Short Communication

A Description of the Possible Etiology of the Cilantro Yellow Blotch Disease

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Abstract

A virus-like disease characterized by foliar yellow blotch symptoms and resembling those described for cilantro yellow blotch disease in California was observed in a 4.05-ha cilantro (*Coriandrum sativum*) cv. Santo field in Hidalgo County, Texas during spring 2019. Disease incidence at harvest was estimated at ~20%, and the affected plants were rendered unmarketable. Foliar systemic chlorosis symptoms were observed on sap-inoculated *Nicotiana occidentalis* plants ($n = 3$) using inocula from symptomatic cilantro. Total RNA aliquots from 11 randomly collected leaf tissue samples (symptomatic = 7, asymptomatic = 4) were pooled into a composite cilantro RNA sample which was analyzed by high throughput sequencing (HTS). Analyses of the obtained 15.7 million raw reads (76 nt each) yielded virus-specific contigs that mapped to the genomes of alfalfa mosaic virus (AMV), beet pseudoyellows virus

(BPYV), and lettuce chlorosis virus (LCV). Virus-specific primers designed from the HTS-derived sequences were used to screen the samples in two-step RT-PCR assays, resulting in the detection of AMV+BPYV in 3 of 7 symptomatic cilantro samples, AMV+LCV in 4 of 7 symptomatic cilantro samples, and AMV alone in the 4 asymptomatic cilantro and sap-inoculated *N. occidentalis* samples. The results represent the first reports of the natural infection of cilantro by BPYV and LCV and implicate the mixed infection of a *Crinivirus* and AMV in cilantro yellow blotch disease.

Keywords: whitefly, aphids, etiology, mixed virus infections, alternative host, *Crinivirus*, *Alfalfa mosaic virus*, *Beet pseudoyellow virus*, *Lettuce chlorosis virus*, cilantro yellow blotch

Cilantro (*Coriandrum sativum* L., family Apiaceae), also known as coriander, Mexican parsley, and Chinese parsley, is an economically important, annual specialty herb and spice grown in different climates, including the subtropical lower Rio Grande valley area of south Texas. Although all parts of the plant are edible, the uniformly green, simple palmately lobed leaves of cilantro and its dried seeds are mostly used in cuisines. Very few diseases have been reported affecting cilantro production. These include bacterial leaf spot caused by *Pseudomonas syringae* pv. *coriandricola*, Fusarium wilt caused by the fungus *Fusarium oxysporum*, carrot motley dwarf disease caused by the coinfection of *Carrot red leaf virus* (genus *Polerovirus*) and *Carrot mottle virus* (genus *Umbravirus*), *Apium virus Y* (ApVY) disease caused by the *Potyvirus* ApVY, and cilantro yellow blotch disease (CYBD) of presumed viral etiology (Smith et al. 2011). The causative viruses of carrot motley dwarf and ApVY diseases are vectored by aphids, hence their management relies primarily on the removal of weed reservoirs, and avoidance of fields with a history of the diseases.

Although CYBD in California was presumed to be caused by a virus, tentatively named cilantro yellow blotch virus, based on the observations of closterovirus-like virions in symptomatic plants (Koike et al. 2007; Mayhew 2002; Smith et al. 2011), the etiological agent(s) of the disease was never determined. During spring 2019, a virus-like disease resembling CYBD was observed in a 4.05-ha cilantro field planted with cv. Santo in Hidalgo County, Texas. The affected plants showed symptoms of foliar yellow blotch discolorations (Fig. 1A) relative to healthy-looking leaves (Fig. 1B). Disease incidence at harvest time was estimated at ~20% and the affected plants were

rendered unmarketable. Due to the symptom resemblance to the California case (Koike et al. 2007; Mayhew 2002; Smith et al. 2011), we hypothesized that the observed disease in Texas was also likely to be of viral etiology. The goal of this study was to identify the putative etiological agents of CYBD.

