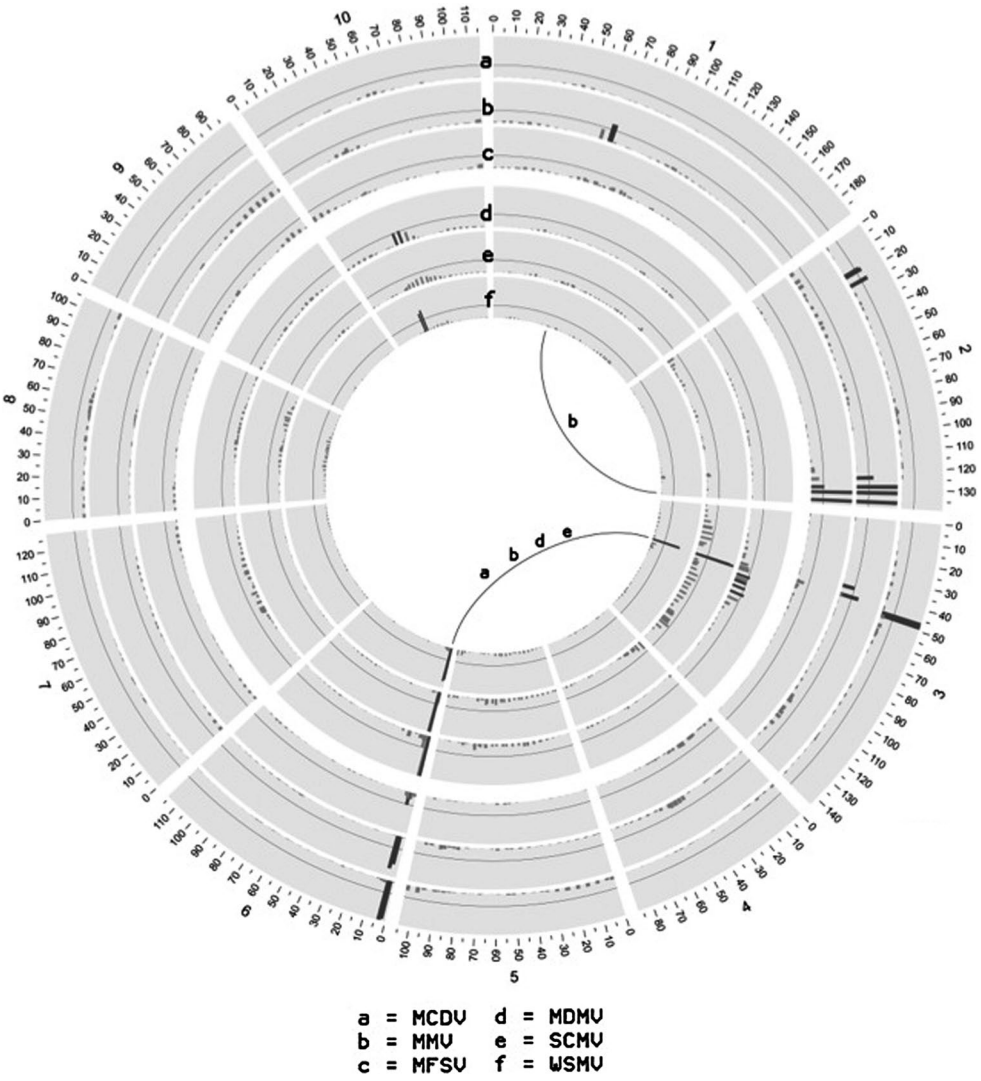


Fig. 4 Genetic location of maize QTLs conferring resistance to virus diseases. The *bars* indicate significant LOD scores ($p < 0.01$) identified by composite interval mapping across the Oh1VI × Oh28 genetic map (cM) for: *a*, *Maize chlorotic dwarf virus* (MCDV); *b*, *Maize mosaic virus* (MMV); *c, Maize fine streak virus* (MFSV); *d*, *Maize dwarf mosaic virus* (MDMV); *e*, *Sugarcane mosaic virus* (SCMV); and *f*, *Wheat streak mosaic virus* (WSMV). The fine *gray circle* within the band for each virus indicates the significance threshold for LOD scores. The *ribbons* link the regions where QTL interactions for some virus diseases were detected ($p < 0.0001$)

segregation ratio (Chi-square $p < 0.05$). These markers were mainly located in chr. 2, 5, 7, 9, and 10.

