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Comparative mapping of ZYMV resistances in cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.)

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Abstract Zucchini yellow mosaic virus (ZYMV) routinely causes significant losses in cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.). ZYMV resistances from the cucumber population 'TMG1' and the melon plant introduction (PI) 414723 show different modes of inheritance and their genetic relationships are unknown. We used molecular markers tightly linked to ZYMV resistances from cucumber and melon for comparative mapping. A 5-kb genomic region (YCZ-5) cosegregating with the *zym* locus of cucumber was cloned and sequenced to reveal single nucleotide polymorphisms and indels distinguishing alleles from ZYMV-resistant (TMG1) and susceptible (Straight 8) cucumbers. A low-copy region of the YCZ-5 clone was hybridized to bacterial artificial chromosome (BAC) clones of melon and a 180-kb contig assembled. One end of this melon contig was mapped in cucumber and cosegregated with ZYMV resistance, demonstrating that physically linked regions in melon show genetic linkage in cucumber. However the YCZ-5 region segregated independently of ZYMV resistance loci in two melon families. These results establish that these sources of ZYMV resistances from cucumber TMG1 and melon PI414723 are likely non-syntenic.

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Introduction

Zucchini yellow mosaic virus (ZYMV) routinely causes significant losses in cucumber (*Cucumis sativus* L) and melon (*C. melo* L.) (Provvidenti et al. 1984; Luis et al. 1998; Yuki et al. 2000). ZYMV resistance in melon USDA plant introduction (PI) 414723 was conditioned by a dominant allele at the *Zym* locus (Pitrat and Lecoq 1984) or by dominant alleles at three complementary loci (*Zym-1, Zym-2*, and *Zym-3*; Danin-Poleg et al. 1997). These two different inheritance modes of ZYMV resistances from the same melon PI were revealed using different strains of the virus (Lecoq and Pitrat 1984; Desbiez and Lecoq 1997; Desbiez et al. 2002; Lecoq et al. 2002). In cucumber, resistances to ZYMV from 'TMG1' and 'Dina' were inherited as recessive alleles at one locus (*zym*) (Provvidenti 1987; Abul-Hayja and Al-Shahwan 1991; Kabelka et al. 1997). Although ZYMV resistances from cucumber and melon have been placed their respective genetic maps (Park et al. 2000; Danin-Poleg et al. 2002), the genetic relationships among ZYMV resistance loci from cucumber and melon are unknown. The *zym* locus of cucumber is especially interesting because it conditioned resistance to all of 173 ZYMV isolates from around the world, whereas melon PI414723 was susceptible to some of these isolates (Lecoq et al. 2002).

Cucumber and melon are distantly related *Cucumis* species (Perl-Treves et al. 1985) with numerous chromosome rearrangements; cucumber possesses 14 chromosomes ($2n=2x=14$) and melon has 24 chromosomes ($2n=2x=24$). No sexual hybrids have been reported (Robinson and Decker-Walters 1997). However, relatively strong signals from cross hybridizations of RFLP probes (Neuhausen 1992) and amplifications from genomic regions by primers for simple sequence repeats (SSR) (Katzir et al. 1996; Danin-Poleg et al. 2001) revealed significant sequence similarities between cucumber and melon. Danin-Poleg et al. (2000) identified nine SSR markers shared between melon and cucumber and proposed that cucumber linkage group B and melon linkage groups E and II were syntenic. High levels of synteny among related species allow genetic information from one species to be used for gene isolation and molecular tagging in other related species (Chen et al. 1998; Paterson et al. 2000). If the ZYMV resistance genes in the cucurbits were orthologous, markers near ZYMV resistance in one cucurbit could be used in marker-assisted breeding of other cucurbits. In this study, molecular markers tightly linked to ZYMV resistances in cucumber and melon were used for comparative mapping of these resistance loci in *Cucumis* and to reveal the relationship between physical linkage in melon and genetic linkage in cucumber.

