Different Haplotypes Encode the Same Protein for Independent Sources of Zucchini Yellow Mosaic Virus Resistance in Cucumber

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Abstract. Cucumber (*Cucumis sativus*) production is negatively affected by Zucchini yellow mosaic virus (ZYMV). Three sources of ZYMV resistance have been commercially deployed and all three resistances are conditioned by a single recessive gene. A vacuolar protein sorting-associated protein 4-like (VPS4-like) gene has been proposed as a candidate for ZYMV resistance from cucumber line A192-18. We analyzed the genomic region across the VPS4-like gene for three independent sources of ZYMV resistance in cucumber (A192-18, Dina-1, and TMG-1) and identified three haplotypes across the coding region and considerable variation in the introns. However, the haplotypes in the coding regions of the VPS4-like gene of A192-18, Dina-1, and TMG-1 encode the same protein sequence, revealing the genetic uniformity for ZYMV resistance from diverse germplasm sources.

The potyviruses (Potyviridae) are especially destructive for cucumber (*Cucumis sativus* L.) and production is often negatively affected by *Watermelon mosaic virus* (WMV), the watermelon strain of the *Papaya ringspot virus* (PRSV-W), and ZYMV. ZYMV is one of the most devastating and economically important virus diseases of cucumber worldwide (Desbiez and Lecoq, 1997; Lecoq et al., 1983; Provvidenti et al., 1984a), and several strains have been differentiated by serological and molecular approaches (Amano et al., 2013; Mahgoub et al., 1997; Nováková et al., 2014; Provvidenti et al., 1984a, 1984b). Resistance to ZYMV has been reported in cucumber. Provvidenti (1987) and Provvidenti et al. (1984a) identified a single recessive gene (*zym*^{TMG-1}) in a selection (TMG-1) from the Chinese cucumber ‘Taichung Mou Gua’, which has been

widely used because it conditioned resistance to all of 173 ZYMV isolates (Lecoq et al., 2002) as well as other potyviruses such as PRSV-W, WMV (Kabelka and Grumet, 1997; Provvidenti, 1985), and *Zucchini yellow fleck virus* (Gilbert-Albertini et al., 1995). The commercial cucumber cultivar Dina-1 (Abul Hayja and Al-Shahwan, 1991; Al-Shahwan et al., 1995) possesses ZYMV resistance that is recessively inherited (*zym*^{Dina-1}) and is allelic or closely linked to *zym*^{TMG-1} (Kabelka et al., 1997). A Japanese slicing cucumber from Saitama Gensyu Iku-seikai Seed Company (A192-18) derived its ZYMV resistance from line G22 and is also inherited as a single recessive gene *zym*^{A192-18} (Amano et al., 2013; Svoboda et al., 2013). Subtle phenotypic differences have been observed for these sources of ZYMV resistance in cucumber. Resistance conditioned by the *zym*^{TMG-1} and *zym*^{Dina-1} alleles constrains systemic movement of the virus through the plant (Ullah and Grumet, 2002). Al-Shahwan et al. (1995) and Provvidenti (1987) reported that ZYMV resistances from TMG-1 and Dina-1

occasionally developed a few chlorotic veins on the lower leaves. Kabelka et al. (1997) found that inoculating cotyledons of Dina-1 resulted in interveinal chlorosis on lower leaves; whereas TMG-1 had no symptoms regardless of inoculant placement. Svoboda et al. (2013) reported A192-18, G22, and TMG-1 as immune to ZYMV-H because no virus was detected by using enzyme-linked immunosorbent assay and electron microscopy.

Amano et al. (2013) proposed a VPS4-like gene as the candidate for *zym*^{A192-18}, which may affect virus movement throughout the plant and it is the first example of association between a vacuolar sorting protein and a recessively inherited virus resistance in plants. In this research, we analyzed sequences of the VPS4-like gene from three sources of ZYMV resistance and two susceptible cucumbers, and revealed that in spite of different haplotypes, all three sources of ZYMV resistance encode the same protein sequence.

