Arch Virol (2016) 161:219–222
DOI 10.1007/s00705-015-2630-9

ANNOTATED SEQUENCE RECORD

Complete genome sequence of a distinct calla lily chlorotic spot virus isolated in mainland China

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Received: 12 June 2015 / Accepted: 25 September 2015 / Published online: 23 October 2015

Abstract The first complete genome sequence of calla lily chlorotic spot virus (CCSV) from Lijiang in northwestern Yunnan Province was obtained using RT-PCR with designed primers. The genome of CCSV isolate LJ-1-Yunnan is tripartite. The small (S) RNA is 3182 nucleotides (nt) in length and encodes a nonstructural protein (NSs, 1383 nt) and a nuclear nucleocapsid (N, 834 nt), separated by an 836-nt intergenic region (IGR). The medium (M) RNA is 4749 nt in length and encodes a nonstructural movement protein (NSm, 930 nt) and a glycoprotein (GnGc, 3,372 nt), also separated by a 349-nt IGR. The large (L) RNA is 8912 nt in length and encodes a predicted RNA-dependent RNA polymerase (RdRp, 8652 nt). The nucleotide sequences of the three viral RNA segments are 92-94 % identical to the published CCSV genome sequence, and the amino acid sequences of the encoded proteins are 96-98 % identical. However, the IGRs of the S and M RNAs are less similar, with 86 and 72 % identity, respectively. Genome sequence comparisons and phylogenetic analysis indicate that the Lijiang CCSV isolate is a unique tospovirus isolate that differs from CCSV isolates in other geographic regions.

Tospovirus is the only genus in the family *Bunyaviridae* whose members infect plants [6, 11, 16]. The genome is composed of three single-stranded RNAs encoding six viral proteins. Tospoviruses infect many crops and flowers, causing serious economic losses [3, 7, 8, 12, 13, 15, 18]. Calla lily chlorotic spot virus (CCSV) was first reported from Taiwan on calla lilies (*Zantedeschia* spp.) in 2001 [4] and was identified and named as a distinct tospovirus in 2005 [1]. It is a member of the watermelon silver mottle virus (WSMoV) serogroup [9]. Previously, four isolates collected from southwest Yunnan Province were identified as CCSV, and their N gene sequences were published: WS8 (HQ115594) and WS5 (HQ115593) from *Nicotiana tabacum*, HSS1 (HQ115591) from *Hymenocallis littoralis*, and CX (HQ115592) from *N. tabacum* [10]. Until now, only one CCSV genome sequence from Taiwan [2] and four CCSV N gene sequences from southeastern Yunnan have been published in GenBank. Here, we report the first complete CCSV genome sequence from mainland China.

In 2014, CCSV LJ-1-Yunnan was isolated from *N. tabacum* plants with typical tospovirus symptoms of tip dieback, rugosity, distorted leaves, and necrotic spots on

Y. Xu and S. Wang contributed equally to this work.

The nucleotide sequence data reported in this manuscript have been deposited at the NCBI under accession numbers (KT004452, KT004453, and KT004454).

Electronic supplementary material The online version of this article (doi:10.1007/s00705-015-2630-9) contains supplementary material, which is available to authorized users.

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Fig. 1 Symptoms of CCSV LJ-1-Yunnan infection on *Nicotiana tabacum* leaves. A and B: rugose, distorted leaves and necrotic spots on veins. C: tip dieback, necrotic spots and marginal necrosis on the leaf laminas, rugosity and distorted leaves

