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BRIEF REPORT

Genetic and serological characterization of chrysanthemum stem necrosis virus, a member of the genus *Tospovirus*

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Abstract Chrysanthemum stem necrosis virus (CSNV) is a member of a tentative tospovirus species. In this study, the complete genomic sequence of the Japanese CSNV isolate TcCh07A was determined. The L RNA is 8960 nt long and encodes the 331.0-kDa RNA-dependent RNA polymerase. The M RNA is 4828 nt long and encodes the 34.1-kDa movement protein (NSm) and the 127.7-kDa glycoprotein precursor (Gn/Gc). The S RNA is 2949 nt long and encodes the 52.4-kDa silencing suppressor protein (NSs) and the 29.3-kDa nucleocapsid (N) protein. The N protein of CSNV-TcCh07A was purified from virus-infected plant tissues and used for production of a rabbit polyclonal antiserum (RAs) and a monoclonal antibody (MAb). Results of serological tests by indirect ELISA and western blotting using the prepared RAs and MAb and a previously produced RAs against the N protein of tomato spotted wilt virus (TSWV) indicated that CSNV-TcCh07A, TSWV, tomato chlorotic spot virus, groundnut ringspot virus, alstroemeria necrotic streak virus and impatiens necrotic spot virus are serologically related.

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Tospoviruses are persistently transmitted by thrips and cause significant losses in yield and quality of many economically important crops worldwide [21]. The genus *Tospovirus*, named for the type species *Tomato spotted wilt virus* (TSWV), is the only genus containing plant-infecting viruses in the family *Bunyaviridae*. The virion of tospoviruses is quasi-spherical in shape and 80–110 nm in diameter. It has a characteristic lipid envelope and possesses a tripartite genome with large (L), medium (M) and small (S) ssRNAs. All three RNAs have a panhandle structure formed by base paring of complementary nucleotides (nt) at the 5′ and 3′ ends [13]. The L RNA is of negative sense and encodes an RNA-dependent RNA polymerase (RdRp) for replication and transcription [27]. Both M and S RNAs are ambisense and contain two open reading frames (ORFs), oriented in opposite directions, that are flanked by an AU-rich intergenic region (IGR). In the viral (v) sense, the M RNA encodes a movement protein named NSm [14]. In the viral complementary (vc) sense, the M RNA encodes a precursor of two glycoproteins, Gn and Gc, which form spikes on the surface of the virion envelope [12] and are responsible for thrips transmission

[29]. In the v sense, the S RNA encodes a nonstructural protein (NSs) that functions as a suppressor of RNA silencing [2, 25] and as an avirulence determinant against the natural resistance gene *Tsw* in *Capsicum annuum* [10]. In the vc sense, the S RNA encodes the nucleocapsid (N) protein, which encapsidates the genomic RNA molecules [8]. The sequence of the N gene is the most important criterion for demarcation of tospovirus species [13]. Classification of tospoviruses based on serological relationships of N proteins is practical in diagnosis and inspection [5].

Chrysanthemum stem necrosis virus (CSNV), a tospovirus causing necrotic lesions surrounded by yellow spots on leaves and necrosis on stems, peduncles and floral receptacles of chrysanthemum, was first found in Atibaia County, Brazil, in 1994 [1]. CSNV has also been reported in European and Asian countries, including the Netherlands [28], the United Kingdom [18], Slovenia [22], Belgium [9], Japan [16] and China, and can also infect tomato [1], aster and Russell prairie gentian [17]. *Frankliniella occidentalis* and *F. schultzei* have been identified as vectors of CSNV [20]. The sequences of the M and S RNAs of CSNV have been reported previously [19, 24, 26].

