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QTL Mapping of *Cucurbit yellow stunting disorder virus* Resistance in Melon Accession PI 313970

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***Abstract. Cucurbit yellow stunting disorder virus* (CYSDV) is a devastating viral disease of melon that can cause significant yield and quality losses. This disease has recently emerged as a major concern in the southwest United States and major melon-growing regions across the world. Coinfection of melon by *Cucurbit chlorotic yellows virus* (CCYV) was recognized in Imperial Valley and neighboring production areas of California and Arizona in 2018, but its importance remains largely unknown. Identifying and deploying CYSDV resistance from elite germplasm is an economical and effective way to manage the disease. A $F_{2:3}$ population was developed from a cross of susceptible 'Top Mark' with CYSDV-resistant PI 313970, which was shown to possess a single recessive gene for resistance to CYSDV. The $F_{2:3}$ population was phenotyped in the field in response to natural, mixed infections by the two viruses, CYSDV and CCYV in the Fall melon seasons of 2018 and 2019. Phenotypic data (foliar yellowing) from both years were not useful for mapping CYSDV resistance quantitative trait loci (QTL), as PI 313970 and CYSDV-resistant $F_{2:3}$ plants exhibited yellowing symptoms from CCYV coinfection. QTL analysis of the relative titer of CYSDV calculated from reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) data identified one locus on chromosome 3 at the physical location of S5-28,571,859 bp that explained 20% of virus titer variation in 2018 but was undetected in 2019. A locus on chromosome 5 between S5-20,880,639 to S5-22,217,535 bp explained 16% and 35% of the variation in CYSDV titer in 2018 and 2019, respectively. One or both of the markers were present in six of 10 putative melon CYSDV resistance sources. Markers flanking the 2019 QTL were developed and can be used in marker-assisted breeding of CYSDV-resistant melons.**

Melon (*Cucumis melo* L.; $2n = 2x = 24$) is an important horticultural crop worldwide and in the United States, with a total production of 872,080 t in 2018, amounting to 2.2% of the total world production (FAOSTAT, 2020). Arizona and California are the major producers of melons in the United States (USDA-NASS, 2020), comprising 89% of reported U.S. cantaloupe production in 2019 (645,850 t). California accounted for 100% of reported U.S. honeydew production in 2019 (143,796 t). Fall season melon production (for October–December harvest) in Arizona and the southern inland portions of California (Imperial Valley, Coachella Valley and Palo Verde Valley) have been devastated by the combination of sweetpotato whitefly (*Bemisia tabaci*) and CYSDV (family *Closteroviridae*, genus *Crinivirus*) since 2006 (Gonzalez et al., 1992; Kuo et al., 2007).

CYSDV infection produces yellowing and interveinal leaf chlorosis. Symptoms generally occur first in older (basal) leaves and spread acropetally and can cause significant yield loss in terms of fruit quantity and quality (Célix et al., 1996; Wintermantel et al., 2009). CYSDV can infect a wide host range in seven plant families, including Cucurbitaceae, of which the perrenial buffalo gourd (*Cucurbita foetidissima*) is native to the southwestern desert United States (Wintermantel et al., 2009). CYSDV is transmitted by sweetpotato whitefly biotypes A, B, and Q (Célix et al., 1996; Wisler et al., 1998) but not transmitted mechanically, through seeds or any other known means.

CYSDV was first identified in melon in 1982 in the United Arab Emirates (Hassan and Duffus, 1990) and then found in Spain (Célix et al., 1996). Since then, it has been reported in 23 other countries in Africa, Asia, Europe, and North America (see https://www.cabi.org/isc/datasheet/17070).

In the Americas, CYSDV was first reported in the United States (Texas) and Mexico in 1999 (Kao et al., 2000). It devastated fall melon fields in California and Arizona in 2006 (Kuo et al., 2007). It occurred in Florida in 2007 and Georgia in 2017 but has not been as severe as in California (Gadhave et al., 2018; Polston et al., 2008).

Cucurbit chlorotic yellows virus (CCYV; genus *Crinivirus*, family *Closteroviridae*) was recently reported to have been present since 2014 in Imperial Valley and neighboring desert melon production areas of Yuma, AZ, and Blythe, CA (Wintermantel et al., 2019). It is vectored by the same species of whiteflies and can coinfect with CYSDV (Wintermantel et al., 2019). Symptoms of these two viruses on melon are virtually identical and require molecular tools to differentiate them in mixed infections. Currently, both viruses are major threats to melon production in the U.S. desert southwest. The undetected presence of CCYV in Imperial Valley confounded field selection for resistance to CYSDV starting in 2014. *Squash vein yellowing virus* (SqVYV; genus *Ipomovirus*, family Potyviridae), a whitefly-transmitted virus, was found in Imperial Valley in Fall 2014 but caused little damage and has not persisted (Batuman et al., 2015).