QTL mapping for virus resistance

QTLs for resistance to virus inoculation mapped to chr. 1, 2, 3, 6, and 10 (Table 4; Fig. 4). Since disease severity (measured as AUDPC) and incidence were highly correlated ($p < 0.0001$) for all six virus diseases, and we did not see major differences in the patterns of the QTLs between incidence and AUDPC, only disease incidence QTLs are shown in Fig. 4. Resistance alleles were all derived from the resistant parent Oh1VI. The statistical associations for all QTLs were confirmed using Kruskal–Wallis test and single marker analysis (results not shown). Clusters of virus resistance QTLs were found on chr. 2, 3, 6, and 10. The largest cluster was located on the short arm of chr. 6, where QTLs for all six viruses were detected. Resistance QTLs for five viruses mapped to the same or nearby regions of chr. 3. Resistance QTLs exclusive to the rhabdoviruses (MFSV and MMV) and potyviruses (MDMV and WSMV) were found on chr. 2 and 10, respectively (Table 4; Fig. 4).

Major QTLs for reduced MFSV incidence and AUDPC were detected between markers PZA02418.2 and bnlg1520 on the long arm of chr. 2. This region explained 60 and 52 % of the phenotypic variance for the two scoring methods, respectively. Additionally, minor QTLs for reduced MFSV incidence and AUDPC were detected between markers PZA03047-12 and PZA00540-3 on the short arm of chr. 6, with this region explaining 3 and 5 % of the variance in incidence and AUDPC, respectively (Table 4).

QTLs for MMV resistance mapped to chr. 1, 2, 3 and 6 (Table 4; Fig. 4), and explained between 41 and 46 % of the total phenotypic variation. Major QTL for reduced MMV incidence and AUDPC between PZA02964-7 and bnlg1520 on the long arm of chr. 2 explained 20 and 32 % of the phenotypic variance, respectively. Minor QTLs for MMV incidence on chr. 1, 3, and 6 explained between 5 and 10 %

each of the phenotypic variance. Similar QTLs on chr. 3 and 6 for reduced disease severity were identified (Table 4).

For MCDV, QTL for reduced incidence and AUDPC were mapped to chr. 2, 3, and 6 (Table 4; Fig. 4). Together, they accounted for 35 % of the total phenotypic variance. QTLs on chr. 2 between markers PHM1511-14 and PHM3309-8 explained 7 and 4 % of the variance for incidence and AUDPC, respectively. QTLs between markers PZA00627-1 and PHM13420-11 on chr. 3, explained 16–18 % of the phenotypic variance, and QTLs on the short arm of chr. 6 explained 12–13 % of the variance, respectively (Table 4).

QTLs for reduced incidence of MDMV and WSMV were located on chr. 3, 6, and 10 (Table 4; Fig. 4). A major QTL for reduced MDMV incidence that mapped to the short arm of chr. 6 explained 79 % of the total phenotypic variance. Minor QTLs each explaining 1 % of the variance mapped to chr. 3 and 10. QTLs for reduced WSMV incidence mapped to the same or nearby regions as those for MDMV incidence, and explained between 7 and 12 % of the phenotypic variance. QTLs for reduced SCMV incidence mapped to the same regions of chr. 3 and 6 as those for WSMV and MDMV incidence, and accounted for 31 % of the phenotypic variance (Table 4).

Interaction between QTLs

Significant QTL interactions between QTLs on chr. 3 and 6 for MMV, MCDV, MDMV and SCMV were detected using orthogonal contrasts ($p < 0.0001$) (Fig. 4). In addition, there was a significant interaction ($p < 0.0001$) between the MMV resistance QTLs located on chr. 1 and 2. According to variance component analysis, none of the interactions explained a higher percentage of the variance than the sum of their single QTL effects, with the exception of the interaction identified for SCMV. Here, the interaction between QTL on chr. 3 and 6 explained up to 28 % more of the total variance than the sum of their individual QTL effects (data not shown).

Discussion

Viral diseases in maize can reduce yield and jeopardize both food security and industrial grain supply (Ali and Yan 2012; Bonamico et al. 2012; Vasquez and Mora 2007; Wangai et al. 2012). The genetics of virus resistance in maize has been studied in diverse germplasm using several different types of populations (reviewed in Redinbaugh and Pratt 2009). Resistance to multiple viruses in the family *Potyviridae* has been characterized in the inbred lines Pa405 and FAP1360A (Jones et al. 2011; Lubberstedt et al. 2006; McMullen and Louie 1989, 1991; McMullen et al. 1994; Stewart et al. 2013), but few lines resistant to phylogenetically diverse viruses have been described. Oh1VI was previously found to be highly resistant to MFSV, MMV, MCDV, MDMV, SCMV, WSMV, MRFV, and *Maize necrotic streak virus* (MNeSV) (Jones et al. 2004; Louie et al. 2000; Redinbaugh et al. 2002; Zambrano et al. 2013). In this study, we report that Oh1VI is also resistant to MMV.

