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The Wheat Dwarf India Virus-Betasatellite Complex Has a Wider Host Range Than Previously Reported

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Abstract

Wheat dwarf India virus (WDIV) and associated alphasatellite and betasatellite have been documented to infect wheat (*Triticum aestivum*). Here, we report, for the first time, presence of WDIV and the associated Ageratum yellow leaf curl betasatellite (AYLCB) in barley (*Hordeum vulgare*) and sugarcane (*Saccharum officinarum*) in addition to wheat. However, surprisingly, the previously reported alphasatellites were not detected in the WDIV-positive

barley, sugarcane, and wheat samples. The virus and betasatellite detected from barley, sugarcane, and wheat showed high levels of sequence identity to the previously reported WDIV and AYLCB. The study shows that WDIV may have much wider host range than previously documented.

Keywords: WDIV, satellites, host range, barley, sugarcane

Members of the genus *Mastrevirus*, family *Geminiviridae*, infect either dicotyledonous (dicot) or monocotyledonous (monocot) hosts and are transmitted by leafhoppers (Boulton 2002). They have a monopartite circular ssDNA genome of 2.5 to 2.7 kb that codes for the movement protein (MP; open reading frame [ORF] V2) and coat protein (CP; V1) on the virion-sense strand and replication-associated proteins (Rep and RepA) on the complementary-sense strand (Boulton 2002). RepA is translated from a nonspliced transcript that spans the C1 and C2 ORFs, whereas Rep is translated from a spliced transcript that fuses C1 and C2 ORFs (Hefferon and Dugdale 2003; Wright et al. 1997). The genes on the virion and complementary strands are separated by the large intergenic region (LIR) and small intergenic region (SIR). The LIR contains a characteristic stem loop structure with the invariant non-nucleotide sequence (TAATATTAC) (Boulton 2002; Lazarowitz et al. 1989).

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Recently, two mastreviruses, wheat dwarf India virus (WDIV) and chickpea chlorotic dwarf virus (CpCDV), have been reported to be associated with satellites (Hamza et al. 2018; Kumar et al. 2014a, 2014b, 2015). Importantly, the associated satellites have been documented to enhance the severity of WDIV and CpCDV disease symptoms (Hamza et al. 2018; Kumar et al. 2014a, 2014b). WDIV is a monocot-infecting mastrevirus and has been reported to be associated with cotton leaf curl Multan alphasatellite (CLCuMuA) and guar leaf curl alphasatellite of the family *Alphasatellitidae* and Ageratum yellow leaf curl betasatellite (AYLCB) of the family *Tolecusatellitidae* (Adams et al. 2017; Briddon et al. 2018; Kumar et al. 2012, 2014a, 2014b, 2015). The presence of WDIV and associated alpha- and betasatellites was documented from several geographical regions in India, which demonstrate a strong association between WDIV and the satellites (Kumar et al. 2015). On the other hand, CpCDV is a dicot-infecting mastrevirus and was found to be associated with cotton leaf curl Multan betasatellite, of the family *Tolecusatellitidae*, and CLCuMuA in field infections of spinach (Hamza et al. 2018).

The present study reports the presence of WDIV and associated betasatellite in symptomatic barley (*Hordeum vulgare*), sugarcane (*Saccharum officinarum*), and wheat (*Triticum aestivum*) samples from India. The symptomatic barley and wheat plants had dwarfing and/or yellowing symptoms, whereas the sugarcane plants showed chlorotic streaks on the leaves (Fig. 1). Leaf samples from six barley, 11 sugarcane, and six wheat plants were collected during 2017 to 2018 from farmers and research fields in India (Meerut, Uttar Pradesh; latitude, 28.96° N; longitude, 77.74° E). Young leaf samples from symptomatic and nonsymptomatic barley, sugarcane, and wheat plants were collected and stored in separate tubes with ethanol at room temperature for 1 week. Then the ethanol was decanted, and the dried leaf samples were stored in a freezer until further use.

FIGURE 1

Wheat dwarf India virus- and Ageratum yellow leaf curl betasatellite-infected barley (A) and wheat (B) plants are shown in red circles. Leaves of an infected sugarcane plant showing chlorotic streak symptoms (C).

