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Molecular mapping of a major gene conferring resistance to maize mosaic virus

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Abstract The objective of this study was to determine the genetic basis of resistance to maize mosaic virus (MMV). Molecular markers were used to map resistance loci to MMV in a set of 91 maize (*Zea mays* L.) recombinant inbred lines (RILs), derived from the cross between Hi31 (a B68 conversion resistant to MMV) and Kil4 (a Thai inbred susceptible to MMV). The RILs were evaluated for MMV resistance in disease nurseries in Hawaii in the winter of 1993 and the summer of 1994. Twenty-eight highly susceptible RILs were chosen for gene mapping using the pooled-sampling approach. Initial evidence from the pooled DNA indicated that restriction fragment length polymorphism (RFLP) probes on chromosome 3 near the centromere were biased to the susceptible parent allele. Analysis of 91 RILs at 103 RFLP loci confirmed the presence of a major MMV resistance gene on chromosome 3. The resistant allele at this locus, previously named *Mv1*, is present in the resistant parent Hi31 and traces back to the Argentine parent used in conferring common rust resistance to B68. We conclude that resistance to MMV in B68 and Caribbean flints involves a major gene *mv1* on chromosome 3 located between RFLP markers *umc102* and *php20508*.

Key words Corn · Restriction fragment length polymorphism · Genetic mapping · Disease resistance

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Introduction

Maize mosaic virus (MMV) causes a major disease of maize (*Zea mays* L.) in Hawaii and many tropical and sub-tropical countries (Brewbaker 1981). The virus is transmitted by the leafhopper *Peregrinus maidis* (Ashmead) in a persistent manner (Carter 1941). The disease occurs coincident with this widely distributed vector throughout humid tropical and sub-tropical regions of the world (Herold 1972). Maize mosaic was reported to be a serious disease of maize in Hawaii as early as 1914 (Kunkel 1921). Symptoms include dwarfing of internodes and husks, and high-contrast chlorotic stripes along lead veins, sheaths, stalks, and husks (Herold 1972). It becomes especially severe when maize is planted on a continuous basis (Brewbaker 1981). Entire fields can be dwarfed below 50 cm in height with no kernels produced under severe epiphytotics (Brewbaker 1979).

Almost all temperate-zone hybrids are susceptible to MMV. Resistance to MMV was first recognized in Cuban flint materials imported into Hawaii. Brewbaker and Aquilizan (1965) concluded that resistance to MMV from the Caribbean materials was monogenic with co-dominant gene action, and they designated the locus *mv1*. The heterozygotes showed intermediate resistance under severe epiphytotics (Brewbaker 1981). Brewbaker (1974) generated near-isogenic lines of resistant inbred Hi27 and concluded that the resistance gene was linked to two morphological markers *nana plant1* (*na1*) and *liguleless2* (*lg2*) on chromosome 3. This gene has been introduced by direct backcrossing into

more than 100 inbreds and cultivars and 130 genetic stocks (Brewbaker 1974).

The advantages of recombinant inbred lines (RILs) in genetic studies have been reviewed by Burr et al. (1991). Recombinant inbred lines have been used for constructing molecular maps in maize (Burr et al. 1989), *Arabidopsis* (Reiter et al. 1992), and tomato (*Lycopersicon esculentum* Mill. (Paran et al. 1995), as well as mapping disease resistance genes in rice (*Oriyza sativa* L.) (Wang et al. 1994) and maize (Kyetere et al. 1995). A pooled-sampling approach can rapidly identify restriction fragment length polymorphism (RFLP) or randomly amplified polymorphic DNA (RAPD) markers linked to disease resistance genes, as reported by Michelmore et al. (1991) for the *Dm5/8* locus in lettuce (*Lactuca sativa* L.) and McMullen et al. (1994) for the *wsm2* and *wsm3* loci in maize. In this paper we describe the mapping of a major gene for resistance to MMV.

Materials and methods

Plant material

Recombinant inbred lines were generated from the cross, Hi31 × Kil4 (Moon and Brewbaker 1995). Hi31 is a dent corn B68 conversion derived from 'Iowa Stiff Stalk Synthetic', and Ki14 is a Thai flint inbred derived from 'Suwan 1'. The cross was made in 1987 at Waimanalo, Hawaii. Two hundred F_2 seeds were randomly selected and planted in the spring of 1990. Each plant was self-pollinated without selection. F_3 seeds from each harvested ear were planted ear to row (5 m long and 0.75 m spacing between the rows). Two plants of each row were randomly selected and self-pollinated. The self-pollinated ear on the first plant of two in the row, when possible, was harvested to advance the line to the next generation. This single seed descent (SSD) procedure was repeated to the F_7. The lines (ears) in the F_7 generation were planted ear-to-row in the winter of 1992 and harvested in the spring of 1993. Ten plants in each RIL were sib-pollinated to given an adequate amount of seed for future experiments.

