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ANNOTATED SEQUENCE RECORD

Molecular characterization of a divergent strain of calla lily chlorotic spot virus infecting celtuce (*Lactuca sativa* var. augustana) in China

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Abstract

Through sequencing and assembly of small RNAs, an orthotospovirus was identified from a celtuce plant (*Lactuca sativa* var. augustana) showing vein clearing and chlorotic spots in the Zhejiang province of China. The S, M, and L RNAs of this orthotospovirus were determined to be 3146, 4734, and 8934 nt, respectively, and shared 30.4-72.5%, 43.4-80.8%, and 29.84-82.9% nucleotide sequence identities with that of known orthotospoviruses. The full length nucleoprotein (N) of this orthotospovirus shared highest amino acid sequence identity (90.25%) with that of calla lily chlorotic spot virus isolated from calla lily (CCSV-calla) [China: Taiwan: 2001] and tobacco (CCSV-LJ1) [China: Lijiang: 2014]. Phylogenetic analyses showed that this orthotospovirus is phylogenetically associated with CCSV isolates and clustered with CCSV, tomato zonate spot virus (TZSV), and tomato necrotic spot-associated virus (TNSaV) in a separate sub-branch. These results suggest that this orthotospovirus is a divergent isolate of CCSV and was thus named CCSV-Cel [China: Zhejiang: 2017].

Viruses classified within the sole genus *Orthotospovirus* of the family *Tospoviridae* cause significant economic losses to many crops worldwide [9]. Orthotospoviruses are characterized by quasi-spherical enveloped particles of 80-120 nm in diameter that have a tripartite antisense or ambisense RNA genome, named L, M, and S according to their size. The first eight nucleotides of the 5′-termini and 3′-termini are highly conserved among the three RNA segments and can form non-covalently closed pseudo-circular structures [2]. With the advent of next-generation sequencing (NGS) technologies for virus discovery, the number of sequenced virus genomes, including orthotospoviruses, is rapidly expanding [11]. At present, at least 29 distinct orthotospoviruses have been identified [9]. Calla lily chlorotic spot virus (CCSV) was first discovered on calla lily (*Zantedeschia* spp.) showing chlorotic spots in Taiwan area of China [4]. Later, it was found on spider lily (*Hymenocallis litteralis*) and tobacco (*Nicotiana tabacum*) in Yunnan province of China [5].

In May 2017, several celtuce plants (*Lactuca sativa* var. augustana) showing chlorotic spots on newly emerged leaves and vein banding on older leaves were collected in Changshang County of Zhejiang Province, China (Fig. 1). A small RNA (sRNA) library was constructed using symptomatic celtuce leaves and sequenced by Hangzhou Lianchuan Biological Technology Co., Ltd. (www.lc-bio.com). A total of 10,313,188 clear sRNA reads were obtained and *de novo* assembled as described earlier [10]. A total of twenty-six contigs with high similarities to the genome of orthotospoviruses, e.g. CCSV and tomato zonate spot virus (TZSV), were identified. No contig or deduced amino acid sequence that was homologous to genomic or protein sequences from other plant-infecting viruses was identified. Using SeqMan Pro 7.1.0 (Lasergene, GATC Biotech), the twenty-six contigs were further assembled into four fragments, which represented the near full length sequence of RNA L (8869 nt) and M (4732 nt), and two small fragments (1684 and 1047 nt) of RNA S, respectively. The total RNA was extracted from symptomatic celtuce leaves using the Plant RNA Purification Kit (Tiangen Biotech, Beijing, China) and reverse transcribed into cDNA using the primer Tosp-AUAP that contains the eight conserved nucleotides among all orthotospoviruses (Supplementary Table 1). The full genome of this celtuce-infecting orthotospovirus was then amplified as

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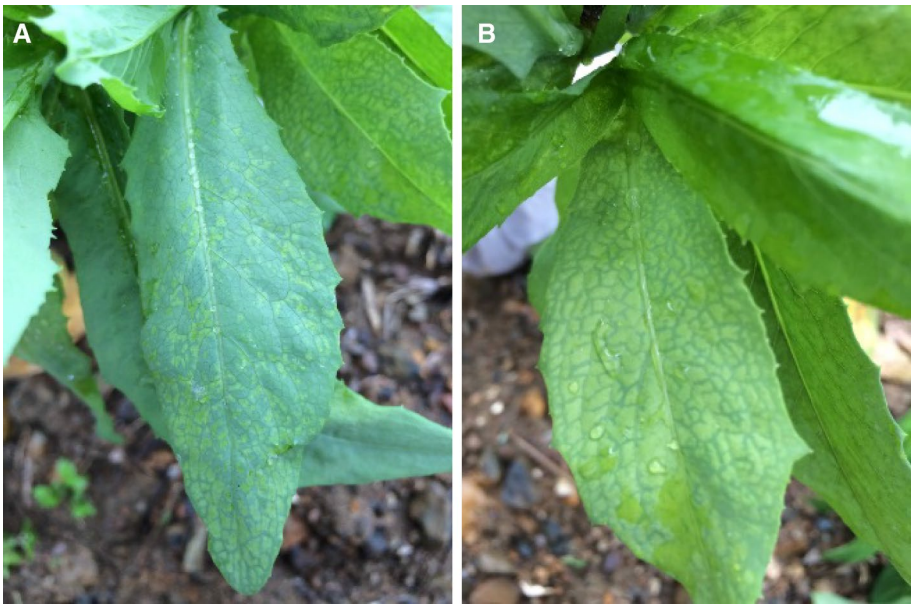

Fig. 1 The symptoms caused by CCSV-Cel on the top (A) or lower (B) leaves of celtuce

described in the Supplementary text using the primers listed in Supplementary Table 1. 5'/3' RACE was used to complete the sequences of the genome segments. The amplified fragments were ligated into the pEASY-Blunt vector (Transgen, Beijing, China) and sequenced. At least 4 clones representing each fragment were sequenced to exclude possible artificial mutations that may have been introduced during PCR amplification.