A total of 11 cilantro leaf tissue samples (symptomatic = 7, Fig. 1A; asymptomatic = 4, Fig. 1B) were randomly collected from the field. Initially, leaf tissue samples from symptomatic cilantro plants were ground in 0.01 M phosphate buffer (pH 7.0) supplemented with 0.1% 2-mercapthoethanol, and the sap was rub-inoculated onto Carborundum-dusted *Nicotiana occidentalis* test plants ($n = 3$). An additional Carborundum-dusted *N. occidentalis* control plant was rub-inoculated with only the phosphate buffer solution. All three *N. occidentalis* test plants produced symptoms of chlorotic lesions in inoculated leaves, followed by chlorotic spots on systemic leaves 7 days postinoculation (Fig. 1C), while systemic leaves of the control plant remained symptomless (Fig. 1D). These results further support the possible viral etiology of CYBD.

Total RNA was extracted from each of the 11 cilantro samples and the three *N. occidentalis* test plants using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO). After quality analyses, complementary DNA (cDNA) was synthesized from approximately 2 µg RNA per sample using the PrimeScript first strand cDNA Synthesis Kit (Takara Bio USA, Inc., Mountain View, CA) according to the manufacturer's protocol. A 2-µl aliquot of cDNA per sample was used in a 25-µl polymerase chain reaction (PCR) volume with the PrimeSTAR GXL DNA Polymerase Rapid PCR Protocol (Takara Bio USA, Inc.). The samples were first screened with generic primers C1For/C1Rev (Ha et al. 2008) and N1b2F and N1b2R (Zheng et al. 2010) targeting conserved regions of potyviruses, polerovirus-specific primers Pol-G-F/Pol-G-R (Knierim et al. 2010), and the primer pair AV494/AC1048 targeting begomoviruses (Wyatt and Brown 1996), with appropriate controls. All the 14 samples (cilantro = 11; *N. occidentalis* = 3) were negative for these viruses.

To further explore the potential viral etiology of CYBD, a composite RNA subsample was made using equimolar amounts of RNA from all 11 cilantro samples for diagnosis by high-throughput

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sequencing (HTS). The RNA subsample was first depleted of its ribosomal (r)RNA contents using the Ribo-Zero rRNA Removal Reagents v2 (Plant Leaf)-Low Input (Epicenter, Madison, WI), according to the manufacturer's instructions. A cDNA library was prepared from the ribo-depleted sample using the ScriptSeq v2 RNA-Seq Library Preparation Kit (Epicenter), according to the manufacturer's instructions. HTS was performed on the cDNA libraries on a NextSeq 500 System (Illumina, Inc., San Diego, CA), and the resulting single-end raw sequence reads (76 nt each) were filtered, trimmed, and further processed as previously described (Al Rwahnih et al. 2017). Briefly, the Illumina reads were adapter-trimmed, followed by their de novo assemblage into contigs of at least 200 bp in length using the CLC Bio Genomic Workstation (v8.5.1; Qiagen, Hilden, Germany). Initially, the tBlastx program version 2.4.0 (Tatusova and Madden 1999) was used to compare the contig sequences against the viral genome division of the National Center for Biotechnology Information (NCBI) RefSeq (<https://www.ncbi.nlm.nih.gov/genome/viruses/>). The contigs matching viral genomes with a combined E-value $\leq 10^{-4}$ were considered candidates; only viruses with a known land plant host (Streptophyta) as per the Viral Host Database (Mihara et al. 2016) were considered further. The reduced list of viral hits was then used to query the protein (nr) and nucleotide (nt) GenBank sequence databases for viral agent identification.

A total of 15.7 million raw reads were obtained from the cilantro cDNA library. Analyses of the cilantro reads resulted in the identification of virus-specific contigs that mapped to the genomes of alfalfa mosaic virus (AMV, *Alfamovirus*; 34 contigs, 205–574 nt), beet pseudoyellows virus (BPYV, *Crinivirus*; 16 contigs, 222–4,500 nt), and lettuce chlorosis virus (LCV, *Crinivirus*; 9 contigs, 231–5,294 nt) (Table 1). The remaining reads were of the plant host origin. Importantly, all the RNA segments of each of the three viruses were represented in the HTS-derived reads (Table 1).