For the six viruses tested, resistance was transmitted to the F_1 and F_2 generations. Few F_1 seedlings developed symptoms after inoculation with MFSV, MMV, MDMV, SCMV and WSMV, suggesting resistance to these viruses is largely dominant (Table 2). In F_1 seedlings that developed symptoms of MFSV or MMV infection, severity was intermediate. In contrast, 88 % of F_1 seedlings inoculated MCDV developed symptoms with a mean severity close to the midparent value, suggesting resistance to this virus is additive. Previously, MCDV resistance in an F_2 population derived from a cross of Oh1VI with the susceptible inbred line Va35 was also found to be additive (Jones et al. 2004). Thus, both dominant and additive genes may be responsible virus resistance in Oh1VI. The ratios of resistant: susceptible phenotypes in F_2 progeny indicated that one or two gene models were sufficient to explain resistance for six of the seven viruses, and that a three gene model is consistent with WSMV resistance (Table 2).

QTL analysis identified one to four loci for resistance to each virus in a RIL population developed from a cross of Oh1VI and Oh28. Resistance QTLs for all six viruses mapped to the short arm of chr. 6, and QTLs for five of the six viruses mapped to the same region on chr. 3 (Table 4; Fig. 4). These same two regions of the maize genome were previously shown to contain resistance genes for the potyviruses MDMV, SCMV, SrMV and JGMV in diverse populations (Ding et al. 2012; Ingvardsen et al. 2010; Jones et al. 2007, 2011; Stewart et al. 2013; Tao et al. 2013; Xia et al. 1999), and the related WSMV (McMullen et al. 1994; Jones et al. 2011). The same region of chr. 3 was previously implicated in MMV (Ming et al. 1997) and MCDV (Jones et al. 2004) resistance. In this study, resistance QTL were identified on chr. 1 for MMV, and on chr. 10 for MDMV and WSMV. Interestingly, the MCDV resistance QTL on chr. 10 identified in the Oh1VI × Va35 population (Jones et al. 2004) was not identified in this population. In addition, novel QTLs for resistance to MMV and MFSV were identified on the long arm of chr. 2, and a QTL for MCDV resistance was found on the short arm of chr. 2 (Fig. 4; Table 4). Together, the segregation and QTL analyses indicate resistance for each of the six viruses in Oh1VI can be explained by one to a few genes or QTL, and are consistent with previous results using a variety of maize populations and virus species (Redinbaugh and Pratt 2009).

Because MDMV, SCMV and WSMV were transmitted to plants mechanically, the QTL detected are likely to confer resistance to the virus per se. However, MCDV, MFSV and MMV were transmitted using vectors, raising the possibility that resistance to the insect plays a role in resistance. However, none of the three viruses infects Oh1VI plants after mechanical transmission using vascular puncture inoculation (Redinbaugh, unpublished results) and high-pressure inoculation protocols similar to those used in this study tend to overcome insect resistance loci (Dintinger et al. 2005). Thus, it is likely that the identified QTL are associated with virus, rather than vector, resistance.

For WSMV and the potyviruses, there was some consistency in the numbers of loci identified in the segregation with QTL analyses carried out here and previously. A 15:1 ratio of WSMV resistant: susceptible F_2 seedlings suggests three dominant genes, consistent with both this QTL analysis and previous results for WSMV resistance in the inbred line Pa405 (McMullen et al. 1994). For SCMV, segregation analysis was consistent with models involving one dominant and one recessive gene (13:3) in 2006 or two dominant interacting (11:5) genes in 2010, and two resistance QTL were identified in this study. Similarly, two dominant, interacting loci in the same regions of chr. 3 and 6 have been described for SCMV resistance in populations derived from the European line FAP1360A (Dussle et al. 2000; Xia et al. 1999), and two resistance loci for SCMV were identified in South American and Chinese germplasm (De Souza et al. 2008; Wu et al. 2007). However, in near isogenic lines derived from the resistant inbred line Pa405, SCMV resistance required the locus on chr. 6 with the locus on chr. 3 acting as a modifier (Jones et al. 2011), consistent with a 3:1 segregation ratio observed in 2010. Although the segregation ratio of 15:1 in 2006 for MDMV resistance suggests two dominant genes, QTL analysis identified one major QTL (chr. 6) and two minor QTL (chr. 3 and 10). In populations based on Pa405, a single dominant gene was identified (McMullen and Louie 1989), but the activity of this gene is enhanced by loci on chr. 3 and 10 in a virus isolate dependent manner (Jones et al. 2011). In 2010, the segregation of resistance for MDMV was not consistent with any one to three gene ratios. The variability in segregation for SCMV and MDMV resistance between years is likely the result of environmental effects on interactions between the loci and the relatively small numbers of F_2 plants screened in a field environment. Segregation and QTL analyses for MFSV resistance both suggest two loci, with either a dominant gene (3:1) or dominant and recessive (13:3) interacting genes identified in segregation analysis, and a major QTL on chr. 2 and a minor QTL on chr. 3.