CYSDV can, in principle, be managed by cultural practices such as removing or burning infected plants, crop rotation, or chemical suppression of whitefly. The most effective method of managing this disease is, however, host resistance. Development of CYSDV-resistant, horticulturally acceptable and adapted germplasm begins with identifying resistance sources, characterizing inheritance of resistance, identifying QTL and markers for selection, and elucidating mechanisms of resistance. The high numbers of whiteflies in the desert southwest United States complicates evaluation of potential CYSDV resistance sources because whitefly feeding by adults and immatures can be sufficient to stunt and kill melon plants in the absence of virus pressure (Gonzalez et al., 1992; McCreight et al., 1995).

No CYSDV-resistant commercial melon cultivars are currently available in the United States. The initial screening of melon varieties and accessions for resistance to CYSDV was carried out in the United Arab Emirates, in a series of four, naturally infected field tests of 1455 lines in 1988 (975 lines) and 1989 (480 lines). It included 968 U.S. Department of Agriculture, National Plant Germplasm System accessions (Hassan et al., 1991). None were highly resistant or immune, although PI 403994 (Meloncillo), which was collected in Colombia, exhibited significantly less yellowing than susceptible 'Ananas'. Expression of resistance in these tests may have been confounded with whitefly feeding damage due to the "high population of viruliferous whiteflies which were

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continuously observed on the plants in all trials'' (Hassan et al., 1991).

Apparently higher level resistance to a Spanish CYSDV strain was first identified in two accessions of African origin, TGR 1551, a.k.a. PI 412420, and TGR 1937, a.k.a. PI 482431 (López-Sesé and Gómez-Guillamón, 2000). Resistance to the California and Arizona CYSDV strain was identified in several accessions of Indian origin, most notably PI 313970 (McCreight and Wintermantel, 2008; McCreight et al., 2016). Resistance in TGR 1551 was attributed to a single dominant gene (López-Sesé and Gómez-Guillamón, 2000), whereas resistance exhibited by PI 313970 was due to a single recessive gene (McCreight and Wintermantel, 2011). Subsequent studies in Texas and Imperial Valley, CA, demonstrated that CYSDV resistance in TGR 1551 was recessive and allelic to that in PI 313970 (McCreight et al., 2017). CYSDV resistance in other potential sources is also single gene recessive (McCreight et al., 2015, 2016), likely allelic with TGR 1551 and PI 313970. Two CYSDV resistance QTL in the same proximity on chromosome 5 were recently identified in TGR 1551 based on disease reaction phenotype and virus titer (Pérez-de-Castro et al., 2020). The QTL identified with phenotypic variation colocalized between S5-6,810,744 and 24,296,585 bp and explained 27% to 62% of the phenotypic variation. The QTL identified with virus titer was localized between 24,791,006 and 27,121,114 bp and accounted for 49% to 53% of the titer variation.

We report here the mapping of resistance QTL against CYSDV in PI 313970, as well as identification of markers closely linked to one of the QTL in PI 313970 and other sources of CYSDV resistance for potential use in marker-assisted selection in the development of CYSDV-resistant melons.

Materials and Methods

Plant materials. 'Top Mark' (TM) and PI 313970 were crossed to develop a $F_{2:3}$ mapping population. 'Top Mark' is a western shipper-type cantaloupe, *C. melo* Group Cantalupensis Subgroup American Western (Pitrat, 2016) susceptible to CYSDV and CCYV. PI 313970, a land race (*C. melo* Group Acidulus) (Pitrat, 2016) from India is resistant to CYSDV (McCreight and Wintermantel, 2011) and susceptible to CCYV (unpublished data).

Disease phenotyping. Virus yellowing was assessed in naturally infected, Fall season field tests at the University of California, Desert Research and Extension Center, Holtville, CA, in 2018 and 2019. The experiments were arranged in a RCBD design with three replications. The plants were grown using standard commercial practices. Irrigation water was provided as needed via subsurface drip irrigation (20 cm depth). Admire (imidacloprid) was applied once at the label rate via drip 5 d after the initial irrigation. Plots were 3 m long on 2-m wide beds, with two seeds per plot, spaced ≈1.5 m apart. Adjacent beds were planted in 2018, whereas every other bed was planted in 2019 to facilitate sampling.

The 2018 experiment was planted on 16 Aug. and evaluated for disease reaction 17–21 Oct., ≈5 weeks postplanting (wpp), and included 200 $F_{2:3}$ TM × PI 313970 families. The 2019 test was planted 14 Aug. and evaluated for disease reaction 20–21 Oct., ≈10 wpp, and included 100 $F_{2:3}$ TM × PI 313970 families. Plants were individually evaluated for virus yellowing symptoms using a visual 1 (0% to 10%) to 10 (91% to 100% symptomatic foliage) scale.