Based on the HTS obtained sequences, primers targeting segments of each virus genome component (Table 2) were designed and used in two-step RT-PCR assays to screen the cilantro and *N. occidentalis*

samples as described above. The newly designed primer pairs AMV-P1F/AMV-P1R, AMV-P2F/AMV-P2R, and AMV-P3F/AMV-P3R produced the expected 542, 335, and 430 bp bands specific to RNA1, RNA2, and RNA3 of AMV, respectively, from all 11 cilantro and 3 *N. occidentalis* test plants. The newly designed primer pairs BPYV-rdrpF/BPYV-rdrpR and BPYV-cpF/BPYV-cpR amplified the expected 760 and 568 bp bands specific to RNA1 and RNA2 of BPYV, respectively, only from 3 of 7 symptomatic cilantro plants; the remaining 8 cilantro and 3 *N. occidentalis* test plants were negative for BPYV. The primer pair LCV-50/LCV-25 and LCV2-F5/LCV2-R8 (Alabi et al. 2017) produced the expected 483 and 3,030 bp bands specific to RNA1 and RNA2 of LCV, respectively, only from 4 of 7 symptomatic cilantro plants; the remaining 7 cilantro and 3 *N. occidentalis* test plants were negative for BPYV. No mixed infection of BPYV and LCV was detected in the same cilantro plant. Thus, 3 of 7 symptomatic cilantro samples were positive for AMV+BPYV, 4 of 7 symptomatic cilantro samples were positive for AMV+LCV, while all 4 asymptomatic cilantro and the 3 inoculated *N. occidentalis* plants were singly infected with AMV. Notably, the yellow blotch symptoms observed on plants positive for AMV+BPYV were visually indistinguishable from those of the AMV+LCV plants. Also, the symptoms observed in the sap-inoculated

Table 1. Virus-specific Illumina contigs derived in this study from a composite sample of cilantro plants affected by cilantro yellow blotch disease

Virus ^a	RNA1 ^b		RNA2 ^b		RNA3 ^b	
	N	Scaf_Length	N	Scaf_Length	N	Scaf_Length
AMV	14	205–574	13	207–522	7	205–396
BPYV	8	250–2,510	8	222–4,500	N/A	N/A
LCV	3	869–5,294	6	231–3,286	N/A	N/A

^a AMV, alfalfa mosaic virus; BPYV, beet pseudoyellows virus; LCV, lettuce chlorosis virus.

^b N, number of contigs; Scaf_Length, minimum to maximum length of each virus-specific contig in nucleotides; N/A, not applicable.

Fig. 1. Symptoms of cilantro yellow blotch disease in cilantro (A) relative to healthy-looking leaf (B). Chlorotic spots induced in systemically infected leaf of sap-inoculated *Nicotiana occidentalis* (C) compared with mock-inoculated healthy plant (D) of the same age.

Table 2. Primers used for the amplification of partial genome component specific fragments in this study

Primer name ^a	Sequence (5' – 3')	(bp)	Target	Source
AMV-P1F	GATCTCGATGTGGGAGAGTTAG	542	RNA 1 P1 protein	This study
AMV-P1R	GCGGAATGAGCATCAGAATTAG			
AMV-P2F	CAGTGAACCTACTGACACTTCC	335	RNA 2 P2 protein	This study
AMV-P2R	CGCTAACTCACGCTCTTCAT			
AMV-P3F	ATGAGGCTACAGGAGAGTTAGT	430	RNA 3 P3 protein	This study
AMV-P3R	ACACTAGGACCTTCGGGAATA			
BPYV-rdrpF	GAACACCAGGGATGGAGATAAG	760	BPYV RdRp gene	This study
BPYV-rdrpR	CGAACCTCTGTCTCGATTATG			
BPYV-cpF	CCAGCTCCGGTTGAGAATAAA	568	BPYV CP gene	This study
BPYV-cpR	TTGTGAAGTCGGAAGTGCTATC			
LCV-50	GAAATCAAACCTTCCTTCG	483	LCV RNA1	Alabi et al. 2017
LCV-25	GGCATCCTGTAAATCTGCA			
LCV2-F5	GTTGACGGTGAACCAACTGCTGATG	3,030	LCV RNA2	Alabi et al. 2017
LCV2-R8	CGGCTAGCTATACTACTAAGTAGTCG			

^a AMV, alfalfa mosaic virus; BPYV, beet pseudoyellows virus; LCV, lettuce chlorosis virus.

Fig. 2. Unrooted minimum evolution phylogenetic trees (1,000 bootstrap replications) depicting evolutionary relationships among genome segment-specific nucleotide sequences of alfalfa mosaic virus (AMV), beet pseudoyellows virus (BPYV), and lettuce chlorosis virus (LCV) derived from cilantro (*Coriandrum sativum*) in this study and corresponding sequences of global isolates of each virus.