In this study, an isolate of CSNV denoted TcCh07A [26], collected from chrysanthemum in Tochigi Prefecture, Japan, was used for whole-genome sequencing. Total RNA was extracted from leaf tissues of CSNV-TcCh07A-infected *Nicotiana benthamiana* plants using a Plant Total RNA Miniprep Purification Kit (GMbiolab, Taichung, Taiwan) according to the manufacturer's instructions. Nucleotide sequences of tospovirus-specific degenerate primers and CSNV-specific primers used in this study are listed in Table S1. Reverse transcription (RT) was performed using 2 μg of total RNA mixed with 200 nM of individual primers and 25 U of Moloney murine leukemia virus (M-MLV) reverse transcriptase (GMbiolab). Reaction mixtures were incubated at 42 °C for 60 min and then inactivated by incubation at 72 °C for 15 min. Subsequently, cDNAs were mixed with 2.5 U of Ex *Taq* DNA polymerase (Takara, Shiga, Japan) and incubated at 94 °C for 2 min for hot start. Polymerase chain reaction (PCR) was performed with 35 cycles of strand separation at 94 °C for 30 s, annealing at 50–60 °C for 30 s (depending on the T_m values of individual primers as indicated in Table S1), and synthesis at 72 °C for 1 min. A final extension step at 72 °C for 7 min was performed. The amplified DNA fragments were analyzed by 1 % agarose gel electrophoresis and then eluted from gels using a Micro-Elute DNA Clean/Extraction Kit (GMbiolab) as per manufacturer's instructions. All amplicons were cloned using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA), and the recombinant plasmids were introduced into *E. coli* DH5α competent cells. Three clones of each amplicon were selected for sequencing using an ABI3730XL DNA Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA), performed by Mission Biotech Company (Taipei, Taiwan).

The tospoviral 5′-terminal consensus sequence (5′-AGAGCAAU-3′) and its complementary 3′-terminal sequence (5′-AUUGCUCU-3′) of the L, M and S RNAs of CSNV-TcCh07A were verified by rapid amplification of cDNA ends (RACE) [15]. First-strand cDNAs were synthesized using SuperScript® III reverse transcriptase (Invitrogen), and oligonucleotide PolyG(11g3a3g) (5′-GGGGGGGGGGGAAAGGG-3′) was ligated at the 3′ end of the cDNA molecules using terminal deoxynucleotidyl transferase (TdT). The tailed cDNA fragments were used as templates for PCR amplification by mixing them with 2.5 U of Ex *Taq* DNA polymerase (Takara), the primer PolyC(3c3t11c) (5′-CCCTTTCCCCCCCCCCC-3′) and one of the primers listed in Fig. S1. Amplified fragments were cloned using a TOPO TA Cloning kit (Invitrogen) for sequencing.

The nt sequences determined for CSNV-TcCh07A were translated into amino acid (aa) sequences using the Six-frame program of Biology Workbench, San Diego Supercomputer Center (SDSC) (http://workbench.sdsc.edu/). The available sequences of individual tospoviruses (Table S2) were obtained from GenBank for sequence analysis. Percent identities of the nt and aa sequences of CSNV-TcCh07A to those of other tospoviruses were calculated using the Gap program of SeqWeb (Accelrys Inc., San Diego, CA). Input data of multiple aa sequence alignments obtained from ClustalW (Biology Workbench, SDSC) were converted to the Phylip format for phylogenetic analysis conducted using the Phylip 3.68 package (University of Washington, Seattle, WA). Bootstrapping was performed with 1,000 repeats to generate multiple reassembled datasets using the Seqboot program. Distance matrices of aa sequences were produced using the Protdist program with PAM matrices and the Dayhoff model. Phylogenetic branches were set by the Neighbor program using the neighbor-joining method. Finally, phylogenetic trees were produced by the Consense program.