Total RNA extraction and complementary DNA synthesis. Yellowing leaves were collected from at least one replication. The 2018 tests were sampled 17–21 Sept., ≈5 wpp. The 2019 test was sampled 23–25 Sept., ≈6 wpp. Leaf samples were stored in a cold room until the leaf samples were collected for RNA extraction. Approximately 100 mg of leaf sample from each $F_{2:3}$ family was placed into a well of a 96-well plate (Denville Scientific Inc., Metuchen, NJ). One stainless steel bead was added to each well and samples were lyophilized for 48 h at –10 °C. The samples were ground to fine powder using 5/32″ (3.97 mm) stainless steel balls in a Retsch Mixer Mill (Retsch, Inc., Newtown, PA). Total RNA was extracted from each sample using the MagMax Plant RNA extraction kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA) in KingFisher Flex Magnetic Particle Processor (ThermoFisher Scientific, Waltham, MA) and the complementary DNA (cDNA) was synthesized using iScript cDNA synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA) following the manufacturer's protocol.

Primer design and RT-qPCR. The CYSDV-specific primers were designed targeting the RNA dependent (RdRp) gene on RNA1 (CYSDV-forward TGATGACGGGAAGGTTAGAGT and CYSDV-Reverse CTTCGGATCGGGTTGGACA) that specifically amplify ≈72 bp. A CYSDV probe was labeled with HEX in the 5′ end (HEX-TGCCAGATGCACAGAGGATGTTCG-BHQ1). The melon ADP gene was used as an internal control for RT-qPCR. All primers were subjected to NCBI BLASTn to verify the specificity and validated against both viruses and healthy melon before use in these studies.

RT-qPCR was conducted in a CFX96 thermal cycler (Bio-Rad Laboratories, Inc.) with cycling parameters: denaturation at 95° for 3 min followed by 40 cycles of 95° for 10 s and 60° for 1 min. Each single qPCR reaction contained 5 µl of 5x PerFecta Multiplex qPCR supermix (Quantabio, Beverly, MA), 0.6 µl forward and reverse primers for CYSDV, 0.6 µl forward and reverse probes, 0.6 µl forward and reverse primers for ADP (Adenosine di-phosphate), 13.6 µl nuclease-free water and 1 µl cDNA for a total reaction of 25 µL in a 96-well PCR plate (Quantabio). Two technical replications were run for each sample. A nontemplate control using nuclease-free water was used for each run. The data were analyzed using Bio-Rad CFX manager software V3.1. The quantification cycle value (Ct) was determined at the default settings. A 5-fold dilution series of CYSDV was generated with known cDNA concentration and run in each plate to construct standard curves by plotting the log value of cDNA against Ct number. The amplification efficiency (e) and the coefficient of determination (R^2) of the primers used in qPCR were calculated automatically by Bio-Rad CFX manager software according to the Ct value generated for the serial dilutions against the corresponding $\log_{10}$ amount of cDNA template.

The average relative virus copy number in the sample was calculated using the following equation for the CYSDV and CCYV positive control.

$$\text{Virus copy number}/\mu l = [\text{cDNA concentration}\,(g/\mu l)/(\text{PCR product in bp} \times 660)] \times 6.022 \times 10^{23}$$

The log10 value of the average relative virus titration was used as phenotypic data for mapping CYSDV resistance QTL.

DNA extraction and genotyping by sequencing. Approximately 100 mg fresh leaf tissue was collected from each of F_2 individual used to generate its respective $F_{2:3}$ family for DNA extraction at Michigan State University and subsequent genotyping by sequencing (GBS) (Elshire et al., 2011) was performed at Cornell University. Briefly, the samples were lyophilized overnight and ground to a fine powder using 5/32″ (3.97 mm) stainless steel balls in a Retsch Mixer Mill. DNA was extracted using the plant DNA DS kit (M1130; Omega Bio-Teck, Norcross, GA). SNP calling was performed in Tassel 5.0 using GBS discovery pipeline (Glaubitz et al., 2014). SNPs were further filtered by removing more than 20% missing and minor allele frequencies less than 5%. All missing data were imputed using LD kNNi algorithm with default setting in Tassel 5.0.

Linkage analysis and QTL mapping. Linkage mapping was performed in MapDisto 2.0 (Lorieux, 2012). The 'Find Groups' command was used to make marker linkage groups with a logarithm of the odds (LOD) value of 3.0 and rmax of 0.3. The 'AutoOrder,' 'AutoCheckInversions,' and 'AutoRipple' commands were used to develop a linkage map at LOD of 3.0, and Kosambi mapping function was used to calculate the genetic distances. Mean virus titrations were used for CYSDV QTL mapping. QTL analysis was performed in QGene 4.0 (Joehanes and Nelson, 2008) using composite interval mapping to identify CYSDV resistance QTL. A permutation test with 1000 iterations was performed to find the LOD threshold at significance level $\alpha = 0.05$ and 0.01.