Materials and methods

Cloning of a cucumber genomic region cosegregating with *zym*

The AFLP (E15/M47-F-197) marker cosegregating with the cucumber *zym* locus was amplified from the ZYMV-resistant recombinant inbred line (RIL) F62 as previously described (Park et al. 2000), extracted from the acrylamide gel (Sambrook et al. 1989), and cloned into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, Calif., USA). Plasmids were isolated (Sambrook et al. 1989), digested with *Eco*RI, and fractionated in 2% agarose gels. Inserts were purified from the gel, radiolabelled with ^{32}P, and hybridized to DNA gel blots (Kennard et al. 1994) carrying DNA samples from ZYMV-resistant and susceptible cucumber RILs (Park et al. 2000) digested separately with *Aat*II, *Alu*I, *Ape*I, *Ava*I, *Bam*HI, *Bgl*II, *Dra*I, *Dde*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Mbo*I, *Msp*I, *Nae*I, *Nco*I, *Nhe*I, *Not*I, *Nsi*I, *Pst*I, *Sac*I, *Spe*I, *Xba*I, and *Xho*I. An RFLP revealed with *Hae*III was used to establish

Table 1 Primer sequences used to amplify genomic regions from cucumber and melon. The sizes of all PCR fragments are given in base pairs. *T* represents the allele from TMG1, *S* the allele from Straight 8. Two fragments resulted from digestion of amplicons by the restriction enzymes *Hae*III (YCZ-CAP-1) or *Xba*I (YCZ-CAP-2)

Region	Primer sequence (5′–3′)	Amplicon	
YCZ-SCAR-1	GGGGAATGAGTGGATGCAAGATG GGGTAGTTGGCGATTGACATTG	335 (T)	425 (S)
YCZ-SCAR-2	GGCTATTGTACCCTATGAACAAC GTAGCACAAATAGGATTTAAGGTC	407 (T)	298 (S)
YCZ-CAP-1	GACCTTAAATCCTATTTGTGCTAC GCGGCTTGGACTTGGCTCAAC	869 (T)	520 + 305 (S) *Hae*III digest
YCZ-CAP-2	CATTCGTTGATGTGGAAGACCTGTC CAGAAGCAGAGCCGTCACTCTCC	482 + 445 (T) *Xba*I digest	922 (S)
M1	GCTTTGGAAAGAATTGTAAACG CAGTTGTAAAAGTGAGAGCTTGG	1,322	
M2	ATTACAAGTTAGGGGACAATGAAAG CGACCTTGGTGAATTAGAGATTAG	1,307	
M3	CACTCTAATCTCTAATTCACCAAGG TGGGGGTTTTCTTGAGAGTT	1,358	
M4	TGTTCTTCAAATCACGTATCCT TGGGCAGAATTTGAACTTGT	1,137	
8164	ATGTGTGATTTGCAGATTTTCATAG ACCTTCCCTGATCGACTCCT	232	
MB-H17-E1	GATGCCCGTTTCACTTCC CTGTATCGCTTCGTTAGTAGACTGAAC	348	
MB-H17-E2	GCACCGTTCGTTCATGGTTATCTCATG CTCTATCAGATCAAGGCCTACCGCGTAGC	352	
MB-H21-E1	AAAGGGCCGAGAACAACATACATAAAAGGA GCTGTTCCAATAAGCATTCCCAAGCAAC	434	
MB-H21-E2	GATAACATTGGTACCACAACAACTTCAGC GACCAAACCCTTCGAGTTCTCTATATC	550	

cosegregation of AFLP clone 1-7 with the cucumber *zym* locus using 49 cucumber RILs (Park et al. 2000). AFLP1-7 fragment was sequenced as previously described (Lilly et al. 2001).