Detection and Analysis of Virus and Satellite Genomes

Total DNA was isolated from the dried leaf samples using a modified sodium dodecyl sulfate method (Edwards et al. 1991) and quantified using a NanoDrop (Thermo Scientific, Waltham, MA). Approximately 100 ng of total DNA was used as a template in polymerase chain reaction (PCR). PCR-based amplification of WDIV and the satellite DNA was performed using specific primers (Table 1), which yielded fragments of the sizes expected for WDIV and betasatellite. A total of three barley, four sugarcane, and two wheat samples tested positive for WDIV. The nine WDIV-positive samples also tested positive for AYLCB. However, the PCR reactions using primers published earlier (Bull et al. 2003; Kumar et al. 2010) and primers designed as part of the study here (Table 1) for alphasatellite did not yield a fragment from any of the samples. The PCR products obtained using WDVKF/R and Beta01/04 primer pairs and representing WDIV and betasatellite genomes, respectively, were cloned in pJET1.2 cloning vector (Thermo Scientific). Three clones for WDIV and AYLCB from each of nine samples were selected for partial sequencing to confirm their identity. Upon confirmation of the identity, one clone for virus and one for betasatellite from each of the two barley, three sugarcane,

and two wheat samples were sequenced in entirety. The full-length sequences of the virus and satellite genomes were assembled using CAP3 program (Huang and Madan 1999). Phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis software, MEGA version 7 (Kumar et al. 2016).

Analysis of the Virus and Satellite Genomes

The virus genome amplified from the samples was 2,783 bp (Table 2) and showed sequence identities of 98 to 99% with WDIV reported previously (Kumar et al. 2012). The phylogenetic analysis showed the newly characterized WDIV sequences isolated from barley, sugarcane, and wheat form a separate cluster with the previously reported WDIV isolates, whereas viruses belonging to other species clustered separately (Supplementary Fig. 1). Based on the nucleotide sequence identity and the phylogenetic analysis, the newly characterized viruses are isolates/variants of WDIV.

ORFs of the WDIV clones were determined using the ORF finder tool (<https://www.ncbi.nlm.nih.gov/orffinder/>). The analysis showed four ORFs (V1, V2, C1, and C2) and two intergenic regions (LIR and SIR), which were conserved in sequence and position between all of the clones. The LIR harbors a predicted stem-loop

structure with the nonanucleotide sequence TAATATTAC as also documented previously (Boulton 2002; Kumar et al. 2014a; Lazarowitz et al. 1989). The complete nucleotide sequences of one clone from each of the two barley, three sugarcane, and two wheat samples were submitted to GenBank (Table 2).

Betasatellite sizes ranged from 1,362 to 1,365 bp (Table 2) and showed sequence identities of 99% with AYLCB reported previously from wheat (Kumar et al. 2014a, 2014b). The phylogenetic tree shows the seven betasatellite sequences determined here to cluster with AYLCB sequences previously isolated from wheat, and

they are distinct from other betasatellites species (Supplementary Fig. 2). Based on the full-length nucleotide sequence identity and the phylogenetic analysis, the newly characterized betasatellites are isolates/variants of AYLCB.

Sequence analysis showed a genome organization typical of betasatellites. The betasatellites contain an adenine-rich region, a β C1 gene, and a sequence of approximately 150 nucleotides—known as a satellite conserved region—conserved between all betasatellites that encompasses a predicted hairpin structure that contains the nonanucleotide sequence TAATATTAC. The nucleotide sequences of betasatellites were submitted to GenBank (Table 2).

Association of Satellites and Host Range

This is the first report on the presence of WDIV and AYLCB in barley and sugarcane in field infections. The WDIV sequences isolated from the barley, sugarcane, and wheat have high levels of nucleotide sequence identity (98 to 99%). This shows that WDIV has a much wider host range than previously documented (Kumar et al. 2012, 2014a, 2015).

In previous studies (Kumar et al. 2014a, 2015), both alphasatellite and betasatellite were found to be nearly ubiquitously associated with WDIV infection across different geographical regions in India. However, the alphasatellite was not detected in barley, sugarcane, and wheat during the present investigation.

