Nucleotide sequence and taxonomy of *Cycas necrotic stunt virus*

Brief Report

S. S. Han1, A. V. Karasev2, H. Ieki1, and T. Iwanami3

1 National Institute of Fruit Tree Science, Tsukuba, Japan
2 Biotechnology Foundation Laboratories, Thomas Jefferson University, Doylestown, Pennsylvania, U.S.A.
3 National Agricultural Research Center for Kyushu and Okinawa Region, Kumamoto, Japan

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Summary. *Cycas necrotic stunt virus* (CNSV) is the only well-characterized virus from gymnosperm. cDNA segments corresponding to the bipartite genome RNAs (RNA1, RNA2) were synthesized and sequenced. Each RNA encoded a single polyprotein, flanked by the 5′ and 3′ non-coding regions (NCR) and followed by a poly (A) tail. The putative polyproteins encoded by RNA1 and RNA2 had sets of motifs, which were characteristic of viruses in the genus *Nepovirus*. The polyproteins showed higher sequence identities to *Artichoke Italian latent virus*, *Grapevine chrome mosaic virus* and *Tomato black ring virus*, all of which belong to subgroup b of the genus *Nepovirus*, than to other nepoviruses. Phylogenetic analysis of RNA dependent RNA polymerase and coat protein also showed closer relationships with these viruses than other viruses. The data obtained supported the taxonomical status of CNSV as a definitive member of the genus *Nepovirus*, subgroup b.

*Cycas necrotic stunt virus* (CNSV), a member of genus *Nepovirus* [26, 30], in the family *Comoviridae*, was found in cycas (*Cycas revoluta*) in Japan [11, 18]. Infected *C. revoluta* plants show twisting on young leaves and chlorotic or necrotic spot on the mature leaves. It is noteworthy that CNSV is the first virus from a gymnosperm to have been characterized. CNSV is efficiently transmitted

*The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the following accession number AB073147 (RNA1) and AB073148 (RNA2).
through seeds [18]. Transmission by nematode vectors has not been demonstrated. CNSV has spherical particles, about 28 nm in diameter [11]. The components of the bipartite single-stranded RNA genome, with Mr’s of 2.5 × 10^6 (RNA1) and 1.5 × 10^6 (RNA2), are separately encapsidated by a single component of coat protein (CP) [11]. Hanada et al. reported that the molecular mass of CP is about 65 K as estimated by SDS-PAGE, which is similar to that of Tomato black ring virus (TBRV), suggesting a relationship between the two viruses [11]. The TBRV isolate used by them had been identified as a beat ringspot strain on the basis of serological relationship [11, 15]. We refer it as Beet ringspot virus (BRSV), following the present classification [30], in this paper. The definite members of the genus Nepovirus are subdivided into three subgroups, on the basis of the estimated size of RNA2 [24, 30], and both CNSV and TBRV were placed in subgroup b [30]. On the other hand, no serological relationship was demonstrated between CNSV and any of the viruses in the genus Nepovirus. In this paper, we report the complete sequences of CNSV RNA1 and RNA2, and compare them with other nepoviruses to elucidate definitive relationships.

Viral RNA was extracted from purified preparation of CNSV particles as reported previously [11]. Synthesis of double-stranded cDNA was carried out, as described previously [10, 27]. The cDNA was blunt-end ligated into the Sma I site of pUC19 plasmids and used to transform Escherichia coli (strain DH5α). The cDNA clones, which were selected by northern hybridization with RNA1 and RNA2, were used for most of the sequencing analysis. Sequencing reaction of the virus specific cDNA clones and their subclones was performed using M13 forward and reverse primers, and a cycle sequencing kit (Applied Biosystems). The sequence was analyzed by an automatic sequencer (Applied Biosystems 373).

Rapid amplification of cDNA ends at the 5′ terminus (5′RACE) [7] was conducted to determine the sequences at the 5′ termini of RNA1 and 2. The nucleotide sequences were compiled using the Genetyx program package (Software Development Co., Tokyo, Japan).

The nucleotide sequence of the CNSV cDNA clone was compared to the sequences of nepoviruses available from the DDBJ, EMBL, and GenBank nucleotide sequence databases. Multiple alignment of the sequences was performed with the CLUSTALX program [29]. Phylogenetic analysis was performed using programs from the PHYLIP package, version 3.5 [6].

The N-terminal sequence of CP was determined by automated Edman degradation of viral coat protein, which were blotted on PVDF membrane after SDS-PAGE [14, 19].

The sequence of RNA1 was 7471 nucleotides in length, excluding the poly (A) tail. Computer analysis revealed a single open reading frame (ORF) in the positive sense. The predicted product from this ORF consisted of 2401 amino acids, and had a calculated molecular mass of 269064. The 5′ and 3′ non-coding regions (NCR) were 156 and 304 nucleotides long, respectively. The amino acid sequence contained conserved motifs for NTP-binding protein, VPg, viral cystein protease and RNA dependent RNA polymerase, which were characteristic of viruses of the genus Nepovirus [24].