Sequence tagged site marker development and validation Two flanking markers, S5-20,880,639 and S5-21,353,819 (0.87 mb apart), that differentiate the parents, 'Top Mark' and PI 313970, were selected. On the basis of the single nucleotide polymorphism

(SNP) between the parents in these markers, parent-specific sequence tagged site markers were developed and served as codominant markers. The primers were designed in specificity to the last SNP base at the 3′ terminus of the primer that are specific to the parents and the PCRs were optimized so the amplicons were specific to either of the parents. For marker S5-20,880,639, the primers et 20880639-F1 (5′ CTGCCTCTCCCATTACA TCCAG 3′) and 20880639-TM-R1 (5′ CAA AGCTAAATGGCGGACAACC 3′) can only produce an amplicon from 'Top Mark', whereas the primer set 20880639-F1 and 20880639-PI313-R1 (5′ CAAAGCTAAATG GCGGACAACT 3′) can only produce an amplicon from PI 313970 (Fig. 1). Similarly, for S5-21,353,819 marker, the primer set S5-21356819-TM-F (5′ TGCTCGTCAAAATT CCTCCCTGAG 3′) and S5-21356819-TM-R (5′ CAATTTTCTCACCAAGGCATCGGCA 3′) can amplify only from 'Top Mark' and S5_21356819-PI-F (5′ CCACCTCGAACCA ATTTGTTCTCG 3′), and S5-21356819-PI-R (5′ CAATTTTCTCACCAAGGCATCGGCG 3′) can only produce amplicon from PI 313970. The PCR amplification parameter was denaturation at 95° for 5 min, 30 cycles of 95° for 30 s, 65° for 30 s, and 72° for 30 s, and final extension of 72° for 5 min for marker S5-20,880,639. For S5-21,356,819 marker, all the PCR parameters were same except the annealing temperature was raised to 66° and the cycle to 32. Marker S5-20,880,639 was used to genotype selected F_2 lines whose $F_{2:3}$ families showed low virus titer in 2019. Both markers were used to genotype several other known CYSDV resistance sources. All PCR amplicons were visualized on 1% agarose gels with GelRed® Prestain Plus 6X DNA Loading Dye (Biotium, Inc., Fremont, CA).

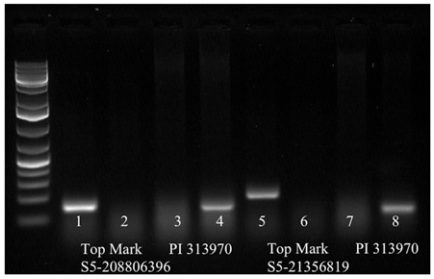

Fig. 1. Genotyping of 'Top Mark' and PI 313970 with two flanking markers (**A**) S5-20880639 and (**B**) S5-21356819. Lanes 1, 3, 5, and 7 were 'Top Mark'-specific primers, and lanes 2, 4, 6, and 8 were PI 313970–specific primers. The 'Top Mark'–specific primers amplified only from 'Top Mark' and PI 313970–specific primers amplified only from PI 313970 in both primer sets.

Results

Virus infection as evidenced by yellowing symptoms was uniform in both years. All $F_{2:3}$ families and the resistant parent PI 313970 exhibited yellowing symptoms in both years due to coinfection by CCYV. PI 313970 and the $F_{2:3}$ population had mean yellowing ratings in rep 1 of 8.5 (n = 5) and 9.9 (n = 303), respectively, in 2018. Their respective means in 2019 were 6.0 (n = 2) and 6.8 (n = 100) (Table 1). CYSDV resistance QTL detection and mapping were not possible using phenotypic data from CCYV coinfected plants with CYSDV and were solely based on the relative titer of CYSDV calculated from RT-qPCR data.

RT-qPCR and virus quantification. In Fall 2018, the relative CYSDV virus in PI 313970 was 201,500,000 copies, which was 9000× less (0.01%) than in susceptible 'Top Mark' with 1,796,690,100,000 copies. CYSDV titer ranged from 1,422,500 to 72,000,032,250,000 copies in the $F_{2:3}$ TM × PI 313970 population (from 0.0001% to 4007.37% of 'Top Mark') (Supplemental Table 1). In Fall 2019, the relative CYSDV virus in PI 313970 was 8,830,000 copies, which was 4300× less (0.02%) than in susceptible 'Top Mark' with 38,159,550,000 copies. CYSDV titer ranged from 5,450,000 to 660,000,000,000 copies in the $F_{2:3}$ TM × PI 313970 population (from 0.01% to 1729.58% of 'Top Mark') (Supplemental Table 2).

QTL mapping. A total of 24,673 SNP markers were identified in GBS SNP calling that passed the quality calls in $F_{2:3}$ TM × PI 313970 population. These SNP markers were further filtered in Tassel 5.2.59 with the criteria of site minimum count of 80, site minimum allele frequency of 5%, site max allele frequency of 95%, the minimum heterozygous proportion of zero, and maximum heterozygous proportion of 1. A total of 4684 SNP markers passed the criteria. Imputation was performed in Tassel 5.0 using LD KNNI with default settings. All markers that were not polymorphic and the markers with segregation distortion were then removed, which left 993 SNP markers for genetic linkage mapping. The resulting map contained 12 linkage groups corresponding to the 12 chromosomes of melon (Supplemental Table 3).

One QTL on chromosome 5 located at a physical position of 22,217,535 bp (interval S5-21,666,550 to S5-22,718,324) was identified that explained ≈16% of the variation in CYSDV titer in Fall 2018 (Fig. 2, Table 2). The significant marker associated with this QTL is S5-22,217,535 bp (P = 0.05). A second QTL was identified on chromosome 3 at a physical position of 28,571,859 (interval S3-28,360,995 to S3-28,568,929, R^2 = 20%, P = 0.01) (Table 2).