Materials and Methods

DNA was isolated from cucumber accessions ZYMV-resistant TMG-1 (Park et al., 2000; Provvidenti, 1987; Provvidenti et al., 1984a) and Dina-1 (Abul Hayja and Al-Shahwan, 1991), ZYMV-susceptible ‘Straight 8’ (St 8), and 49 F₆ recombinant inbred lines (RILs) from a cross of TMG-1 and St 8 (Park et al., 2000) from bulked leaf tissue from at least five plants using the DNeasy® Plant Maxi Kit protocol (Qiagen, Valencia, CA). We genotyped the candidate gene for *zym* in St 8, TMG-1, and the 49 RILs using the cleaved amplified polymorphic (dCAPS-G99A) marker developed by Amano et al. (2013). Digested amplicons were visualized after running through 8% polyacrylamide gels and silver stained. Then, we compared the mean disease severity index scores of RILs (Park et al., 2000) carrying the resistant or susceptible genotype at the CAPS marker using a linear model in analysis of variance in RStudio (2016) version 1.0.136.

Single-end, 100 base pair (bp) sequencing runs for St 8 and TMG-1 were performed on the HiSeq2000 sequencer, version 1, SBS chemistry (Illumina, San Diego, CA). Raw reads were filtered for base quality ≥28 and length ≥41 nucleotides. Reads were mapped to scaffold03904 on chromosome 6 of the Gy 14 reference (Cavagnaro et al., 2010) with Bowtie2, version 2.1.0, (Langmead and Salzberg, 2012)

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Disclaimer: Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1. Four haplotypes across the eight exons of the proposed candidate gene vacuolar protein sorting-associated protein 4-like for the Zucchini yellow mosaic locus relative to the reference sequence of Gy 14.

Haplotype number	Line ^z	Zucchini yellow mosaic virus phenotype ^y	Single-nucleotide polymorphism variant ^x
#1	Gy 14	S	Reference
#2	St 8	S	A90G, G136T, A337C
#3	TMG-1	R	T86C, A90G, G99A, A337C, T1150C
#4	Dina-1	R	T86C, A90C, G99A, A337C
	A192-18	R	

^zGy 14 is the reference sequence.

^yResistant (R) and susceptible (S).

^xSequence alignment is provided in Supplemental Fig. 1.

Table 2. Three amino acid variants across the eight exons of the proposed candidate gene vacuolar protein sorting-associated protein 4-like for the *Zucchini yellow mosaic* locus relative to the reference sequence of Gy 14.

Variant number	Line ^z	<i>Zucchini yellow mosaic virus</i> phenotype ^y	Amino acid variant ^x
#1	Gy 14	S	Reference
#2	St 8	S	E46-*
#3	TMG-1	R	F29S, M33I
	Dina-1	R	
	A192-18	R	

^zGy 14 is the reference sequence.

^yResistant (R) and susceptible (S).

^xAmino-acid alignment is provided in Fig. 1.

and converted to sorted bam format with Samtools version 0.1.18 (Li, 2011).

Polymorphisms relative to the reference were visualized with Integrative Genome Viewer, version 2.3 (Robinson et al., 2011; Thorvaldsdottir et al., 2013). Sequences of St 8 [Sequence Read Archive (SRA) SRX1091587], TMG-1 (SRA SRX1091638), A192-18 (AB819729), and Dina-1 (KY775581) were downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/>) and translated into amino acid (AA) sequences. Alignments of the

TMG-1	MYSNFKEQAIEYVKQAVHEDNAGNYAKA SPLYI NALEYFKTHLKY EKNPKIKEAITQKFT
Dina-1	MYSNFKEQAIEYVKQAVHEDNAGNYAKA SPLYI NALEYFKTHLKY EKNPKIKEAITQKFT
A192-18	MYSNFKEQAIEYVKQAVHEDNAGNYAKA SPLYI NALEYFKTHLKY EKNPKIKEAITQKFT
Gy 14	MYSNFKEQAIEYVKQAVHEDNAGNYAKA FPLYM NALEYFKTHLKY EKNPKIKEAITQKFT
St 8	MYSNFKEQAIEYVKQAVHEDNAGNYAKA FPLYM NALEYFKTHLKY *KNPKIKEAITQKFT

TMG-1	EYLRRAEEIRAVLDDGGPGPASNGDAAVATKPKTKPKDGEGGDGEDPEQAKLRAGLNSAI
Dina-1	EYLRRAEEIRAVLDDGGPGPASNGDAAVATKPKTKPKDGEGGDGEDPEQAKLRAGLNSAI
A192-18	EYLRRAEEIRAVLDDGGPGPASNGDAAVATKPKTKPKDGEGGDGEDPEQAKLRAGLNSAI
Gy 14	EYLRRAEEIRAVLDDGGPGPASNGDAAVATKPKTKPKDGEGGDGEDPEQAKLRAGLNSAI
St 8	EYLRRAEEIRAVLDDGGPGPASNGDAAVATKPKTKPKDGEGGDGEDPEQAKLRAGLNSAI