Cucumber genomic regions carrying AFLP1-7 were cloned from two partial genomic DNA libraries from ZYMV-resistant (F62) and susceptible (F12) RILs. DNAs were digested with *Bgl*II and run on a 1% agarose gel. Fragments of 4–6 kb were purified from the gel and cloned into *Bgl*II-digested and dephosphorylated LITMUS28 plasmid vector (New England Biolabs, Beverly, Mass., USA). Transformed DH108 bacteria (Invitrogen) were spread on LB plates with ampicillin and colonies transferred onto C/P Lift Membranes (Biorad, Hercules, Calif., USA) according to the manufacturer's instructions. Membranes were hybridized with the ^{32}P-labelled AFLP1-7 clone (Kennard et al. 1994). Clones from the resistant (YCZ-5R) and susceptible (YCZ-5S) RILs were isolated and cosegregation with *zym* confirmed. Clones were sequenced using EZ:TNTM (Epicentre, Madison, Wis., USA) following the manufacturer's instructions. Sequences of the YCZ-5R and YCZ-5S inserts were aligned and primers designed to amplify across two major insertion-deletion (indel) regions and two restriction site polymorphisms. PCR reactions were performed in a 50-μl volume containing 50 ng of genomic DNAs, 0.2 μM each of the primers, 0.2 mM dNTP, 5 μl of 10× buffer containing 20 mM $MgCl_2$, 1.25 U of Takara Ex *Taq* polymerase with cycling parameters of one cycle of 3 min at 94°C, and 35 cycles of 30 s at 94°C, 1 min at 60°C, and 45 s at 72°C. Cucumber populations and breeding lines (Table 2) were evaluated for the indels in the YCZ-5 region.

Isolation of melon BAC clones carrying the YCZ-5 region from cucumber

Four primer sets (M1–M4 in Table 1) were designed to amplify different regions from the YCZ-5R clone. PCR products were radiolabelled with ^{32}P and hybridized to blots containing genomic DNA of melon PI414723 digested with either *Dra*I or *Hin*dIII. The cucumber subclone from nucleotides 3,900 to 5,037 hybridized to a low-copy region in melon and was hybridized to high-density filters of the *Hin*dIII and *Eco*RI melon BAC libraries synthesized from the cultivar 'MR-1' (Luo et al. 2001). Single colonies of selected candidate BAC clones were cultured overnight in LB with chloramphenicol and plasmids isolated using the QIAprep Spin Miniprep Kit. For fingerprinting of BAC clones, 3 μg of purified plasmid DNAs were digested with either *Eco*RI or *Hin*dIII, run through a 1% agarose gels, blotted, and hybridized with the YCZ-5R clone. Two BAC clones (MB-H17 and H21) with unique banding patterns were selected, digested separately with three enzymes (*Msp*I, *Pst*I, and *Pvu*II), and fractionated in 0.4% agarose gels. A contig was manually constructed assuming that DNA fragments of the same sizes carried overlapping sequences.

The ends of BAC clones MB-H17 and H21 were sequenced as previously described. Four primer sets (MB-H17-E1 and -E2 and MB-H21-E1 and -E2 in Table 1) were designed to amplify 350–550 bp fragments from the terminal regions of each BAC clone. PCR reactions were conducted with one cycle of 3 min at 94°C, 35 cycles of 30 s at 94°C, 1 min at 62°C, and 45 s at 72°C, and one cycle of 15 min at 72°C. Amplified fragments were purified, radiolabelled with ^{32}P, and hybridized to the blots carrying DNA samples from ZYMV-resistant and susceptible RILs digested separately with 18 restriction enzymes (*Aat*II, *Alu*I, *Apa*I, *Bam*HI, *Ban*I, *Bgl*II, *Dra*I, *Eco*RI, *Hae*III, *Hin*dIII, *Msp*I, *Nsi*I, *Pst*I, *Pvu*II, *Sac*I, *Sal*I, *Xba*I, and *Xho*I). Enzymes revealing restriction-site polymorphisms were used to digest the DNAs from the 49 cucumber RILs and hybridizations of the BAC end clones placed the RFLPs on the cucumber genetic map (Park et al. 2000).

Mapping in melon of the cucumber YCZ-5 region

Two segregating families of melon were used for comparative mapping of ZYMV resistance loci. The first was 112 F_2 plants from the cross of resistant PI414723 with the susceptible 'Dulce' previously evaluated for ZYMV resistance (Danin-Poleg et al. 1997, 2002). The second family consisted of 64 F_6 RILs from the cross of susceptible 'Vedrantais' with PI414723. These RILs were developed by single seed descent without selection from individual F_2 plants; F_4 lines were the gift of Dr. Michel Pitrat (INRA, Montfavet, France) to Seminis Seed Company and were advanced to the F_6 generation. For ZYMV evaluations, cotyledons were inoculated with ZYMV pathotype 0NF (Lecoq and Pitrat 1984) using 10 g of infected leaf tissue ground in 50 ml of 0.03 M Na_2HPO_4 buffer with 0.2% sodium diethyldithiocarbamate trihydrate and 3 g carborundum. After inoculation, plants were maintained in the greenhouse at 20–28°C (days) and 19–22°C (nights). Fourteen days after inoculation, plants were scored for ZYMV reactions using a 1, 3, and 5 scale, where 1 indicated no symptoms, 3 showed some systemic chlorosis and necrosis, and 5 showed systemic vein-clearing, chlorosis, mosaics, and stunting. Scores of 1 and 3 were considered as resistant, 5 as susceptible.