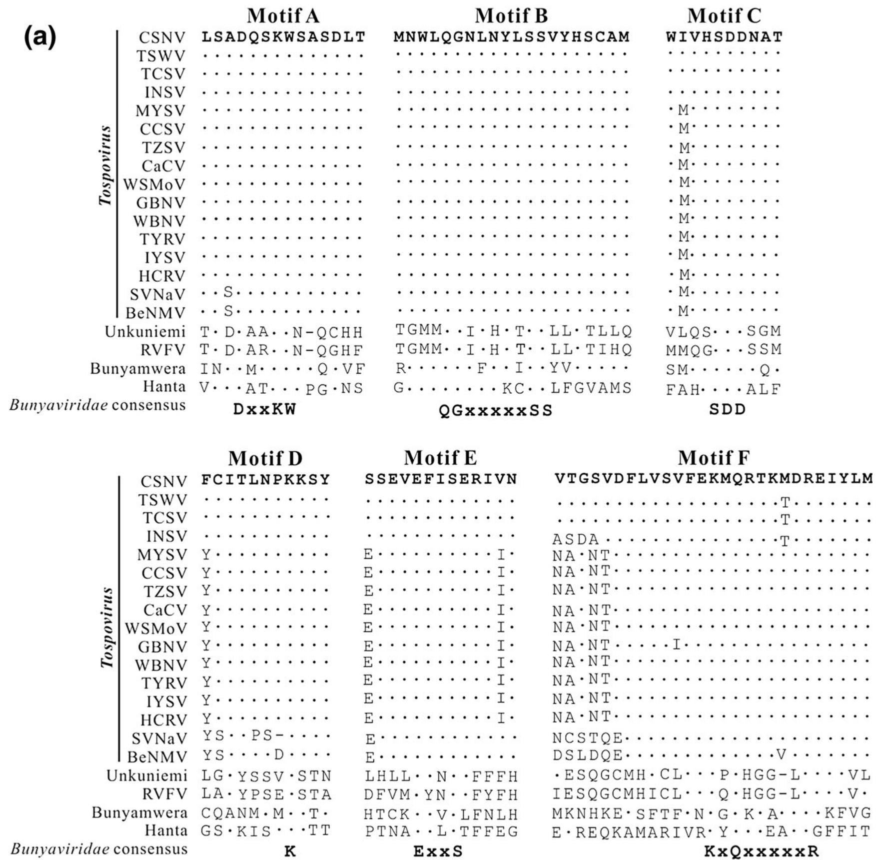
Our results showed that the full-length sequence of CSNV-TcCh07A L RNA is 8960 nt in length (accession no. KF493773), encoding a large ORF of 8625 nt for a RdRp of 2874 aa (331.0 kDa) (Fig. S1a). The 5′ and 3′ untranslated regions (UTRs) contain 33 nt and 302 nt, respectively. The RdRp ORF of CSNV-TcCh07A shares highest nt and aa identities of 69.6–78.3 % and 70.9–88.3 %, respectively, with those of impatiens necrotic spot virus (INSV), tomato chlorotic spot virus (TCSV) and TSWV and nt and aa identities of 40.0–56.1 % and 45.3–47.4 %, respectively, with those of other reported tospoviruses (Table 1). Essential motifs, including motifs A (DxxKW, aa 1367–1371), B (QGxxxxxSS, aa 1455–1463), C (SDD, aa 1493–1495), D (K, aa 1540), E

Table 1 Nucleotide (nt) and amino acid (aa) sequence identities (%) of the individual coding sequences of the chrysanthemum stem necrosis virus (CSNV) TcCh07A isolate to those of other tospoviruses

Virus	RdRp		NSm		Gn/Gc		NSs		N	
	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
ZLCV	–	–	75.7	71.0	76.5	82.5	73.1	73.0	76.6	78.8
TSWV	78.3	88.3	80.1	87.4	78.5	85.6	79.0	85.4	75.1	76.0
PNSV	–	–	–	–	–	–	79.6	85.7	75.4	75.2
TCSV	77.4	86.6	76.6	84.5	74.7	80.2	–	–	71.7	71.7
ANSV	–	–	–	–	–	–	–	–	75.0	74.0
GRSV	–	–	77.9	82.5	75.0	80.1	76.6	80.3	72.8	73.6
MeSMV	–	–	–	–	–	–	65.3	56.6	65.3	61.8
INSV	69.6	70.9	68.9	70.3	65.3	64.3	61.0	54.5	59.2	56.3
BeNMV	55.2	45.3	53.8	41.5	51.8	40.7	42.3	22.6	49.8	40.6
SVNaV	40.0	45.3	52.9	41.4	49.6	39.3	44.3	25.6	50.7	39.0
PolRSV	–	–	51.9	40.9	50.3	37.6	43.2	24.4	48.1	35.2
IYSV	55.8	47.4	52.2	39.5	50.3	38.3	42.1	25.8	47.9	34.9
HCRV	57.5	46.0	56.9	38.4	52.5	33.2	42.3	25.7	49.5	33.7
TYRV	56.1	47.3	52.3	39.7	49.7	37.9	44.1	27.3	49.4	33.6
MYSV	55.1	46.5	53.8	42.2	49.9	36.5	40.9	23.3	47.3	34.0
CaCV	55.9	47.0	51.3	40.2	50.1	37.6	42.1	24.0	47.7	32.1
GBNV	55.6	47.1	53.8	42.4	49.7	37.8	42.4	23.2	46.2	33.7
WBNV	55.9	47.0	53.2	40.5	49.8	37.2	41.3	22.3	47.7	32.1
WSMoV	56.0	46.9	54.9	39.9	50.4	38.1	40.1	23.0	47.7	33.3
TZSV	55.4	46.2	52.8	41.9	49.8	38.1	44.4	26.8	46.2	30.8
TNRV	–	–	50.9	41.9	49.1	36.4	40.6	20.4	46.1	31.1
CCSV	55.4	46.1	54.8	41.9	50.0	37.3	44.2	25.3	46.2	30.0
LNRV	–	–	–	–	–	–	42.9	21.8	45.4	30.7
GYSV	–	–	–	–	–	–	40.4	19.8	45.4	25.6
GCFSV	–	–	–	–	–	–	37.7	28.6	46.5	27.7
PCSV	–	–	–	–	–	–	45.1	19.4	43.9	24.4