TMG-1	IREKPDVKWNDVAGLESAKQALQEAVILPVKFPQFFTGKRRPWRAFLLYGPPGTGKSYLA
Dina-1	IREKPDVKWNDVAGLESAKQALQEAVILPVKFPQFFTGKRRPWRAFLLYGPPGTGKSYLA
A192-18	IREKPDVKWNDVAGLESAKQALQEAVILPVKFPQFFTGKRRPWRAFLLYGPPGTGKSYLA
Gy 14	IREKPDVKWNDVAGLESAKQALQEAVILPVKFPQFFTGKRRPWRAFLLYGPPGTGKSYLA
St 8	IREKPDVKWNDVAGLESAKQALQEAVILPVKFPQFFTGKRRPWRAFLLYGPPGTGKSYLA

TMG-1	KAVATEADSTFFSISSSDLVSKWMESEKLVSNLFQMARDSAPSIIFIDEIDSLCGQRGE
Dina-1	KAVATEADSTFFSISSSDLVSKWMESEKLVSNLFQMARDSAPSIIFIDEIDSLCGQRGE
A192-18	KAVATEADSTFFSISSSDLVSKWMESEKLVSNLFQMARDSAPSIIFIDEIDSLCGQRGE
Gy 14	KAVATEADSTFFSISSSDLVSKWMESEKLVSNLFQMARDSAPSIIFIDEIDSLCGQRGE
St 8	KAVATEADSTFFSISSSDLVSKWMESEKLVSNLFQMARDSAPSIIFIDEIDSLCGQRGE

TMG-1	GNESEASRRIKTELLVQMVGHNQKVLVLAATNTPYALDQAIARRRFDKRIYIPLPDLK
Dina-1	GNESEASRRIKTELLVQMVGHNQKVLVLAATNTPYALDQAIARRRFDKRIYIPLPDLK
A192-18	GNESEASRRIKTELLVQMVGHNQKVLVLAATNTPYALDQAIARRRFDKRIYIPLPDLK
Gy 14	GNESEASRRIKTELLVQMVGHNQKVLVLAATNTPYALDQAIARRRFDKRIYIPLPDLK
St 8	GNESEASRRIKTELLVQMVGHNQKVLVLAATNTPYALDQAIARRRFDKRIYIPLPDLK

TMG-1	ARQHMFVKVHLGDTPHNLTEADFNELARKTDGFSGSDISVCVKDVLFEFVRKTQDAMFFIM
Dina-1	ARQHMFVKVHLGDTPHNLTEADFNELARKTDGFSGSDISVCVKDVLFEFVRKTQDAMFFIM
A192-18	ARQHMFVKVHLGDTPHNLTEADFNELARKTDGFSGSDISVCVKDVLFEFVRKTQDAMFFIM
Gy 14	ARQHMFVKVHLGDTPHNLTEADFNELARKTDGFSGSDISVCVKDVLFEFVRKTQDAMFFIM
St 8	ARQHMFVKVHLGDTPHNLTEADFNELARKTDGFSGSDISVCVKDVLFEFVRKTQDAMFFIM

TMG-1	TPDGMWVPCGPKQQGAVQISMQELAAKGLASKILPPPITRTDFDKVLARQRPTVSKSDLE
Dina-1	TPDGMWVPCGPKQQGAVQISMQELAAKGLASKILPPPITRTDFDKVLARQRPTVSKSDLE
A192-18	TPDGMWVPCGPKQQGAVQISMQELAAKGLASKILPPPITRTDFDKVLARQRPTVSKSDLE
Gy 14	TPDGMWVPCGPKQQGAVQISMQELAAKGLASKILPPPITRTDFDKVLARQRPTVSKSDLE
St 8	TPDGMWVPCGPKQQGAVQISMQELAAKGLASKILPPPITRTDFDKVLARQRPTVSKSDLE

TMG-1	IHERFTKEFGEEG
Dina-1	IHERFTKEFGEEG
A192-18	IHERFTKEFGEEG
Gy 14	IHERFTKEFGEEG
St 8	IHERFTKEFGEEG

Fig. 1. CLUSTAL O (version 1.2.4) alignment of predicted amino-acid sequences of five cucumber accessions. TMG-1, Dina-1, and A192-18 are resistant to *Zucchini yellow mosaic virus*, and Gy 14 and St 8 are susceptible. Polymorphisms are shown with bold text.

coding and AA sequences of Gy 14 (Cavagnaro et al., 2010), St 8, TMG-1, A192-18, and Dina-1 were performed using CLUSTAL O, version 1.2.4 (Li et al., 2015).