Primer set 8164 (Table 1) was designed from end sequences of melon BAC clone MB-H21-E2 and used to amplify genomic fragments from Dulce, Vedrantais, and PI414723. PCR was performed with 25 ng of melon DNA using one cycle at 94°C for 2 min; 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min; one cycle of 72°C for 30 min, and then held at 4°C. Fragments of 431 and 348 bp were amplified from PI414723 and Dulce, respectively, and were sequenced to confirm homologies. Polymorphic amplicons were scored using 2% agarose gels and DNA from the PI414723 by Dulce segregating family and placed on the melon map (Danin-Poleg et al. 2002) using Mapmaker (Lander et al. 1987) and the Kosambi mapping function. Amplicons from both Ve-

drantais and PI414723 were 431 bp in size. Eleven microlitres of the PCR reactions were digested with *Ava*II, *Cla*I, *Dde*I, *Dpn*II, *Eco*RI, *Hae*III, *Hha*I, *Hinc*II, *Hin*dIII, *Hin*fI, *Hpa*II, *Rsa*I, *Sau*3A, *Taq*I, or *Tru*9I, fractionated in 2.0% agarose gels, stained with EtBr, and visualized under UV light. Segregations of a polymorphic *Hpa*II site and ZYMV resistance were tested using the RILs from Vedrantais by PI414723 and chi-square analysis.

Results and discussion

Cloning of the cucumber genomic region cosegregating with the *zym* locus

The AFLP (E15/M47-F-197) marker cosegregating with the cucumber *zym* locus (Park et al. 2000) was cloned and sequenced to yield a 193-bp fragment. This clone (AFLP1-7) revealed an RFLP with *Hae*III digests of the ZYMV-resistant and susceptible cucumber RILs that cosegregated with *zym* (Park et al. 2000). Hybridization of AFLP1-7 to *Bgl*II-digested genomic DNA revealed 5-kb fragments in both the resistant and susceptible cucumber RILs. These fragments were cloned from partial genomic libraries of the ZYMV-resistant and susceptible RILs F62 and F12, respectively. The genomic clone from the resistant RIL (YCZ-5R) was 5,161 bp (GenBank accession number AY254907) and from the susceptible RIL (YCZ-5S) was 5,133 bp (AY254908). The YCZ-5R and YCZ-5S clones were hybridized to *Hae*III-digested DNAs from the cucumber RILs and cosegregation with the *zym* locus confirmed. The sequence of the YCZ-5 region was AT rich (66.8±1.3%) with multiple tandem repeats. The *zym*-linked AFLP was located on the YCZ-5R clone (Fig. 1); a single nucleotide polymorphism (SNP) located in the selective nucleotides of primer *Mse*I-CAA conditioned the absence of the AFLP. No sequence homology across the YCZ-5 region was found in GenBank using BLASTN searches.

Sequence comparisons between the YCZ-5 alleles from susceptible and resistant cucumber RILs revealed three major indels and a number of SNPs. Primer sets (Table 1) were designed to amplify across two indels [YCZ-SCAR-1 and -2 (Fig. 1)] and to reveal two cleaved amplified polymorphisms (YCZ-CAP-1 and -2); all four polymorphisms cosegregated with *zym* (Fig. 1). We next evaluated a diverse set of cucumber populations for these PCR-based polymorphisms and found that 31 out of 38 cucumber populations possessed the allele of the resistant population TMG1, two PIs possessed the allele of the susceptible population Straight 8, and five PIs possessed both alleles (Table 2). The ZYMV-susceptible population WI 2757 also possessed the marker alleles from TMG-1. Thus although we successfully converted the *zym*-linked AFLP into codominant PCR-based markers, these markers may not be polymorphic between TMG1 and some ZYMV-susceptible cucumbers.