Tests for resistance to MMV

Ninety-six RILs were planted for MMV resistance evaluation in a lattice design in the winter of 1993 at Waimanalo, Hawaii. Ninety-one RILs were grown in a randomized complete block design in the same screening site in the summer of 1994. Twenty sub-lines (SSD from parental line) each were grown of resistant parent Hi31 and susceptible parent Ki14. The two trials with two replications each were evaluated for the disease under natural infection in a field where susceptible corn was planted successively for a year to increase the virus and leafhoppers. Test entries were planted in one-row plots 5 m long with 0.75-m between-row spacing. Two rows of susceptible sweet corn were planted every eight test entries, and three rows were planted around the blocks to enhance the natural inoculation. The first ten plants from each line were rated for disease severity using a 1–9 scale (1 = symptomless, 9 = severe stunting with no ear formed).

RFLP analysis

Plant DNA was extracted from lyophilized, ground leaf tissue as described by McMullen and Louie (1989). To determine the probe-enzyme combinations revealing polymorphisms between Hi31 and Ki14, we digested parental DNA and pooled DNA from 28 highly susceptible RILs with eight restriction enzymes, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sac*I, and *Xba*I, in a total volume of 300 µl with 3 units of restriction enzyme/µg DNA for 4 h at 37°C. DNA was precipitated, resuspended, electrophoresed in 0.7% agarose gels, and transferred to nylon membranes with 25 m*M* $NaPO_4$, pH 6.5. After transfer overnight, membranes were briefly washed in 2 × SSC, dried, and baked at 80°C for 2 h, DNA was UV cross-linked to the membrane with a Stratalinker according to the manufacturer's recommendations (Stratagene, San Diego, Calif.). Hybridization and wash conditions were as described by McMullen et al. (1994). One hundred and seventeen RILs, including the 91 RILs tested for MMV resistance, were selected for RFLP analysis.

Linkage analysis

The RFLP loci were mapped using MAPMAKER version 3.0 (Lincoln et al. 1993). The segregation of alleles into genotypic classes at each locus was checked against the expected 1:1 ratio for an RIL population by using a chi-square test with a significance level of 5%. Linkage groups were based on the University of Missouri at Columbia (UMC) 1993 maize RELP map (Coe 1993), but marker order and distances were obtained using the RILs algorithm in the MAPMAKER program, based on the segregation data of the F_7 RILs. To determine the associations between molecular markers and the MMV resistance, we conducted single-factor analysis of variance using the GLM procedure in SAS (SAS Institute 1989). MAPMAKER/QTL version 1.1 (Lincoln et al. 1993) was used to identify putative loci affecting MMV resistance based on interval analysis.

Results and discussion

Disease resistance test

From the disease nursery in the winter of 1993, 46 RILs were symptomless in two replications, while 50 RILs showed MMV-induced symptoms. Among these 50 RILs, 32 RILs exhibited susceptible plants in both replications, and 18 RILs showed susceptible symptoms in one replication. Due to limited disease pressure, not all of the plants of each susceptible RIL were infected. After continued planting of susceptible corn in the field, 91 RILs were replanted March, 1994, with severe disease pressure; all of the plants of the 20 sublines from the susceptible parent exhibited infection. Fifty RILs were classified as susceptible with 90% or more of the plants infected; 41 RILs were classified as resistant with a disease score of 2 or below (Fig. 1). Sublines of resistant parent Hi31 and susceptible parent Ki14 averaged 1.3 and 7.2, respectively. Due to low virus severity ratings in some susceptible RILs, the distribution of the phenotypic data was skewed toward the resistant phenotype. The results for individual RILs from two seasons were generally consistent. The mean virus severity ratings of two replications from the summer of 1994 were used for mapping MMV resistance. The distribution did not deviate significantly from a 1:1 segregation ratio, as was expected from the