Although the reason is unclear, WDIV seems to prefer to associate with betasatellites as compared with alphasatellites. Betasatellites have been reported to be involved in mitigating antiviral resistance, enhanced virus accumulation, pathogenicity determination, and movement inside the host plant (Saeed et al. 2005; Saunders et al. 2004; Tahir and Mansoor 2011). On the other hand, things are less clear for alphasatellites. Some of the alphasatellites have been shown to have transcriptional and posttranscriptional gene silencing and to enhance symptoms and accumulation of the virus, whereas others have been reported to be associated with reduction of betasatellite and viral DNA accumulation and attenuation of the disease symptoms (Abbas et al. 2019; Idris et al. 2011; Kumar et al. 2014a; Mar et al. 2017; Nawaz-ul-Rehman et al. 2010; Vinoth Kumar et al. 2017; Wu and Zhou 2005).

Because betasatellites enhance symptoms, the presence of a betasatellite with WDIV in infected wheat, barley, and sugarcane

TABLE 1
List of primers used during the present study^a

Primer name	Nucleotide sequence (5'-3')	Target
WDVHF*	<u>AAGCTT</u> AGGAAGCTCCCGGT	WDIV
WDVHR*	<u>AAGCTT</u> GGTCGCCGATCCTTT	WDIV
WDVKF*	<u>GGTACCT</u> GAAGGTTGAAGACGA	WDIV
WDVKR*	<u>GGTACCG</u> CAGTAATAACTGCTT	WDIV
Beta01**	GAAACCACTACGCTACGCAGC	Beta
Beta04**	ACCCTCCCAGGGGTACACACCG	Beta
Nanofor**	AAGTGGGTCCTGGTTCTA	Alpha
Nanorev**	CTGTACAGGTCTCTGGC	Alpha
DNA101**	<u>CTGCAGATA</u> ATGTAGCTTACCAG	Alpha
DNA102**	<u>CTGCAGAT</u> CCTCCACGTGTATAG	Alpha
AlphaPstF*	<u>CTGCAGATA</u> AVGTWYVRTWD	Alpha
AlphaPstR*	<u>CTGCAGAT</u> CCWCKKSMWW	Alpha
Alpha1F*	GTAATTCGAAGTCCGGTGAGA	Alpha
Alpha1R*	AGATTCTCGTTGTCGTCGTT	Alpha
Alpha2F*	ACGAAGCTCGGGATTACTGTA	Alpha
Alpha2R*	CGGTCTTACGCGCACGTTGTT	Alpha
Alpha3F*	GACGACTCGACGACGCCACT	Alpha
Alpha3R*	GCGGACTCTTCTCTCGCCA	Alpha

^a Underlined nucleotides represent restriction sites. Primers with “*” were designed as a part of the present study, whereas the primers with “**” were designed by Kumar et al. (2010) and Bull et al. (2003). WDIV = wheat dwarf India virus; Beta = betasatellite; and Alpha = alphasatellite.

TABLE 2
Names, accession numbers, and gene coordinates of the virus and betasatellites^a

Virus/satellite name (acronym)	Host	Sample number	Size of the clone (bp)	Accession number	Gene coordinates				
					MP	CP	RepA	Rep	β C1
Wheat dwarf India virus (WDIV)	Barley	Bar2	2,783	MN273439	(V-strand)	(V-strand)	(C-strand)	(C-strand) join 1,435 to	NA
		Bar5	2,783	MN273440	175 to	540 to	1,728 to	1,810 and 1,900 to	
	Sugarcane	Sug4	2,783	MN273441	495	1,283	2,597	2,597	
		Sug6	2,783	MN273442					
		Sug11	2,783	MN273443					
	Wheat	Wh2	2,783	MN273444					
Ageratum yellow leaf curl betasatellite (AYLCB)	Barley	Wh5	2,783	MN273445					
		Bar2	1,364	MN273446	NA	NA	NA	NA	(C-strand)
	Sugarcane	Bar5	1,364	MN273447					180 to
		Sug4	1,364	MN273448					596
		Sug6	1,362	MN273449					
		Sug11	1,362	MN273450					
	Wheat	Wh2	1,365	MN273451					
		Wh5	1,364	MN273452					

^a NA = not applicable; V = virion; C = complementary; MP = movement protein; CP = coat protein; RepA = replication associated protein-A; Rep = replication associated protein; and β C1 = C1 protein of betasatellite.

has serious implications. More severe symptoms will lead to greater crop losses and economic impact. Considering the findings present here, it will be of interest to examine further barley, sugarcane, wheat, and other plant species to determine the geographical and host range of this mastrevirus-betasatellite complex.

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