Isolation and mapping of melon BACs carrying the cucumber YCZ-5 region

Hybridizations of the YCZ-5R region between nucleotides 3,900 and 5,037 to DNA gel blots of melon revealed simple patterns (autoradiogram not shown). This region was used to screen filters of melon BAC libraries (Luo et al. 2001). Two clones (MB-H17 and H21) from the *Hin*dIII library showed the most unique patterns after restriction enzyme digests and were used to build a contig of 185.4 kb with 105.5 kb of overlapping sequence and 43.4 and 36.5 kb of flanking sequences. End sequences of BACs MB-H17 and MB-H21 [GenBank accessions BZ892968 (MB-H17-E1), BZ892969 (MB-H17-E2), BZ892970 (MB-H21-E1), and BZ892971 (MB-H21-E2)] were used to design primers amplifying the ends (Table 1). All four ends were AT rich (56–70%) and BLASTN searches revealed no similar sequences in GenBank. Melon BAC amplicons were hybridized to blots carrying cucumber DNAs from the resistant and susceptible RILs digested with several enzymes. The MB-H17 end sequences were highly repeated within the cucumber genome, whereas MB-H21 ends revealed low-copy regions. Probe MB-H21-E1 revealed RFLPs with *Bgl*II and *Pst*I, and this region cosegregated with ZYMV resistance in cucumber (autoradiogram not shown). The MB-H21-E1 region is at least 36.5 kb away from the YCZ-5 homologous region in melon, which is located somewhere within the 105.5 kb overlapping region of the contig. This result demonstrates that physically linked

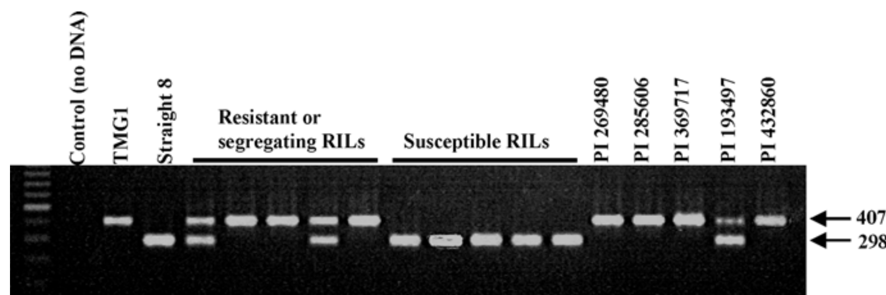

Fig. 1 Agarose gel showing amplicons across a 129-bp indel (YCZ-SCAR-2 in Table 1) in cucumber. TMG 1 and Straight 8 are resistant and susceptible, respectively, to zucchini yellow mosaic virus. RILs are F_6 recombinant inbred lines from TMG1 by Straight 8. PIs are ZYMV-susceptible cucumber plant introductions. Fragment sizes in base pairs are shown on *right*

Table 2 Cucumber accessions evaluated and allele(s) observed for the YCZ-SCAR-2 polymorphism. The accession sources were either commercial seed companies or public institutions. *UW* University of Wisconsin-Madison, *NCRPIS* north central regional plant introduction station, Ames, Iowa. *T* allele from ZYMV-resistant population TMG1, *S* allele from ZYMV-susceptible cultivar Straight 8, *H* Heterozygous