Similarly, in 2019, a QTL was identified on chromosome 5 that is colocalized with the 2018 QTL, which is ≈1.3 mb away from significant marker S5-22,217,535 of 2018 QTL (Fig. 3, Table 2). The significant marker associated with this QTL, S5-20880639 (S5-20,833,260 to S5-21,666,437), explained ≈35% of the variation in CYSDV titer (Fig. 2, Table 2, P = 0.01). The positions of the 2018 and 2019 QTL on chromosome 5 are close when the flanking markers are considered.

Marker S5-20,880,639 was used to genotype selected F_2 families with low virus titer, and all the lines tested were homozygous for the PI 313970 marker (Fig. 4). In a further attempt to validate markers S5-20,880,639 and S5-21,356,819, we tested for their presence in TGR 1551 and nine other accessions (Table 3) identified as putative sources of CYSDV resistance (McCreight et al., 2016). PI 122847, PI 145594, and PI 614185 were identical to PI 313970 for both flanking markers. PI 614213 was identical to PI 313970 for S5-20880639 and identical to 'Top Mark' for S5-21356819. TGR 1937 was heterozygous for S5-20880639, whereas TGR 1551 was identical to 'Top Mark'. Neither TGR 1551 nor TGR 1937 had the 'Top Mark' or PI 313970 marker at S5-21356819.

Discussion

Commercial melon cultivars resistant to CYSDV and adapted to the desert southwest United States are not currently available. To be successful in the desert southwest United States, CYSDV-resistant cultivars must hold up to the combination of CYSDV, CCYV, and whitefly feeding pressure. Palomares-Rius et al. (2018) recently released 'Carmen', a Yellow Canary (*C. melo* group Ibericus Subgroup Amarillo) breeding line for Spain (Pitrat, 2016). CYSDV has been devastating each year to the melon production since it was detected in California and Arizona in 2006 (Kuo et al., 2007). CYSDV-resistant melon cultivars are of utmost importance in the United States and

Table 1. *Cucurbit yellow stunting disorder virus* symptom severity (foliar yellowing) and titer in susceptible 'Top Mark', resistant PI 313970, and $F_{2:3}$ Top Mark × PI 313970 population; Imperial Valley, CA, 2018 and 2019.

	2018		2019	
Entry	Yellowing (n)[z]	Virus titer[y]	Yellowing (n)	Virus titer
Top Mark	10.0 (1)	1×10^{12}	7.5 (2)	38×10^{9}
PI 313970	8.5 (5)	201×10^{6}	6.0 (2)	9×10^{6}
$F_{2:3}$	9.0 (303)	—	6.8 (100)	—

[z]Virus induced foliar yellowing symptoms evaluated on a 1 (0% to 10%) to 10 (91% to 100 symptomatic foliage) scale. Yellowing attributed to a combination of *Cucurbit yellow stunting disorder virus* and *Cucurbit chlorotic yellows virus* (Wintermantel et al., 2019).
[y]Five (2018) or six (2019) weeks postplanting.