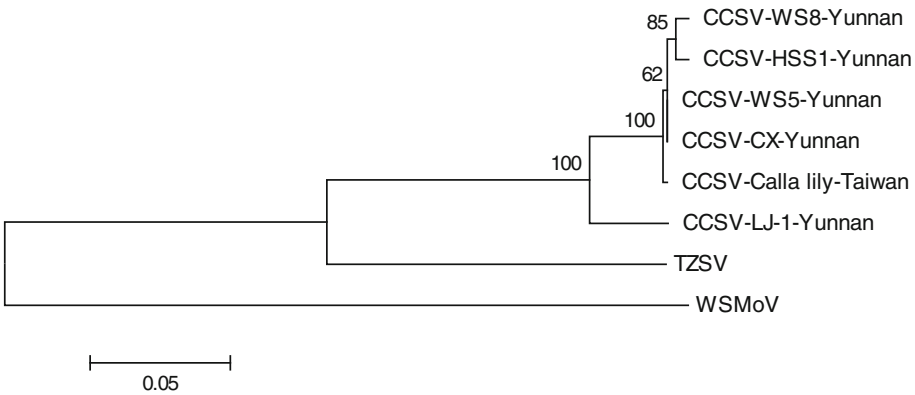

Fig. 2 Phylogenetic tree based on N nucleotide sequences. The tree was constructed using the maximum-likelihood method in the MEGA 5 program, with 1,000 bootstrap replications. The sequences used in the analyses were obtained from NCBI, with the following accession numbers: watermelon silver mottle virus, WSMoV, EU177876; tomato zonate spot virus, TZSV, KM452917; WS8-Yunnan, HQ115594; HHS1-Yunnan, HQ115591; WS5-Yunnan, HQ115593; CX-Yunnan, HQ115592; calla lily-Taiwan, AY867502; LJ-1-Yunnan, KT004452

Table 1 Comparison of genomic segments of calla lily chlorotic spot virus, isolate LJ-1-Yunnan, with those of the calla lily-Taiwan isolate

Genomic segment	S RNA (AY867502)			M RNA (FJ822961)			L RNA (FJ822962)
Nucleotide sequence identity (%)	93			92			94
Proteins/IGR	NSs	IGR	N	NSm	IGR	GnGc	RdRp
Nucleotide sequence identity (%)	95	86	94	93	72	93	94
Amino acid sequence identity (%)	96		96	98		96	98

leaves and veins (Fig. 1). This was the first case of CCSV isolated from the high-altitude region of northwest Yunnan. A PCR assay using degenerate primers suggested that the pathogen was CCSV [5]. *Nicotiana tabacum* was mechanically inoculated with CCSV LJ-1 Yunnan, and total RNAs were extracted from leaves of the infected plants. Fifteen primers were designed (Supplemental Table S1) for use in RT-PCR assays. RT-PCR, cloning, and sequencing were performed as described previously [17].

CCSV LJ-1-Yunan contained three RNA segments (Supplemental Table S2). The S RNA (KT004452) was 3182 nt in length with two open reading frames (ORFs). One ORF in the sense orientation encoded a predicted 460-amino-acid (aa) non-structural protein (NSs). The other ORF was in the antisense direction and encoded a predicted 277-aa nucleocapsid (N) protein. The NSs and N ORFs were separated by an 836-nt intergenic region (IGR). The M RNA (KT004454) was 4,749 nt in length and had two ORFs. The sense ORF encoded a predicted 309-aa non-structural protein (NSm). The antisense ORF encoded a predicted 1123-aa glycoprotein precursor (GnGc) that was that was predicted to be processed into two virion surface proteins. NSm and GnGc were separated by a 349-nt IGR. The L RNA (KT004453) was 8912 nt in length and had one ORF in the antisense orientation. The 8652-nt ORF encoded a 2883-aa predicted RNA-dependent RNA polymerase (RdRp).

Phylogenetic analysis of the CCSV N proteins was performed using the maximum-likelihood method in MEGA 5.05 (http://www.megasoftware.net/) [14]. The resulting phylogenetic tree showed that CCSV LJ-1-Yunan was distinct from other isolates from Taiwan and the lower altitudes of Yunnan (Fig. 2). A comparison between LJ-1-Yunnan and the only other complete genome sequence, a CCSV isolate from calla lily in Taiwan (S RNA: AY867502, M RNA: FJ822961, L RNA: FJ822962) [2], showed that the IGRs of the S and M segments shared 86 % and 72 % identity, respectively. The sequences of the complete viral RNA segments shared 92 to 94 % identity, and the amino acid sequence identity between the viral protein sequences of the two isolates was 96 to 98 % (Table 1).

The isolates from southeast Yunnan caused systemic chlorotic and necrotic spot symptoms on *N. tabacum* and *H. litteralis*. However, the LJ-1-Yunnan isolate from northwest Yunnan caused tip dieback, rugosity, distortion, and necrotic spots on leaves and veins of *N. tabacum.* The comparison of the available sequence data, when combined with phylogenetic analysis, suggests that LJ-1-Yunnan is a unique CCSV isolate. This is the first report of the complete genome sequence of a CCSV isolate from mainland China.

Acknowledgments This work was supported in part by grants from the National Science Foundation of China (Grant No. 31260451, 31471828, 30960224), Key New Product Development Projects in Yunnan Province (2014BB005), Reserve Personnel Fund for Young and Middle-Aged Academic Technical Leaders of Yunnan Province (2013HB071), and Academic Award for Up-and-Coming Doctoral Candidate of Yunnan Province.

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