Cultivar or germplasm	Origin	Source	Allele
RZ-1	The Netherlands	Rijk Zwaan	T
RZ-4	The Netherlands	Rijk Zwaan	T
23833	The Netherlands	Leen de Mos	T
23835	The Netherlands	Leen de Mos	T
Dugan	The Netherlands	Nunhems	T
Sandra	The Netherlands	Nunhems	T
9303	The Netherlands	De Ruiter	T
9314	The Netherlands	De Ruiter	T
Gador	The Netherlands	Zaadunie	T
Hallando	The Netherlands	Zaadunie	T
RS-7	The Netherlands	Royal Sluis	T
RS-60	The Netherlands	Royal Sluis	T
Windemoor wonder	USA	UW	T
GY-14	USA	UW	H
Poinsett 86	USA	UW	S
Dasher II	USA	Seminis	H
WI 2757	USA	UW	T
Corona	The Netherlands	De Ruiter	T
Baltus	The Netherlands	De Ruiter	T
Passandra	The Netherlands	Enza Zaden	T
Zudm 1	The Netherlands	UW	T
PI 193497	Ethiopia	NCRPIS	H
PI 200815	Myanmar	NCRPIS	H
PI 267746	India	NCRPIS	H
PI 269480	Pakistan	NCRPIS	T
PI 284699	Sweden	NCRPIS	T
PI 285606	Poland	NCRPIS	T
PI 432860	China	NCRPIS	T
PI 209264	USA	NCRPIS	T
PI 257486	China	NCRPIS	T
PI 18749	Egypt	NCRPIS	T
PI 369717	Poland	NCRPIS	T
PI 183127	India	NCRPIS	S
PI 200818	Myanmar	NCRPIS	T
PI 137836	Iran	NCRPIS	T
PI 227013	Iran	NCRPIS	T
AMES 20664	India	NCRPIS	T
AMES 20881	India	NCRPIS	H
AMES 21026	India	NCRPIS	T

regions in melon show genetic linkage in cucumber, at least near the cucumber *zym* locus.

Comparative mapping of ZYMV-resistance loci in cucumber and melon

Primer set 8164 (Table 1) designed from the melon BAC-end sequence MB-H21-E2 amplified highly similar 431-bp fragments from the original BAC and PI414723. This primer set amplified 348-bp and 431-bp fragments from Dulce and PI414723, respectively, and this size polymorphism mapped in the Dulce by PI414723 population to linkage group III (LOD > 3.0) between markers CMTC160 (19.6 cM) and 427_1.2 (3.4 cM), independently of ZYMV resistance (Danin-Poleg et al. 2002). Linkage group III from the Dulce by PI414723 population corresponds to group XI on the reference map of Perin et al. (2002) near the *Fusarium* resistance gene *Fom–2*. Primer set 8164 (Table 1) amplified 431-bp fragments from both ZYMV-susceptible Vedrantais and PI414723. Restriction enzyme digestions revealed a polymorphic *Hpa*II site that segregated independently of the *Zym* locus using the RILs from Vedrantais and PI414723.

A recessive allele at the *zym* locus conditions ZYMV resistance in cucumber (Provvidenti 1987). ZYMV resistance in melon PI414723 is conditioned by a dominant allele at one locus (*Zym*) (Pitrat and Lecoq 1984) or by dominant alleles at three complementary loci (*Zym-1, Zym-2* and *Zym-3*, Danin-Poleg et al. 1997). These contrasting inheritance modes in melon were established using strains E15 (pathotype 0) and NAT (pathotype 1), respectively, of ZYMV (Lecoq and Pitrat 1984; Desbiez and Lecoq 1997; Lecoq et al. 2002). Our comparative mapping studies establish that cucumber *zym* locus from TMG1 and the *Zym* and *Zym-1* loci from melon PI414723 are likely non-syntenic. Because inoculations of the segregating populations of cucumber (Park et al. 2000) and melon (Pitrat and Lecoq 1984; Danin-Poleg et al. 1997, 2002) were conducted with different ZYMV isolates, the cucumber and melon ZYMV-resistance loci could be specific to different virus strains. However, this is unlikely given that Lecoq et al. (2002) inoculated TMG1 and PI414723 with 173 isolates of ZYMV and observed that TMG1 was resistant to all isolates, whereas PI414723 showed susceptibility to some of these isolates. Lecoq et al. (2002) proposed that the cucumber *zym* locus is a durable source of ZYMV resistance. Independent inheritance of the cucumber *zym* and the melon *Zym*, and *Zym-1* loci may also be the result of genomic rearrangements during the evolution of *Cucumis* species (Schmidt 2002). If the *zym* and *Zym-1* are paralogous loci, there may exist melon loci orthologous to the cucumber *zym* locus. Regardless, the relatively small nuclear genome of cucumber (Arumuganathan and Earle 1991) and the strength of the *zym* locus (Lecoq et al. 2002) make this recessive virus resistance gene an ideal candidate for map-based cloning.

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