The polymerase chain reaction (PCR) protocol used to amplify the ~5-kb genomic region from Dina-1 carrying the candidate gene for *zym* consisted of two separately prepared solutions, one which had the template DNA and the other with Taq polymerase. For a single reaction, solution 1 contained 4 µL of 2.5 mM dNTPs, 2 µL of combined forward and reverse primers (5'-TTCTTTGCTTGATTGAGATAGTCAAAG and 5'-CAACTCTGATGGGTGTATGCA) (20 µM/L), 17 µL of double-distilled (dd) H₂O, and 2 µL of 20 ng/µL template DNA. Solution 2 had 19.25 µL of dd H₂O, 5 µL of 10× Expand High Fidelity Buffer with 15 mM MgCl₂, and 0.75 µL of Expand High Fidelity Enzyme Mix (Roche, Madison, WI). The two mixes were combined into a single tube with PCR conditions of 94 °C for 2 min, 30 cycles of 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 4.2 min, and 72 °C for 7 min. Amplicons were visualized in 0.6% agarose gels and remaining reaction was purified to a concentration of 50 ng/µL using the NucleoSpin® PCR clean-up kit (Macherey-Nagel, Düren, Germany). Amplicons were Sanger sequenced using forward primers (Supplemental Table 1) and the reverse primer listed previously by Functional Biosciences (Madison, WI), and reads were analyzed using SnapGene software version 3.3.3 (GSL Biotech, Chicago, IL).

Results and Discussion

The dCAPS-G99A marker (Amano et al., 2013) was significantly ($P = 2.2 \times 10^{-16}$) associated with reactions to ZYMV across the RILs from St 8 × TMG-1 (Park et al., 2000), indicating that *zym*^{TMG-1} and *zym*^{A192-18} are alleles at the same locus or tightly linked loci. Kabelka et al. (1997) previously reported that *zym*^{Dina-1} and *zym*^{TMG-1} are also allelic or very closely linked. Together these results indicate that these three independent sources of ZYMV resistance may be one in the same.

Amano et al. (2013) reported that the candidate VPS4-like gene has eight exons and seven introns. We conducted a BLAST search using the Cucurbit Genomics Database (<http://www.icugi.org/>) of both the genomic and coding regions of the candidate VPS4-like gene in cucumbers 9930 and Gy 14, and only one highly similar sequence was identified and no evidence of a duplicated gene was found. However, other gene(s) with minor effects on ZYMV resistance could exist. Further evaluation of the sequences revealed substantial variation in the VPS4-like gene among the cucumber accessions Gy 14, Dina-1, St 8, and TMG-1, with most polymorphisms in the intronic regions. Across the eight exons, six single-nucleotide polymorphisms (SNPs) define four haplotypes corresponding to Gy 14; St 8; TMG-1 and Dina-1; and A192-18 (Table 1). Across the seven introns, we identified 29 SNPs and four indels (insertions or deletions). SNPs were

found in all introns (one, twelve, three, four, two, one, and six SNPs in introns one through seven, respectively). In the first intron, Dina-1 has a 37-bp duplication. In the third intron, there are four copies of a seven-bp region in Dina-1 vs. three copies in the other accessions. There are also deleted regions in intron 3, a deletion of 63-bp in Dina-1 and a 33-bp deletion at the same position in TMG-1. Intron seven carries a 3-bp deletion in Dina-1, St 8, and TMG-1 relative to Gy 14.

The four haplotypes in the coding region for the VPS4-like gene (Table 1; Supplemental Fig. 1) encode three protein variants (Table 2; Fig. 1). The two key SNPs (T86C and G99A), which translate into two AA changes (F29S and M33I) in *zym*^{A192-18} allele (Amano et al., 2013), are also present in the *zym*^{TMG-1} and *zym*^{Dina-1} alleles (Table 2). ZYMV-susceptible Gy 14 and St 8 do not possess these two key SNPs, and St 8 has a unique SNP (G136T) that results in a premature stop codon (E46-*) (Table 2). Although A192-18, Dina-1, and TMG-1 possess different haplotypes at the VPS4-like candidate gene for *zym* and do not trace back to the same source population, all three haplotypes encode the same protein sequence. The most parsimonious explanation would be that the two key SNPs (T86C and G99A) occurred in a common progenitor of the original sources of ZYMV resistance introduced into Dina-1, G22, and TMG-1. Later polymorphisms may have accumulated in the coding and/or intronic regions of the gene to produce the haplotype diversity that we uncovered. Our research documents that independently identified and introgressed sources of ZYMV resistance in cucumber possess the same genetic basis and undesirable genetic uniformity exists in cucumber for potyvirus resistance that could potentially be overcome by the viruses.