The RNA S, M, and L of this celtuce-infecting othotospovirus are 3146, 4734, and 8934 nt, respectively (accession nos: MG252780-MG252782). The RNA S has 30.4-72.5% overall nucleotide sequence identities with other orthotospoviruses (Supplementary Table 3). The NSs and N proteins share 15.31-89.13% and 16.55-90.25% amino acid (aa) sequence identities with that of known orthotospoviruses, respectively (Table 1). Moreover, the N protein has 91.9-93.27% aa sequence identities with the partial N protein of four other CCSV isolates (Table 1). With reference to the N protein criterion from the International Committee on Taxonomy of Viruses (ICTV) [1, 6], the N protein of this celtuce-infecting orthotospovirus has ≥ 90% aa sequence identity with that of CCSV; therefore, it should be classed as a divergent strain of CCSV, which we have named CCSV-Cel [China: Zhejiang: 2017]. The CCSV-Cel RNA M and L segments share 43.4-80.8% and 29.84-82.9% nucleotide sequence identities with the respective segments from known orthotospoviruses, respectively (Supplementary Table 3). The deduced amino acid sequence of the NSm and G precursor proteins share 35.24-89.32% and 28.99-89.94% aa sequence identities with that of other orthotospoviruses (Table 1). The amino acid sequence of the CCSV-Cel RdRp shares the highest aa sequence identity with of the corresponding sequence from CCSV-LJ1 [China: Yunnan: 2014] (93.55%) and the lowest identity with the RdRp from bean necrotic mosaic virus (BeNMV) (41.39%; Table 1). Phylogenetic analyses were also performed with the N, NSs, NSm, G, and RdRp protein sequences of CCSV-Cel as well as other orthotospoviruses (Supplementary Table 2) using MEGA 6 software [8]. In the reconstructed Neighbor-Joining phylogenetic trees, CCSV-Cel is closely associated with CCSV isolates and was clustered with CCSV, TZSV, and tomato necrotic spot-associated virus (TNSaV) in a sub-branch (Supplementary Figure 1). These results further confirmed that CCSV-Cel is phylogenetically related to CCSV.

Orthotospoviruses are characterized by a wide host range. For instance, the tomato spotted wilt virus (TSWV) can infect more than 800 species [7]. To date, including celtuce more than 27 species of plants have been reported to be susceptible to CCSV [3–5, 12]. Considering the wide geographic distribution and high degree of genetic diversity of CCSV, special attention should be paid to the damage that it may cause.

Table 1 Relative protein similarities for CCSV-Cel RdRp, NSm, G, NSs, and N when compared to other orthotospoviruses and other CCSV strains

	RNA L	RNA M		RNA S	
	RdRp	NSm	G	NSs	N
AINSV	-	-	-	-	26.07
BeNMV	41.39	33.86	32.14	18.57	26.67
CaCV	76.70	74.19	71.80	62.39	63.9
CCSV-Calla [China: Taiwan: 2001]	93.55	89.32	89.94	89.13	90.25
CCSV-LJ1 [China: Yunnan: 2014]	93.58	89.97	89.49	89.13	90.25
CCSV-HSS1 [China: Yunnan: 2012]	-	-	-	-	91.90*
CCSV-CX [China: Yunnan: 2012]	-	-	-	-	93.27 *
CCSV-WS5 [China: Yunnan: 2012]	-	-	-	-	93.27 *
CCSV-WS8 [China: Yunnan: 2012]	-	-	-	-	92.83 *
CSNV	45.07	38.59	34.08	20.37	25.36
GBNV	76.32	72.82	73.4	61.74	64.26
GCFSV	36.31	35.24	28.99	16.44	18.79
GRSV	45.45	39.87	35.23	18.92	26.79
GYSV	-	-	-	15.31	16.55
HCRV	66.83	60.26	60.58	48.05	39.50
INSV	44.4	38.14	33.42	18.16	21.91
IYSV	65.96	62.94	61.14	50.00	46.59
LNRV	-	-	-	16.63	17.25
MSMV	45.13	37.30	34.03	18.26	27.66
MVBaV	77.42	75.08	73.62	58.7	60.22
MYSV	73.44	59.81	64.72	46.28	56.63
PCSV	72.40	64.65	66.10	50.87	54.61
PNSV	-	-	-	18.92	26.79
PoILRV	67.24	61.54	60.77	46.97	40.57
SINaV	41.63	33.54	30.33	18.78	29.55
TCSV	45.45	40.19	34.86	19.71	24.64
TNRV	-	65.5	63.97	52.39	56.03
TNSaV	84.14	79.61	83.27	75.43	80.14
TSWV	43.48	36.98	34.34	16.8	27.14
TYRV	66.72	62.5	62.08	49.57	41.28
TZSV	88.34	88.03	88.87	81.96	79.93
WBNV	76.39	69.58	72.15	60.87	63.54
WMSoV	76.84	67.83	71.62	61.96	63.54
ZLCV	45.26	34.08	34.08	18.09	25.36

Protein sequences were aligned by ClustalW and calculated using the Sequence Manipulation Suite [8]. Note: * the identities between CCSV-Cel [China: Taiwan: 2001] and CCSV-HSS1 [China: Yunnan: 2012], CCSV-CX [China: Yunnan: 2012], CCSV-WS5 [China: Yunnan: 2012], or CCSV-WS8 [China: Yunnan: 2012] were calculated based on partial N protein sequences

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Compliance with ethical standards

Conflict of interest All authors declare that no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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