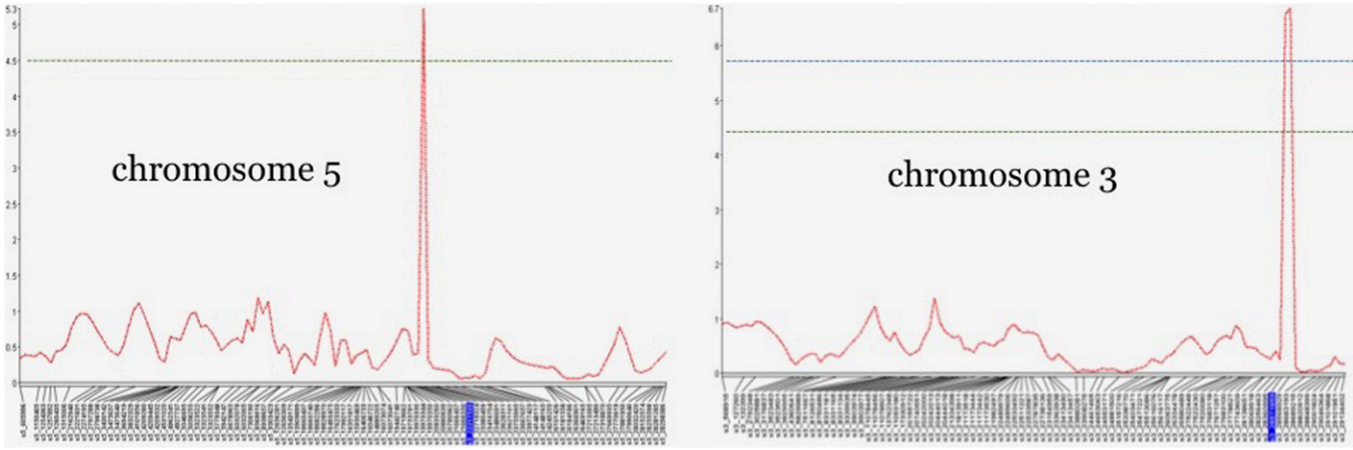

Fig. 2. Composite interval mapping of melon chromosomes 5 and 3 showing significant *Cucurbit yellow stunting disorder virus* resistance quantitative trait locus (QTL) in a $F_{2:3}$ Top Mark × PI 313970 population in Fall 2018. The x-axis represents the markers, and the y-axis represents the logarithm of the odds (LOD) values. LOD threshold was calculated by 1000 permutations at $P = 0.05$ and $P = 0.01$ significance levels and are denoted by blue and green lines, respectively. The LOD and R^2 values for chromosome 5 QTL are 5.74 and 16%. The LOD and R^2 values for chromosome 3 QTL are 6.7 and 20%. The significant marker on chromosome 5 was S5-22217535, which is located at physical position of 20880639 bp, and the significant marker on chromosome 3 was S3-28571859 located at physical position of 28571859.

Table 2. *Cucurbit yellow stunting disorder virus* resistance quantitative trait locus identified on chromsomes (Chr.) 3 and 5 in naturally infected field tests of $F_{2:3}$ Top Mark × PI 313970; Imperial Valley, CA, 2018 and 2019.

Chr.	Yr	Significant markers	Interval (cM)	R^2	Physical position (bp)	LOD	LOD threshold	Additive effect[z]
5	2019	S5-20880639**	145.31	35	20833260–21666437	8.12	5.0*, 7.76*	1.054
5	2018	S5-22217535*	149.15	16	21666550–22718324	5.37	4.51*	0.883
3	2018	S3-28571859**	218.81	20	28360995–28568929	6.73	4.51*, 5.74**	–0.69

[z]A positive value indicates that the allele effect is from Top Mark and a negative value indicates trait value from PI 313970.
LOD = logarithm of the odds.
Significant at $\alpha_{0.05}$ (*) or $\alpha_{0.01}$ (**).

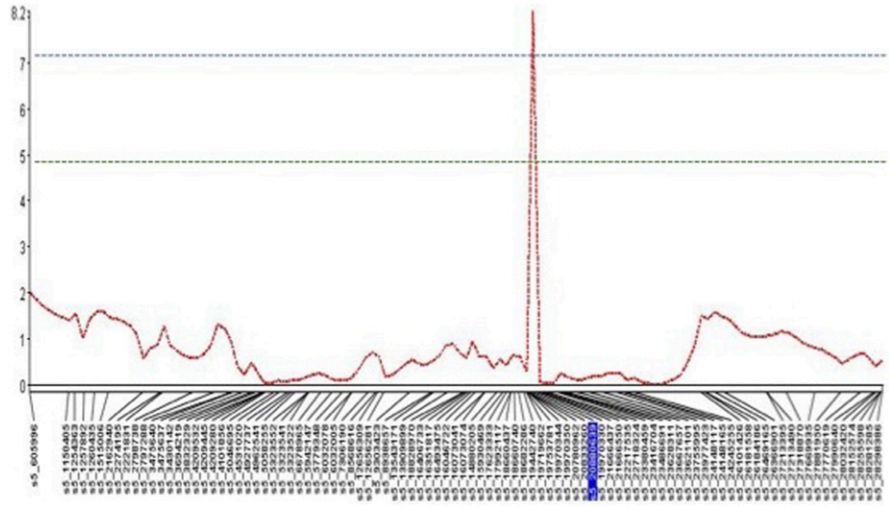

Fig. 3. Composite interval mapping of melon chromosome 5 showing significant *Cucurbit yellow stunting disorder virus* resistance quantitative trait locus (QTL) in a $F_{2:3}$ Top Mark × PI 313970 population in Fall 2019. The x-axis represents the markers, and the y-axis represents the logarithm of the odds (LOD) values. LOD threshold was calculated by 1000 permutations at $P = 0.05$ significance level is denoted by blue line and at $P = 0.01$ is represented by green line. The LOD and R^2 values for chromosome 5 QTL was 8.12% and 35%. The significant marker S5-20880639 is located at 20880639 bp on chromosome 5.

other melon growing regions of the world to reduce yield loss from this disease.

Developing virus-resistant genotypes in a breeding program is a difficult task as the resistance sources are often from exotic germplasm, viral symptoms take time to express, and disease escape is always possible in controlled or natural inoculation tests. Screening of potential CYSDV resistance sources is made difficult in the U.S. desert southwest due to the presence of high numbers of whiteflies because they can kill melon plants in the absence of virus pressure (Gonzalez et al., 1992; McCreight et al., 1995). The situation is further exacerbated by the recent appearance of another whitefly-transmitted virus, CCYV, that can coinfect the same host and produce identical symptoms (Wintermantel et al., 2019). Molecular markers linked with CYSDV resistance gene(s)/QTL are essential for plant breeders to introgress the resistance into elite, breeding lines, and cultivars.