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Supplemental Table 1. List of primers used for sequencing of the vacuolar protein sorting-associated protein 4-like gene.

Name	Forward
AR1.5	CCGGCTTCCAATGGAGATG
AR3	GAGGCTGACTCGACATTTTCA
AR3.5	GTCGTCATTGCAATTCTTTTGTCT
AR5	CTTGAACTTTTACGGTTCCTGGC
AR5.5	CTCATTGCTGCTGATCTACATCC
AR7	GGGCTTAGGGGCTGTAACT
AR7.5	TCGTCACAAGTGCGTGATTC
AR9	CCATGAACTTTGCTAATATTCGTAGAT

TMG-1	ATGTATAGCAATTTCAAGGAGCAAGCTATTGAATACGTGAAGCAGGCGGTACATGAAGAT
Dina-1	ATGTATAGCAATTTCAAGGAGCAAGCTATTGAATACGTGAAGCAGGCGGTACATGAAGAT
A192-18	ATGTATAGCAATTTCAAGGAGCAAGCTATTGAATACGTGAAGCAGGCGGTACATGAAGAT
Gy 14	ATGTATAGCAATTTCAAGGAGCAAGCTATTGAATACGTGAAGCAGGCGGTACATGAAGAT
St 8	ATGTATAGCAATTTCAAGGAGCAAGCTATTGAATACGTGAAGCAGGCGGTACATGAAGAT

TMG-1	AATGCTGGCAATTACGCAAAAGCCT CTCCG CTGTATAT AAACGC CTTGGAGTACTTCAAA
Dina-1	AATGCTGGCAATTACGCAAAAGCCT CTCCG CTGTATAT AAACGC CTTGGAGTACTTCAAA
A192-18	AATGCTGGCAATTACGCAAAAGCCT CTCC C CTGTATAT AAACGC CTTGGAGTACTTCAAA
Gy 14	AATGCTGGCAATTACGCAAAAGCCT TTCC A CTGTATAT GAACGC CTTGGAGTACTTCAAA
St 8	AATGCTGGCAATTACGCAAAAGCCT TTCCG CTGTATAT GAACGC CTTGGAGTACTTCAAA
	***** ** *
TMG-1	ACTCATTTGAAGTAT GAGAAAAA TCCCAAGATCAAGGAAGCTATTACCCAGAAATTCACC
Dina-1	ACTCATTTGAAGTAT GAGAAAAA TCCCAAGATCAAGGAAGCTATTACCCAGAAATTCACC
A192-18	ACTCATTTGAAGTAT GAGAAAAA TCCCAAGATCAAGGAAGCTATTACCCAGAAATTCACC
Gy 14	ACTCATTTGAAGTAT GAGAAAAA TCCCAAGATCAAGGAAGCTATTACCCAGAAATTCACC
St 8	ACTCATTTGAAGTAT TAGAAAAA TCCCAAGATCAAGGAAGCTATTACCCAGAAATTCACC

TMG-1	GAGTATTTGCGCCGGGCTGAGGAGATACGTGCTGTTTTGGATGATGGCGGTCTGGTCCG
Dina-1	GAGTATTTGCGCCGGGCTGAGGAGATACGTGCTGTTTTGGATGATGGCGGTCTGGTCCG
A192-18	GAGTATTTGCGCCGGGCTGAGGAGATACGTGCTGTTTTGGATGATGGCGGTCTGGTCCG
Gy 14	GAGTATTTGCGCCGGGCTGAGGAGATACGTGCTGTTTTGGATGATGGCGGTCTGGTCCG
St 8	GAGTATTTGCGCCGGGCTGAGGAGATACGTGCTGTTTTGGATGATGGCGGTCTGGTCCG