For the other viruses, some discrepancies were observed between the numbers of loci identified in segregation and QTL analyses. The 13:3 segregation ratio observed for MCDV resistance was consistent with a two-gene model involving one dominant and one recessive gene, but three QTL (chr. 2, 3 and 6) were identified. It should be noted that the Chi-square p value for this segregation was marginal (0.084) given the large number of tests carried out (Table 2). Because of this and the relatively small number of F_2 plants used for the segregation analysis, the number of loci identified by QTL analysis is likely more robust. In the F_2 population derived from Oh1VI × Va35, two major additive QTL on chr. 3 and 10 were identified, along with minor QTL on chr. 6 and 4 (Jones et al. 2004). These results indicate a further effect of the susceptible parent on QTL identification for resistance to MCDV, but also suggest that loci on chr. 3, 6 and 10 that are linked to the potyvirus resistance loci are important for MCDV resistance. A resistance segregation ratio of 9:7 suggests two complementary genes are involved in MMV resistance, while QTL analysis identified a major QTL on chr. 2 with other QTL on chr. 1, 3 and 6. The locus on chr. 2 co-localized with the major resistance QTL identified for the other rhabdovirus, MFSV, and the minor QTL on chr. 3 and 6 co-localize with loci for potyvirus and MCDV resistance. In Hawaiian germplasm, a major QTL for MMV resistance was identified in the same region of chr. 3 (Ming et al. 1997), and the QTL on chr. 1 is in a similar location to QTL for MRCV and MSV resistance identified in other germplasm (Di Renzo et al. 2004; Kyetere et al. 1999; Welz et al. 1998). Discrepancies between the segregation and QTL analyses are not unexpected, and could be the result of phenotypes being controlled by complex interactions among several resistance genes similar to potyvirus resistance in *Arabidopsis* (Cosson et al. 2012), to environmental effects on resistance genes, or to sample size effects on segregation analysis (Loesch and Zuber 1967; McMullen and Louie 1991). Resolution of these discrepancies requires development and testing of near isogenic lines carrying the QTL alone and in combination (Jones et al. 2011; Lubberstedt et al. 2006).

The potential for interactions among resistance QTL was identified using Cockerham's model for epistatic effects (Kao and Zeng 2002). Significant interactions ($p < 0.0001$) between resistance QTLs on chr. 6 and 3 were observed (Fig. 4). Previously, interactions between these loci were reported for SCMV and MDMV resistance (Jones et al. 2011; Wu et al. 2007; Xia et al. 1999), and interactions between the resistance loci on chr. 6 and 10 were reported for MDMV and MCDV (Jones et al. 2004, 2011). These results suggest that the gene(s) on chr. 6 interact with other loci. The interaction observed between the MMV resistance QTLs located on chr. 1 and 2 is noteworthy since major resistance QTLs to MRCV and MSV have been reported in the same region of chr. 1 (Di Renzo et al. 2004; Kyetere et al. 1999; Welz et al. 1998).