A limited number of CYSDV resistance sources have been reported, beginning with TGR 1551 and TGR 1937 from Zimbabwe (López-Sesé and Gómez-Guillamón, 2000). Resistance in TGR 1551 was initially reported to be controlled by a single dominant gene based on controlled inoculation tests (López-Sesé and Gómez-Guillamón, 2000). Subsequent studies in Texas and Imperial Valley, CA, in open greenhouse and field tests showed the resistance in TGR 1551 to be recessive in nature (McCreight et al., 2017). PI 313970 from India was reported as a source of recessive resistance to CYSDV in an open field test (McCreight and Wintermantel, 2011). This variation in expression of resistance, either dominant or recessive, might be due to the timing of disease assessment or could be due to the experimental environment (controlled or field test). CYSDV resistance in other potential sources was expressed as a single recessive gene (McCreight et al., 2015,

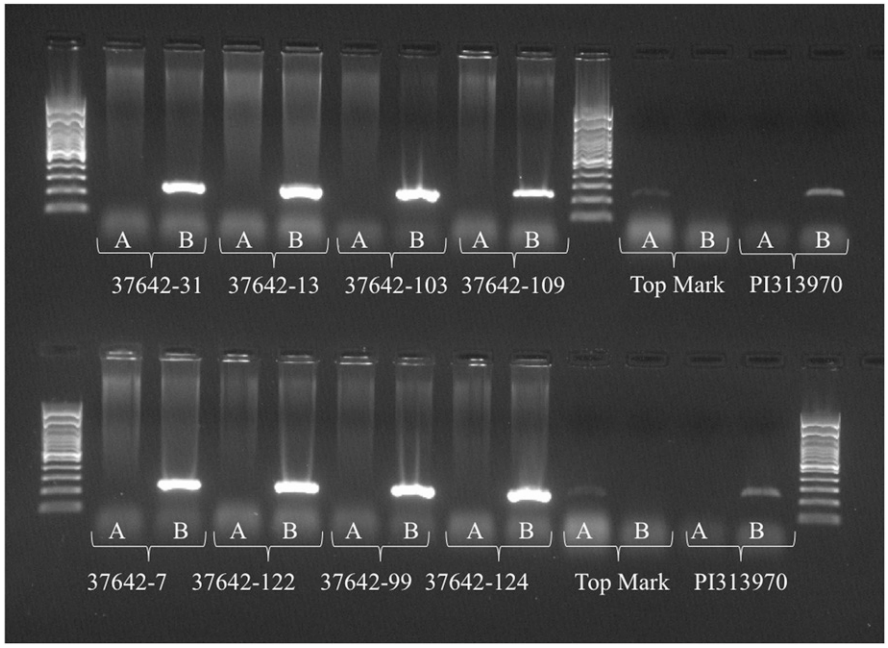

Fig. 4. Genotyping of eight F_2 lines whose $F_{2:3}$ progenies exhibited low virus titer in 2019. All $F_{2:3}$ lines resembled the *Cucurbit yellow stunting disorder virus*–resistant parent PI 313970 for the two flanking markers. (**A**) 'Top Mark'–specific and (**B**) PI 313970–specific markers, respectively.

Table 3. Genotypes for two molecular markers of 10 putative *Cucurbit yellow stunting disorder virus* (CYSDV) resistance sources and three F_1 and one backcross progenies with the two markers flanking the quantitative trait locus on chromosome 5. Resistance markers in CYSDV-resistant PI 313970 (PI), susceptible markers in 'Top Mark' (TM), or heterozygous for the two markers (PI/TM).

	Marker	
Source/progeny	S5-20880639	S5-21356819
Resistance source		
Ames 26704	TM	TM
PI 116482	TM	TM
PI 122847	PI	PI
PI 123496	TM	TM
PI 145594	PI	PI
PI 145596	TM	PI/TM
TGR 1551 (PI 482420)	TM	—
TGR 1937 (PI 482431)	PI/TM	—
PI 614185	PI	PI
PI 614213	PI	TM
F_1 and backcross progenies		
$\{S_1$[Impac × (F_2(Top Mark × PI 614486))]} × Top Mark	PI/TM	TM
F_1 PI 123496 × PI 145594	PI/TM	PI/TM
F_1 PI 145594 × Impac	PI/TM	PI/TM
F_1 Top Mark × PI 123496	TM	TM
Susceptible cultivar		
Impac	PI/TM	TM

2016), and likely allelic with resistances in TGR 1551 and PI 313970.

In the present study, we identified two CYSDV resistance QTL on chromosomes 3 and 5 in PI 313970. The QTL on chromosome 3 was localized at the physical location of 28,571,859 bp (interval S3-28360995 to S3-28568929) and explained 20% of the phenotypic variation. This chromosome 3 QTL has not been reported before, so it could be a novel resistance QTL/gene. This 3H QTL harbors 23 predicted proteins of which 22 were annotated and none were associated with disease resistance genes (Supplemental Table 4). Because it was detected only in the Spring 2018 data, and without any disease resistance–related gene in this QTL region, it must be confirmed with further studies. We sought to do so in Spring 2020, but the virus complex present was dominated by CCYV with very low levels of CYSDV as determined by RT-qPCR (data not shown).