TMG-1	GCTTCCAATGGAGATGCAGCTGTTGCAACTAAACCCAAGACGAAACCTAAGGATGGAGAA
Dina-1	GCTTCCAATGGAGATGCAGCTGTTGCAACTAAACCCAAGACGAAACCTAAGGATGGAGAA
A192-18	GCTTCCAATGGAGATGCAGCTGTTGCAACTAAACCCAAGACGAAACCTAAGGATGGAGAA
Gy 14	GCTTCCAATGGAGATGCAGCTGTTGCAACTAAACCCAAGACGAAACCTAAGGATGGAGAA
St 8	GCTTCCAATGGAGATGCAGCTGTTGCAACTAAACCCAAGACGAAACCTAAGGATGGAGAA

TMG-1	GGAGGAGATGGAGAGGATCCAGAACAGGCTAAGTT ACGGG CTGGTCTTAATTCCGCAATC
Dina-1	GGAGGAGATGGAGAGGATCCAGAACAGGCTAAGTT ACGGG CTGGTCTTAATTCCGCAATC
A192-18	GGAGGAGATGGAGAGGATCCAGAACAGGCTAAGTT ACGGG CTGGTCTTAATTCCGCAATC
Gy 14	GGAGGAGATGGAGAGGATCCAGAACAGGCTAAGTT AAGGG CTGGTCTTAATTCCGCAATC
St 8	GGAGGAGATGGAGAGGATCCAGAACAGGCTAAGTT ACGGG CTGGTCTTAATTCCGCAATC

TMG-1	ATAAGGGAGAAACCGGATGTTAAGTGGAAACGATGTTGCTGGATTGGAGAGCGCCAAGCAG
Dina-1	ATAAGGGAGAAACCGGATGTTAAGTGGAAACGATGTTGCTGGATTGGAGAGCGCCAAGCAG
A192-18	ATAAGGGAGAAACCGGATGTTAAGTGGAAACGATGTTGCTGGATTGGAGAGCGCCAAGCAG
Gy 14	ATAAGGGAGAAACCGGATGTTAAGTGGAAACGATGTTGCTGGATTGGAGAGCGCCAAGCAG
St 8	ATAAGGGAGAAACCGGATGTTAAGTGGAAACGATGTTGCTGGATTGGAGAGCGCCAAGCAG

TMG-1	GCGTTGCAAGAGGCTGTAATATTACCCGTTAAGTTCCCCAGTTCTTTACTGGTAAGAGG
Dina-1	GCGTTGCAAGAGGCTGTAATATTACCCGTTAAGTTCCCCAGTTCTTTACTGGTAAGAGG
A192-18	GCGTTGCAAGAGGCTGTAATATTACCCGTTAAGTTCCCCAGTTCTTTACTGGTAAGAGG
Gy 14	GCGTTGCAAGAGGCTGTAATATTACCCGTTAAGTTCCCCAGTTCTTTACTGGTAAGAGG
St 8	GCGTTGCAAGAGGCTGTAATATTACCCGTTAAGTTCCCCAGTTCTTTACTGGTAAGAGG

Supplemental Fig. 1. CLUSTAL O (version 1.2.4) alignment of nucleotide coding sequences of five cucumber accessions. TMG-1, Dina-1, and A192-18 are resistant to *Zucchini yellow mosaic virus*, and Gy 14 and St 8 are susceptible. Polymorphisms are shown with bold text.

TMG-1	CGACCATGGCGAGCTTTCTTGTTATATGGGCCTCCTGGAAGTGGAAAGTCATACTTGGCA
Dina-1	CGACCATGGCGAGCTTTCTTGTTATATGGGCCTCCTGGAAGTGGAAAGTCATACTTGGCA
A192-18	CGACCATGGCGAGCTTTCTTGTTATATGGGCCTCCTGGAAGTGGAAAGTCATACTTGGCA
Gy 14	CGACCATGGCGAGCTTTCTTGTTATATGGGCCTCCTGGAAGTGGAAAGTCATACTTGGCA
St 8	CGACCATGGCGAGCTTTCTTGTTATATGGGCCTCCTGGAAGTGGAAAGTCATACTTGGCA

TMG-1	AAGGCTGTTGCGACAGAGGCTGACTCGACATTTTTCAGCATTTCTTCTTCGGACCTTGTC
Dina-1	AAGGCTGTTGCGACAGAGGCTGACTCGACATTTTTCAGCATTTCTTCTTCGGACCTTGTC
A192-18	AAGGCTGTTGCGACAGAGGCTGACTCGACATTTTTCAGCATTTCTTCTTCGGACCTTGTC
Gy 14	AAGGCTGTTGCGACAGAGGCTGACTCGACATTTTTCAGCATTTCTTCTTCGGACCTTGTC
St 8	AAGGCTGTTGCGACAGAGGCTGACTCGACATTTTTCAGCATTTCTTCTTCGGACCTTGTC