(ExxS, aa 1550–1553) and F (KxQxxxxxR, aa 1286–1294) are present in the deduced aa sequence (Fig. 1a). We noticed that motifs A to E are identical within the RdRps of CSNV, TSWV, TCSV and INSV. The same was also observed for melon yellow spot virus (MYSV), calla lily chlorotic spot virus (CCSV), tomato zonate spot virus (TZSV), capsicum chlorosis virus (CaCV), watermelon silver mottle virus (WSMoV), groundnut bud necrosis virus (GBNV), watermelon bud necrosis virus (WBNV), tomato yellow ring virus (TYRV), iris yellow spot virus (IYSV) and hippeastrum chlorotic ringspot virus (HCRV), as well as soybean vein necrosis-associated virus (SVNaV) and bean necrosis mosaic virus (BeNMV). This suggests a co-evolutionary relationship within distinct tospovirus groups.

The complete M RNA sequence of CSNV-TcCh07A is 4828 nt in length (accession no. KF493772), consisting of two ORFs, NSm and Gn/Gc, in an opposite orientation, separated by an AU-rich IGR (Fig. S1b). The sequences of the 5′-UTR, 3′-UTR and IGR of the M RNA consist of 101 nt, 83 nt and 324 nt, respectively. The NSm ORF is 912 nt in length, encoding a protein of 303 aa (34.1 kDa), and the Gn/Gc ORF is 3408 nt in length, encoding a glycoprotein precursor (GP) of 1135 aa (127.7 kDa). The NSm ORF shares 99.3 % nt and aa sequence identity with the original Brazilian isolate, and the Gn/Gc ORF shares 97.6 % nt sequence identity and 98.9 % aa sequence identity with the Brazilian Chry-1 isolate (Table S3). Additionally, the NSm ORF shares nt and aa sequence identities of 68.9–80.1 % and 70.3–87.4 %, respectively, with those of groundnut ringspot virus (GRSV), TCSV, TSWV, zucchini lethal chlorosis virus (ZLCV) and INSV, and lower nt and aa sequence identities of 50.9–54.9 % and 38.2–42.4 %, respectively, with those of other tospoviruses (Table 1). The Gn/Gc ORF shares nt and aa sequence identities of 74.7–78.5 % and 80.1–85.6 %, respectively, with those of GRSV, TCSV, TSWV and ZLCV; 65.3 % nt sequence identity and 64.3 % aa sequence identity with that of INSV; and lower nt and aa sequence identities of 38.7–51.8 % and 32.0–40.7 %, respectively, with those of other tospoviruses (Table 1).