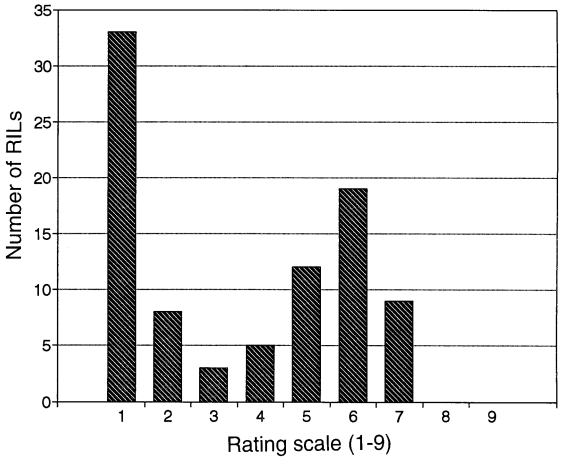

Fig. 1 Mean disease rating of 91 RILs derived from Hi31 × Ki14 for reactions to maize mosaic virus under natural infection in the summer of 1994. Rating scale, 1 = no symptom, 9 = plant completely collapsed and no ear formed

previous study reporting single-gene-controlled MMV resistance (Brewbaker 1981).

Since MMV can not be mechanically inoculated, being transmitted only by leafhoppers, the reliability of the field test under natural infection is subject to challenge. The use of RILs rather than other types of progeny provides advantages in this situation, as the disease reading for a genotype could be confirmed by multiple plants within each homozygous RIL as well as across seasons and replications.

Screening for parental polymorphisms

Among 163 probes tested, 114 were polymorphic between Hi31 and Ki14. Since the RILs were derived from unrelated, temperate inbred Hi31 and tropical inbred Ki14, the overall level of polymorphism detected was high (69.3%). This can compared with values of 60% previously reported for temperate germplasm (Gardiner et al. 1993).

Genotyping of RILs

The 114 RFLP markers showing polymorphisms between the two parents were used for mapping and analysis in the RILs. Since duplicated loci were exhibited from 13 probes, a total of 127 loci were genotyped for these RILs. For 19 probes, non-parental band mobilities were observed and coded as missing data. The average frequency of non-parental alleles was 3.2%.

Of the 117 RILs, 4 lines were eliminated due to too many missing data points (2 lines) or too many heterozygous loci (2 lines). For the remaining 113 RILs, 83 RILs fit the 1:1 segregation ratio with a slight bias to the Hi31 alleles (54.7%), 24 RILs were skewed to Hi31 alleles (68.7%), and the other 6 RILs were skewed to the Ki14 alleles (66.3%). The overall average of the Hi31 alleles in 113 RILs was 56.5%, suggesting that the RILs favored Hi31 alleles during their development.

Skewed segregation favoring Hi31 alleles (average of 71.6%) was observed for 48 RFLP markers. At 14 RFLP loci there was an excess of Ki14 alleles (average of 64.5%) represented in the RIL population. The remaining 65 RFLP markers fit the 1:1 expected segregation ratio with an average of 50.6% Hi31 alleles.

Non-parental alleles might come from the residual heterozygosity of the parental lines. By examination of the presence of the non-parental alleles in the RILs, the 113 RILs could be easily divided into two groups, 56 RILs with six or less than six non-parental alleles and 57 RILs with more than six non-parental alleles. In the low non-parental allele group (56 RILs), the overall average of the parental alleles fitted the 1:1 segregation ratio with 50.3% Hi31 alleles; but in the high non-parental allele group (57 RILs), 39 RFLP markers were almost completely biased to Hi31 alleles with 1 marker was biased to Ki14 alleles. The extremely skewed distribution represents a significant problem in constructing the RFLP map.

The overall average parental allele frequency did not deviate significantly from the 1:1 segregation ratio despite the distorted segregation. Compared with the theoretical 1.56% heterozygosity of the F_7 generation (Falconer 1989), the observed 1.7% heterozygous allele frequency was acceptable.

Map construction

Only after deleting the biased alleles in 57 RILs with a high frequency of non-parental alleles, was an acceptable map constructed. The primary linkage groups were based on the UMC RFLP map (Coe, 1993). A total of 103 RFLP loci were mapped, with total length 1624.7 cM and average interval between markers 15.8 cM. No major disagreements were found with the order derived from the immortalized F_2 population described by Gardiner et al. (1993).