TMG-1	TCGAAATGGATGGGTGAAAGCGAGAAGCTAGTTTCAAATCTTTTCAAATGGCTCGTGAT
Dina-1	TCGAAATGGATGGGTGAAAGCGAGAAGCTAGTTTCAAATCTTTTCAAATGGCTCGTGAT
A192-18	TCGAAATGGATGGGTGAAAGCGAGAAGCTAGTTTCAAATCTTTTCAAATGGCTCGTGAT
Gy 14	TCGAAATGGATGGGTGAAAGCGAGAAGCTAGTTTCAAATCTTTTCAAATGGCTCGTGAT
St 8	TCGAAATGGATGGGTGAAAGCGAGAAGCTAGTTTCAAATCTTTTCAAATGGCTCGTGAT

TMG-1	AGCGCGCCTTCTATCATCTTTATTGATGAAATAGATTTCGTTGTGTGGTCAACGAGGTGAA
Dina-1	AGCGCGCCTTCTATCATCTTTATTGATGAAATAGATTTCGTTGTGTGGTCAACGAGGTGAA
A192-18	AGCGCGCCTTCTATCATCTTTATTGATGAAATAGATTTCGTTGTGTGGTCAACGAGGTGAA
Gy 14	AGCGCGCCTTCTATCATCTTTATTGATGAAATAGATTTCGTTGTGTGGTCAACGAGGTGAA
St 8	AGCGCGCCTTCTATCATCTTTATTGATGAAATAGATTTCGTTGTGTGGTCAACGAGGTGAA

TMG-1	GGTAATGAAAGTGAAGCTTCAAGACGCATTAAGACTGAACTTCTTGTGCAATGCAGGGT
Dina-1	GGTAATGAAAGTGAAGCTTCAAGACGCATTAAGACTGAACTTCTTGTGCAATGCAGGGT
A192-18	GGTAATGAAAGTGAAGCTTCAAGACGCATTAAGACTGAACTTCTTGTGCAATGCAGGGT
Gy 14	GGTAATGAAAGTGAAGCTTCAAGACGCATTAAGACTGAACTTCTTGTGCAATGCAGGGT
St 8	GGTAATGAAAGTGAAGCTTCAAGACGCATTAAGACTGAACTTCTTGTGCAATGCAGGGT

TMG-1	GTAGGACACAATGATCAAAAGGTTCTTGTTCTTGCTGCAACAAATACTCCCTATGCTTTG
Dina-1	GTAGGACACAATGATCAAAAGGTTCTTGTTCTTGCTGCAACAAATACTCCCTATGCTTTG
A192-18	GTAGGACACAATGATCAAAAGGTTCTTGTTCTTGCTGCAACAAATACTCCCTATGCTTTG
Gy 14	GTAGGACACAATGATCAAAAGGTTCTTGTTCTTGCTGCAACAAATACTCCCTATGCTTTG
St 8	GTAGGACACAATGATCAAAAGGTTCTTGTTCTTGCTGCAACAAATACTCCCTATGCTTTG

TMG-1	GATCAGGCCATTTCGTCGACGATTTCGACAAGCGGATATACATTCTCTACCTGATTTGAAA
Dina-1	GATCAGGCCATTTCGTCGACGATTTCGACAAGCGGATATACATTCTCTACCTGATTTGAAA
A192-18	GATCAGGCCATTTCGTCGACGATTTCGACAAGCGGATATACATTCTCTACCTGATTTGAAA
Gy 14	GATCAGGCCATTTCGTCGACGATTTCGACAAGCGGATATACATTCTCTACCTGATTTGAAA
St 8	GATCAGGCCATTTCGTCGACGATTTCGACAAGCGGATATACATTCTCTACCTGATTTGAAA

TMG-1	GCCAGACAGCACATGTTCAAAGTGCATCTGGGGGATACACCACATAATTTAACAGAAGCA
Dina-1	GCCAGACAGCACATGTTCAAAGTGCATCTGGGGGATACACCACATAATTTAACAGAAGCA
A192-18	GCCAGACAGCACATGTTCAAAGTGCATCTGGGGGATACACCACATAATTTAACAGAAGCA
Gy 14	GCCAGACAGCACATGTTCAAAGTGCATCTGGGGGATACACCACATAATTTAACAGAAGCA
St 8	GCCAGACAGCACATGTTCAAAGTGCATCTGGGGGATACACCACATAATTTAACAGAAGCA