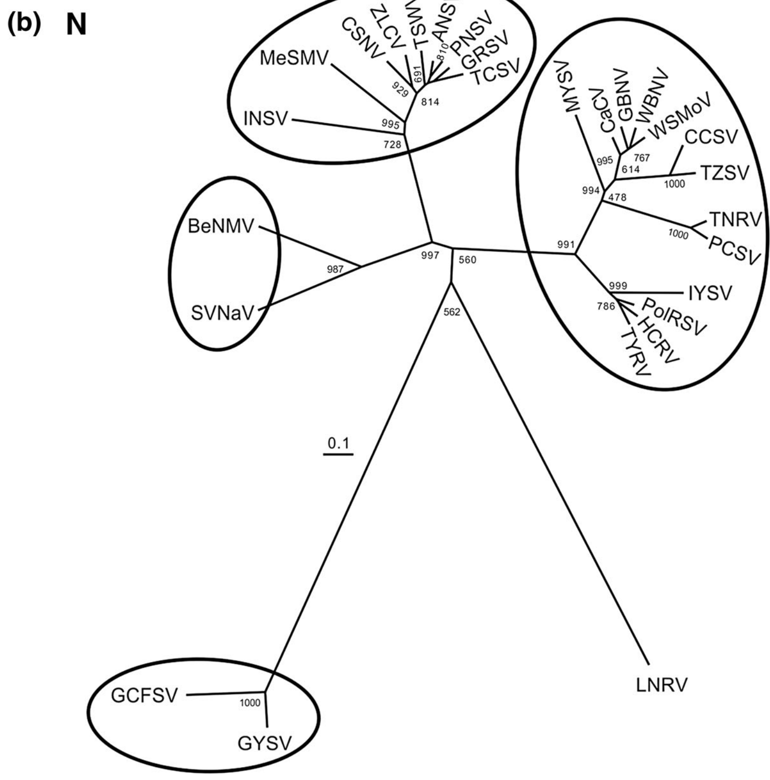


Fig. 1 Analysis of the RNA-dependent RNA polymerases (RdRps) and nucleocapsid (N) proteins of tospoviruses. (**a**) Conserved motifs of RdRps of tospoviruses are aligned. Consensus sequences within individual motifs of members of the family *Bunyaviridae* are indicated. Accession codes of the analyzed sequences of tospoviruses are listed in Table S2. The phleboviruses Uukuniemi virus (Uukuniemi, D10759) and Rift Valley fever virus (RVFV, X564664), the orthobunyavirus Bunyamwera virus (Bunyamwera, X14383), and the hantavirus Hantaan virus (Hanta, X55901) were used for comparison. (**b**) Phylogenetic dendrogram of tospoviruses based on the N protein using the neighbour-joining algorithm with 1000 bootstrap replicates. Viruses with a close relationship are circled

The S RNA sequence of CSNV-TcCh07A was found to be 2949 nt in length (accession no. KF493771), consisting of NSs and N ORFs in opposite directions flanked by a 531-nt AU-rich IGR (Fig. S1c). The 5′- and 3′-UTRs of the S RNA are 79 nt and 152 nt long, respectively. The NSs ORF is 1404 nt in length, encoding a 467-aa (52.4-kDa) protein, and the N ORF is 783 nt in length, encoding a 260 aa (29.3 kDa) protein. The nt sequence of CSNV-TcCh07A S RNA was compared to that of another Japanese isolate, HiCh06A L1 (accession no. AB600873) [26], and it was found that these two CSNV isolates share a high degree of identity (98.2 %). The NSs ORFs of both TcCh07A and HiCh06A L1 isolates share 97.9 % and 91.3 % nt and aa sequence identity, respectively. In addition, the NSs protein of TcCh07A is 3 aa longer than that of HiCh06A L1. Different host responses upon infection with TcCh07A and HiCh06A L1 have been reported previously [26]. Whether the NSs protein plays a role in host range determination remains to be investigated. The N ORF of CSNV-TcCh07A shares 98.5–99.0 % and 98.8–100 % nt and aa sequence identity, respectively, with those of different isolates of CSNV available in GenBank (Table S3). The N ORF shares nt and aa sequence identities of 71.7–76.6 % and 71.7–78.8 %, respectively, with those of ZLCV, TSWV, pepper necrotic spot virus (PNSV) [6], alstroemeria necrotic streak virus (ANSV), GRSV and TCSV; 59.2–65.3 % and 56.3–61.8 %, respectively, with those of melon severe mosaic virus (MeSMV) and INSV; and 43.9–50.7 % and 24.4–40.6 %, respectively, with those of other known tospoviruses (Table 1). The NSs ORF shares 73.1–79.6 % nt and 73.0–85.7 % aa sequence identity with those of PNSV, TSWV, GRSV and ZLCV; 61.0–65.3 % nt and 54.5–56.6 % aa sequence identity with those of MeSMV and INSV; and 37.7–44.4 % nt and 19.8–28.6 % aa sequence identity with those of other tospoviruses (Table 1).

Phylogenetic relationships based on the RdRp, Gn/Gc, NSm, NSs and N proteins showed that the current tospoviruses can be clustered into five evolutionary clades (Fig. 1b and Fig. S2). The first group is designated as the TSWV-related (T) clade. The second group is regarded as the WSMoV-related (W) clade. The third group is referred to as the groundnut yellow spot virus (GYSV)-related (G) clade. The fourth group is denoted as the SVNaV-related (S) clade. Lisianthus necrotic ringspot virus (LNRV) [23] is distantly related to other tospoviruses and thus referred as the fifth (L) clade. To date, at least 27 tospoviruses have been characterized (Table 1), and most of them belong to the T and W clades.