N. occidentalis plants are consistent with those previously described for mechanically inoculated AMV-positive tobacco species (Hershman and Varney 1982).

At least two virus segment-specific DNA fragments were cloned into the pJET1.2 plasmid vector (Takara Bio USA, Inc.) and sequenced by the Sanger method. In pairwise comparisons, the obtained segment-specific AMV nt sequences (RNA1: MN395624-27, RNA2: MN395628-33, and RNA3: MN395634-39) were 96 to 100% identical to each other and 91 to 99% identical to corresponding sequences of several AMV isolates in the GenBank database. In pairwise comparisons, the obtained segment-specific BPYV nt sequences (RNA1: MN395640-42, RNA2: MN395643-46) were 100% identical among themselves and 99% identical to corresponding sequences of BPYV-MI from Japan (RNA1: LC100131, RNA2: LC100132). In pairwise comparisons, the obtained segment-specific LCV nt sequences (RNA1: MN395647-51, RNA2: MN395652-55) were 99 to 100% identical among themselves and 99 to 100% identical to corresponding sequences of LCV-PTX from Texas (RNA1: KY271955, RNA2: KY271956). As expected, closer phylogenetic relationships were observed among virus segment-specific sequences derived from cilantro in this study relative to sequences obtained from other hosts from different regions of the world (Fig. 2). These results provide further confirmations for the occurrence of AMV, BPYV, and LCV in cilantro plants affected by CYBD in Texas. Whereas AMV has been reported previously from cilantro in western Oregon (Pscheidt and Ocamb 2019), this is the first report of the natural occurrence of BPYV and LCV in cilantro, to the best of our knowledge. Our results implicate possible synergism between AMV and a *Crinivirus* in coinfecting, CYBD-affected cilantro plants in Texas. The results add to the repertoire of documented synergistic outcomes of coinfections involving criniviruses and viruses belonging to other genera as documented for instance for *Sweet potato chlorotic stunt virus* + *Sweet potato feathery mottle virus* in sweet potato (Karyeija et al. 2000), *Blackberry yellow vein-associated virus* + *Blackberry virus Y* in blackberry (Susaimuthu et al. 2008), *Lettuce infectious yellows virus* + *Turnip mosaic virus* in *N. benthamiana* (Wang et al. 2009), and LCV + *Papaya ringspot virus* in papaya (Alabi et al. 2017). However, we cannot exclude the possibility that CYBD may also be induced by either *Crinivirus* species since none of the disease-affected cilantro plants evaluated in this study was singly infected by BPYV or LCV.

AMV is a multipartite ss(+)RNA virus that is nonpersistently transmitted by several aphid species, in addition to being sap transmissible to herbaceous host plants (Hershman and Varney 1982). Seed transmission of AMV has also been reported for alfalfa (Frosheiser 1974; Hemmati and McLean 1977), although it is unclear if this transmission pathway applies to cilantro and other hosts of the virus. In contrast, the bipartite ss(+)RNA BPYV and LCV are semi-persistently transmitted by whiteflies and both are not known to be seed or mechanically transmissible. Therefore, the only plausible explanation for the co-occurrence of these disparate viruses in cilantro is their vector-mediated transmission from alternative host plants within the landscape or the infestation of viruliferous aphids and whiteflies from external sources. Several documented crop hosts of all three viruses (e.g., alfalfa, lettuce, and beet) are routinely cultivated in the study area, and they could be sources of inoculum for vector-mediated spread, in addition to potential evergreen weed reservoirs of these viruses. Hence, the results of this investigation underscore the need for growers to take these known and potential host plants into consideration when planning their crop rotation and field management strategies.

Since the primary symptoms observed in CYBD-affected cilantro plants in this study were similar to those described for cilantro yellow blotch disease in California (Koike et al. 2007; Mayhew 2002; Smith

et al. 2011), it is conceivable that the California case may also be associated with similar mixed virus infection combinations. Hopefully, this report will stimulate further studies to revisit the etiology of the disease-affected cilantro plants in California. Although results obtained in this study revealed possible synergistic interactions between the criniviruses BPYV or LCV, and AMV in the induction of CYBD, studies that utilize infectious clones of each virus will be necessary to conclusively affirm the outcome of this interaction and to determine the specific virus and host encoded factors involved.

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