Fig. 1. continued

TMG-1	GATTTTAAAACTTAGCACGCAAGACAGATGGTTTCTCGGGTTCAGATATTTAGTTTGT
Dina-1	GATTTTAAAACTTAGCACGCAAGACAGATGGTTTCTCGGGTTCAGATATTTAGTTTGT
A192-18	GATTTTAAAACTTAGCACGCAAGACAGATGGTTTCTCGGGTTCAGATATTTAGTTTGT
Gy 14	GATTTTAAAACTTAGCACGCAAGACAGATGGTTTCTCGGGTTCAGATATTTAGTTTGT
St 8	GATTTTAAAACTTAGCACGCAAGACAGATGGTTTCTCGGGTTCAGATATTTAGTTTGT

TMG-1	GTGAAGGATGTGCTCTTTGAACCTGTACGTAAAACTCAAGATGCTATGTTCTTCATTATG
Dina-1	GTGAAGGATGTGCTCTTTGAACCTGTACGTAAAACTCAAGATGCTATGTTCTTCATTATG
A192-18	GTGAAGGATGTGCTCTTTGAACCTGTACGTAAAACTCAAGATGCTATGTTCTTCATTATG
Gy 14	GTGAAGGATGTGCTCTTTGAACCTGTACGTAAAACTCAAGATGCTATGTTCTTCATTATG
St 8	GTGAAGGATGTGCTCTTTGAACCTGTACGTAAAACTCAAGATGCTATGTTCTTCATTATG

TMG-1	ACTCCTGATGGTATGTGGGTACCTTGTGGACCAAAGCAACAAGGAGCTGTCCAAATTAGC
Dina-1	ACTCCTGATGGTATGTGGGTACCTTGTGGACCAAAGCAACAAGGAGCTGTCCAAATTAGC
A192-18	ACTCCTGATGGTATGTGGGTACCTTGTGGACCAAAGCAACAAGGAGCTGTCCAAATTAGC
Gy 14	ACTCCTGATGGTATGTGGGTACCTTGTGGACCAAAGCAACAAGGAGCTGTCCAAATTAGC
St 8	ACTCCTGATGGTATGTGGGTACCTTGTGGACCAAAGCAACAAGGAGCTGTCCAAATTAGC

TMG-1	ATGCAAGAGCTAGCAGCCAAAGGACTTGCCTCAAAGATTCTTCCTCCACCAATTACAAGA
Dina-1	ATGCAAGAGCTAGCAGCCAAAGGACTTGCCTCAAAGATTCTTCCTCCACCAATTACAAGA
A192-18	ATGCAAGAGCTAGCAGCCAAAGGACTTGCCTCAAAGATTCTTCCTCCACCAATTACAAGA
Gy 14	ATGCAAGAGCTAGCAGCCAAAGGACTTGCCTCAAAGATTCTTCCTCCACCAATTACAAGA
St 8	ATGCAAGAGCTAGCAGCCAAAGGACTTGCCTCAAAGATTCTTCCTCCACCAATTACAAGA

TMG-1	ACAGATTTTGACAAGGTCTTAGCTAGACAAAGGCCTACGGTGAGTAAATCTGATCTGGAA
Dina-1	ACAGATTTTGACAAGGTCTTAGCTAGACAAAGGCCTACGGTGAGTAAATCTGATCTGGAA
A192-18	ACAGATTTTGACAAGGTCTTAGCTAGACAAAGGCCTACGGTGAGTAAATCTGATCTGGAA
Gy 14	ACAGATTTTGACAAGGTCTTAGCTAGACAAAGGCCTACGGTGAGTAAATCTGATCTGGAA
St 8	ACAGATTTTGACAAGGTCTTAGCTAGACAAAGGCCTACGGTGAGTAAATCTGATCTGGAA

TMG-1	ATTCATGAAAGGTTTACAAAAGAGTTTGGGGAGGAAGGTTGA
Dina-1	ATTCATGAAAGGTTTACAAAAGAGTTTGGGGAGGAAGGTTGA
A192-18	ATTCATGAAAGGTTTACAAAAGAGTTTGGGGAGGAAGGTTGA
Gy 14	ATTCATGAAAGGTTTACAAAAGAGTTTGGGGAGGAAGGTTGA
St 8	ATTCATGAAAGGTTTACAAAAGAGTTTGGGGAGGAAGGTTGA

Fig. 1. continued