Although no virus resistance genes have been isolated to date, fine mapping of the *Scmv1* and *Scmv2/Rscmv2* resistance genes on chr. 6 and 3, respectively, has narrowed the genomic regions encoding these dominant genes (Ding et al. 2012; Ingvardsen et al. 2010; Tao et al. 2013). The region of the B73 genome homologous to the *Scmv1* region encodes ten candidate genes, including genes previously associated with pathogen and abiotic stress responses such as putative thioredoxin h and cycloartenol synthase genes (Tao et al. 2013). Twenty ESTs with identity to portions of the *Scmv2* region were identified (Ingvardsen et al. 2010). Of these four with similarity to heat shock protein 70, general vesicular transport factor p115, RhoGTPase activating protein, and syntaxin/t-SNARE proteins were considered candidate genes based on their involvement in plant responses to various stimuli. Similarly, the *Rscmv2* region encodes 19 genes, two of which were considered candidate genes based on their possible roles in the resistance response: a RhoGTPase activating protein and an auxin binding protein-1 gene (Ding et al. 2012). These two genes are also contained in the *Scmv2* region. Interestingly, no genes homologous to the NBS-LRR containing class of R genes, which includes dominant genes conferring to *Tomato mosaic virus* and *Potato virus X* (Bendahmane et al. 1999; Gururani et al. 2012; Lanfermeijer et al. 2003), were found in either genomic region.

MCDV resistance was mainly additive in Oh1VI, and some of the gene models suggested by segregation analysis implicate recessive genes. Additive virus resistance genes remain to be isolated, but recessive genes are implicated in about 40 % of known virus resistance in plants (Diaz-Pendon et al. 2004). This resistance is related primarily to host susceptibility factors that are required for virus replication, especially translation factors such as eukaryotic initiation factor 4E (eIF4E) (Gomez et al. 2009; Gururani et al. 2012; Truniger et al. 2008). To determine whether any translation factors underlie QTL identified in this study, the positions of sequences encoding eIF3c, eIF4E, eIFiso4E, eIF4G (CBP80), eIFiso4G, eIF4A and eEF1α, which were all identified as part of the cap-binding complex (Lazaro-Mixteco and Dinkova 2012), were mapped in the B73 v.2 genome using POPCorn BLAST (Cannon et al. 2011). Sequences encoding genes for all of the factors were identified in the B73 genome, and their positions were compared with the positions of QTL identified in this study (data not shown). Two eIF4e genes were found on chr. 3, one between 132,459,978 and 132,646,651 Mb and the other between 145,182,409 and 145,184,767 Mb. Both genes are within the interval defining the virus resistance QTL on chr. 3 (Table 4), raising the possibility that eIF4E plays a role in virus resistance in maize. However, this gene was not contained in the *Scmv2/Rscmv2* regions identified in the fine-mapping studies (Ding et al. 2012; Ingvardsen et al. 2010). In addition, a homolog of eIF4G maps to chr. 1 at 59,061,226–59,087,208 Mb, within the region of the QTL for MMV resistance (Table 4).

It is not clear if a single pleiotropic gene or a cluster of genes are responsible for the multiple virus-resistance loci. About 80 % of plant viruses have positive-strand RNA genomes with overlapping replication, movement or gene regulation strategies (Gomez et al. 2009). A single maize gene targeting one of these common mechanisms could be sufficient to partially or completely suppress virus replication or movement. For example, the *A. thaliana RTM1* gene restricts long distance movement of *Tobacco etch virus* (TEV) and other potyviruses by the accumulation of a jacalin-like protein in the phloem tissue (Chisholm et al. 2001). On the other hand, maize genome regions with resistance QTL for multiple viruses are known to contain clusters of resistance genes for multiple taxonomic groups of pathogens (Redinbaugh and Pratt 2009; Wisser et al. 2006), in addition to defense response and resistance gene homologs (Wang et al. 2007; Xiao et al. 2007). The tendency of resistance genes to cluster is widely described in maize and other plants, and seems to occur via gene duplication, unequal crossing-over, transposon insertion, and divergence through time by selection and recombination (Friedman and Baker 2007; Gururani et al. 2012; Lozano et al. 2012). Further characterization and cloning of the maize genes conferring resistance to virus diseases will provide a better understanding of the biological and molecular processes that are important for the development of virus-resistant plants.

In summary, the multi-virus resistance observed in Oh1VI was controlled by one or few genes. Dominant, recessive, additive, and epistatic gene effects were responsible for the multiple-virus resistance observed in the inbred line Oh1VI. In addition, clusters of QTLs conferring resistance to a diverse set of virus diseases were mapped on chr. 3, 6, and 10 at the same regions where virus resistance has been reported before, and a novel major resistance QTL that is effective against negative sense RNA viruses was identified on chr. 2. The identification of clusters of genes conferring resistance to multiple virus diseases in Oh1V1 suggests that this line offers potential for breeding programs seeking to protect the crop through improved resistance. For instance, introgressing QTL regions on chr. 2, 3, 6, and/or 10 into elite cultivars could improve resistance to both potyviruses and MMV, which cause significant losses.

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