A major QTL identified on chromosome 5 in the same region in both years explained 16% and 35% of titer variation in 2018 (interval S5-21,666,550 to S5-22,718,324) and 2019 (S5-20,833,260 to S5-21,666,437), respectively. The two estimates were 1.3 mb apart but very close when flanking markers are considered. This could be due to the difference in virus titer in the mapping population in 2018 and 2019. Therefore, it is likely a single QTL present on chromosome 5. Pérez-de-Castro et al. (2020) reported CYSDV resistance QTL in TGR 1551 on chromosome 5 in the same physical region using phenotypic (S5-6,810,744 to S5-24,296,585 bp) and virus titer data from RT-qPCR (S5-24,791,006 to S5-27,121,14 bp). Our results confirmed the presence of a major CYSDV resistance QTL on chromosome 5. Markers flanking this QTL can be used in marker-assisted breeding of CYSDV-resistant melons. The chromosome 5 CYSDV resistance QTL (S5-20,833,260 to S5-21,666,437) region contains 96 predicted genes, of which only 73 were annotated (Supplemental Table 5). Twelve of the annotated candidate genes were related with disease resistance genes that include serine/threonine-protein kinase-like gene, aldose 1-epimerase family protein, defensin-like gene, clathrin assembly protein, and subtilisin-like protease. Identification of the polymorphic markers within these regions is essential to further saturate the QTL region or fine mapping of the CYSDV resistance gene. Molecular markers will be useful in developing CYSDV-resistant cultivars in the presence of CCYV because their symptoms are nearly identical.

PI 313970 is susceptible to CCYV that was first detected in California and Arizona in 2018 (Tamang et al., 2019; Wintermantel et al., 2019). The CYSDV resistant parent PI 313970 and resistant $F_{2:3}$ TM × PI 313970 families exhibited yellowing reactions (susceptible) due to coinfection by CCYV. The phenotypic data collected in field were, thus, not useful for QTL analysis. We, therefore, used, RT-qPCR to quantify the relative amount of virus in each sample of $F_{2:3}$ population for QTL mapping. Pérez-de-Castro et al. (2020) used a relative virus titer to identify the CYSDV resistance QTL in TGR 1551. Neither TGR 1551 nor PI 313970 is completely immune to CYSDV, as lower levels of CYSDV were detected in RT-qPCR.

Viruses often occur in mixed infections (coinfection) in cucurbits (Gil-Salas et al., 2012). Titers of CCYV and CYSDV in mixed infection were reduced compared with single virus-infected plants (Abrahamian and Abou-Jawdah, 2014; Wintermantel et al., 2019) in which they observed a greater reduction in CCYV than CYSDV in mixed infection. We observed a greater reduction in CCYV titer in both parents, 'Top Mark' and PI 313970, when coinfected with CYSDV in the 2018 Fall, but CCYV titer was higher in both parents in 2019 Fall. CCYV dominated CYSDV in Spring plantings of 2018 and 2019 (data not shown). The Spring data were, therefore, not useful for QTL mapping. The reasons for the higher CCYV titers remain unknown but may be related to seasonal differences that result in fewer whiteflies, larger and more established plants when infested by small numbers of whiteflies, and differences in reservoir hosts in the spring season.

Chemical insecticides have been used to control the whitefly population in most parts

of the world, but they are considered hazardous to human and animal health and to the environment. Host resistance to whitefly could help to reduce whitefly populations, thereby reducing the amount of chemicals used for their control. Such resistance may also serve to reduce spread of viruses in the field (Kennedy, 1976). PI 313970 is a potential source of resistance to whitefly, *Bemisia tabaci* (Boissot et al., 2003), although the genetic basis for resistance to whitefly is unknown. Introgression of CYSDV and whitefly resistance from PI 313970 to elite commercial cultivars through breeding would be an ideal combination in a CYSDV-resistant cultivar.

There are other traits that can be harnessed from PI 313970. It is resistant to other pathogens, such as powdery mildew, *Podosphaera xanthii* races 1, 2, 2U.S., 3, 3.5, 4.5, 5, and S (McCreight, 2003; McCreight and Coffey, 2011; Pitrat and Besombes, 2008; Shishkoff, 2000), *Cucurbit leaf crumple virus* (CuLCrV) (McCreight et al., 2008), as well as other insect pests such as melon aphid, *Aphis gossypii* (Boissot et al., 2008; Boissot et al., 2000), agromyzid leafminer, *Liriomyza sativae* Blanchard (Kennedy et al., 1978), and melon worm, *Diaphania hyalinata* L. (Boissot et al., 2000), and it may be a source of salt tolerance (Shannon et al., 1984).

A QTL region that conferred resistance to CYSDV in melon line PI 313970 was identified in this study, which confirmed the QTL regions on chromosome 5 of TGR 1551 that were recently reported by (Pérez-de-Castro et al., 2020). The markers closely linked with the CYSDV resistance QTL in PI 313970 can be used to expedite the development of CYSDV-resistant elite breeding lines and cultivars for the desert southwest United States.

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