Mapping MMV resistance

Initial evidence for a resistance gene on chromosome 3 was obtained from the pooled-sampling approach as probes on chromosome 3 near the centromere were biased to the susceptible parent allele. Single-factor analysis of variance was used to determine the significance of correlations between RFLP marker genotype classes and MMV resistance (Table 1). Nine markers on chromosome 3 were significantly ($P < 0.05$) associated with resistance. The marker with the highest

Table 1 Loci significantly associated with MMV resistance from single-factor analysis of variance

Locus	Chromosome bin[a]	R^2	Probability[b]
umc121	3.02	0.0615	0.0206
csu16	3.02	0.1983	0.0031
php20024	3.03	0.2409	0.0020
umc50	3.04	0.2249	0.0002
umc102	3.04	0.3202	0.0001
php20508	3.05	0.4169	0.0001
csu30	3.05	0.3768	0.0001
umc26	3.05	0.4014	0.0001
bn15.37	3.05	0.1491	0.0003

[a] Chromosome bins were based on the UMC 1995 RFLP map
[b] *P* values of *F*-test

F-value was *php20508* ($F = 57.19$, $P < 0.0001$), with an $R^2 = 0.417$ (fraction phenotypic variation explained).

The peak value from interval mapping (MAPMAKER/QTL) placed the MMV resistance gene approximately 6 cM from *php20508* and 4 cM from *umc102* on chromosome 3, and accounted for 74.4% of the phenotypic variation with an LOD score of 14.1 (Fig. 2). The resistant allele at this locus is present in the resistant parent Hi31 and presumably traces back to the Argentine parent used in conferring common rust resistance to B68. The results confirmed Brewbaker's suggestion from near-isogenic conversions that a co-dominant gene for resistance to MMV designated *mv1* is linked to *lg2* and *na1* on chromosome 3 (Brewbaker 1974).

Several RFLP markers on chromosomes 4, 7, and 9 were marginally significant for MMV resistance from the results of SAS/GML but not MAPMAKER/QLT (data not shown).

The co-dominant resistant allele *Mv1* was linked to both flanking RFLP markers from Hi31 in 34 of the 41 lines showing complete resistance in the population. Five lines had *Mv1* linked to the Hi31 allele at either *umc102* or *php*20508, and 2 lines were eliminated due to missing RFLP data. Among the 50 susceptible lines in the RIL population, 42 had the Ki14 allele for either *umc102* or *php20508*, or both, but 8 lines had the Hi31 allele for both markers.

The MMV resistance locus *mv1* was mapped near the centromere of chromosome 3 between RFLP markers *umc102* and *php20508*. This map position placed *mv1* close to the position of *wsm2*, a gene for resistance to wheat streak mosaic virus on chromosome 3 in Pa405 (McMullen et al. 1994), and *rp3*, a gene for resistance to *Puccinia sorghi* (Schwein.) (Sanz-Alferez et al. 1995). Since Pa405 is susceptible to MMV, the dominant resistance allele *Wsm2* and co-dominant allele *Mv1* should not be the same gene. It is unlikely that the fungal disease resistance gene *rp3* and the viral disease resistance gene *mv1* are allelic. Since *mv1* was from an Argentine parent used to confer common rust resistance to B68, these two genes must be tightly linked. These results support the suggestion that a possible clustering of genes for resistance to maize pathogens is located in this region (McMullen and Simcox 1995).

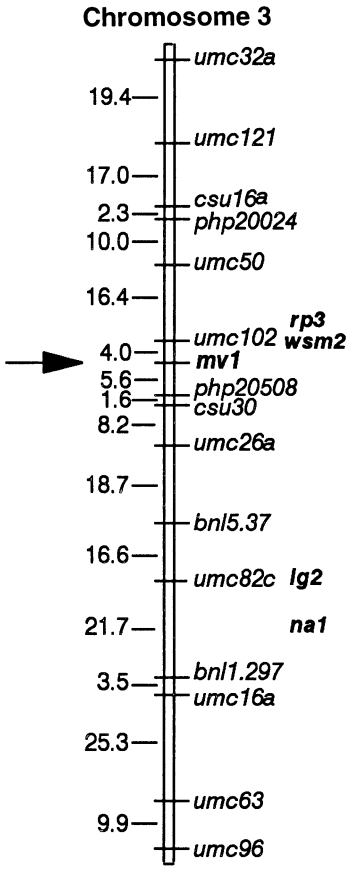

Fig. 2 Genetic map of the region around the *mv1* locus (*arrow*), on chromosome 3. Genetic distances are shown in CentiMorgans to the *left*. The map was generated from the analysis of 117 RILs derived from Hi31 × Ki14. The relative map positions of *rp3*, *wsm2*, *lg2*, and *na1* and shown to the *right*

Selection for resistance to obligatory insect-vectored viruses is often difficult, a problem that could be aided by indirect selection with RFLP markers. With the traditional method, the screening materials must be planted in the area where the virus occurs epidemically and reproducibly high disease pressure is problematic. With the RFLP marker-assisted selection approach, breeding can be carried out without field testing. Work is currently under way to transfer the *mv1* gene into 30 susceptible maize inbreds using the two RFLP markers flanking MMV resistance, thus reducing the cost and time required to develop MMV-resistant lines.

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References

Brewbaker JL (1974) Continuous genetic conversions and breeding of corn in a neutral environment. In: Proc 29th Annu Corn Sorghum Conf. Am Seed Trade Assoc Washington, D.C., pp 118–133

Brewbaker JL (1979) Disease of maize in the wet lowland tropics and the collapse of the classic Maya civilization. Econ Bot 33:101–118

Brewbaker JL (1981) Resistance to maize mosaic virus. In: Gordon DT, Knoke JK, Scott GE (eds) Virus and viruslike diseases of maize in the United States. So Coop Ser Bull Vol. 247 OARDC, Wooster, Ohio pp 145–151

Brewbaker JL, Aquilizan F (1965) Genetics of resistance in maize to mosaic-stripe virus transmitted by *Peregrinus maidis*. Crop Sci 5:412–415

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Carter W (1941) *Peregrinus maidis* Ashm., and the transmission of corn mosaic. Ann Entomol Soc Am 34:551–556

Coe EH (1993) Gene list and working maps. Maize Genet Coop Newsl 67:133–166

Falconer DS (1989) Introduction to quantitative genetics, 3rd edn. John Wiley and Sons, New York

Gardiner JM, Coe EH, Melia-Hancock S, Hoisington DA, Chao S (1993) Development of a core RFLP map in maize using an immortalized F_2 population. Genetics 134:917–930

Herold R (1972) Maize mosaic virus. CMI/AAB description of plant viruses no. 94. Commnow. Mycol Inst Assoc Appl Biol, Kew, Surrey, UK

Kunkel LO (1921) A possible causative agent for the mosaic disease of corn. Hawaii Sugar Plant Assoc Bot Ser 3:44–58

Kyetere DT, Ming R, McMullen MD, Pratt RC, Brewbaker JL, Musket TA, Moon HG (1995) Monogenic tolerance to maize streak virus mapped on the short arm of chromosome 1. Maize Genet Coop News1 69:136–137

Lincoln SE, Daly MJ, Lander ES (1993) Mapping genes controlling quantitative traits using MAPMAKER/QTL version 1.1. Whitehead Institute, Cambridge, Mass.

McMullen MD, Louie R (1989) The linkage of molecular markers to a gene controlling the symptom response in maize to maize dwarf mosiac virus. Mol. Plant-Microbe Interact 2:309–314

McMullen MD, Simcox KD (1995) Genomic organization of disease and insect resistance genes in maize. Mol Plant-Microbe Interact 8:811–815

McMullen MD, Jones MW, Simcox KD, Louie R (1994) Three genes control resistance to wheat streak mosaic virus in the maize inbred pa405. Mol Plant-Microbe Intereact 7:708–712

Michelmore RW, Paran I, Kesselli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Moon HG, Brewbaker JL (1995) Recombinant inbred lines from tropical × temperate single crosses. Maize Genet Coop News1 69:59–60

Paran I, Goldman I, Tanksley SD, Zamir D (1995) Recombinant inbred line for genetic mapping in tomato. Theor Appl Genet 90:542–548

Pratt RC, Anderson RJ, Louie R, McMullen MD, Knoke JK (1994) Maize responses to a severe isolate of maize chlorotic dwarf virus. Crop Sci 34:635–641

Reiter RS, Williams JKG, Feldmann KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Accd Sci USA 89:1477–1481

Sanz-Alferez S, Richter TE, Hulbert SH, Bennetzen JL (1995) The *RP3* disease resistance gene of maize: mapping and characterization of introgressed alleles. Theor Appl Genet 91:25–32

SAS Institute (1989) SAS/STAT user's guide, version 6, 4th edn. SAS Insitute, Cary, N.C.

Wang GL, Mackill DJ, Bonman M, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421–1434