The N protein of CSNV-TcCh07A was purified from virus-infected leaf tissues of *Chenopodium quinoa* plants by a previously described method [30]. The expected band corresponding to the N protein was visualized by preparative gel electrophoresis after the gel was submerged in cold (4 °C) 0.25 M KCl solution. The N protein in gel slices was eluted from the polyacrylamide gel using a Model 442 Electro-Eluter (Bio-Rad, Hercules, CA). The yield of purified N protein was estimated using the software Spot Density (AlphaInnotech Corporation, San Leandro, CA) by comparison with known amounts of bovine serum albumin (BSA).

One hundred fifty micrograms of the purified N protein dissolved in 1 ml of phosphate-buffered saline (PBS) was emulsified with an equal volume of Freund's complete adjuvant (Difco Laboratories, BD, NJ) for subcutaneous injection of a New Zealand white rabbit. Subsequently, the same amount of immunogen in 1 ml of PBS emulsified with an equal volume of Freund's incomplete adjuvant (Difco Laboratories) was administered weekly into the immunized rabbit for three weeks. The antiserum, denoted as RAs-CSNV-N, was collected weekly from the marginal veins of the ear for three months, starting one week after the fourth injection.

For preparation of a monoclonal antibody, 6- to 8-week-old BALB/cByJ mice were first immunized with 50 µg of the purified N protein (in 250 µl of PBS) emulsified with an equal volume of Freund's complete adjuvant (Difco Laboratories) by intraperitoneal injection. The same amount of immunogen emulsified with Freund's incomplete adjuvant (Difco Laboratories) was used for two additional intraperitoneal injections at weekly intervals. Mice were sacrificed three days after the fourth intraperitoneal injection without adding adjuvant to collect splenocytes that were then fused with Spll/0-ag/14 myeloma cells. Hybridoma cells were screened by indirect enzyme-linked immunosorbent assay (ELISA). The selected hybridoma cell lines were further cloned via limiting dilution. Finally, one stable hybridoma cell line (named S217D4C10) was obtained. The monoclonal antibody, denoted MAb-CSNV-N, was obtained from ascitic fluids of the Pristane-primed BALB/cByJ mice injected with S217D4C10 hybridoma cells.

The titer and sensitivity of RAs-CSNV-N and MAb-CSNV-N were determined by indirect ELISA as described previously [4]. Crude extracts of virus-infected plant tissues at a 1/50 dilution in coating buffer (50 mM Na_2CO_3, pH 9.6, containing 0.01 % NaN_3) or 10-fold serial dilutions (1 pg to 1 mg) of the purified N protein of CSNV-TcCh07A dissolved in coating buffer were incubated with RAs-CSNV-N or MAb-CSNV-N at 37 °C for 30 min. The alkaline phosphatase (AP)-conjugated goat anti-rabbit or anti-mouse IgGs (Jackson Immuno Research Laboratories, Inc., West Grove, PA) were diluted 1/5000 in conjugate buffer (PBST containing 2 % PVP-40 and 0.2 % ovalbumin) to react with RAs-CSNV-N and MAb-CSNV-N, respectively. The absorbance at 405 nm was recorded

using a Model 680 microplate reader (Bio-Rad) 1–2 h after the addition of enzyme substrate ρ-nitrophenyl phosphate disodium hexahydrate (ρ-NPP-Na) (GMbiolab). The results showed that the endpoint dilution of RAs-CSNV-N was 1/32000. When RAs-CSNV-N was used at a 10^{-3} dilution, the sensitivity was determined to be 10 ng of purified N protein. The endpoint dilution of MAb-CSNV-N was determined to be 10^{-6}, and 10^{-4}-diluted MAb-CSNV-N was used in further assays. One nanogram of purified CSNV N protein was detected by MAb-CSNV-N at the 10^{-4} dilution.

Western blotting was also conducted for serological assays of RAs-CSNV-N and MAb-CSNV-N following previously published procedures [11]. Leaves of virus-infected *N. benthamiana* plants were ground in protein sample buffer (12.5 mM Tris-HCl, pH 6.8, 10 % glycerol, 2 % SDS, 2 % β-mercaptoethanol and 0.001 % bromophenol blue) at a 1/50 dilution. RAs-CSNV-N and MAb-CSNV-N were used at 10^{-3} and 10^{-4} dilutions, respectively. A rabbit antiserum against the bacterial-expressed N protein of TSWV, denoted RAs-TSWV-N [3], was used at a 1/4000 dilution for comparison. The corresponding secondary antibodies were used at a 1/5000 dilution. The tospoviruses ANSV, CSNV-TcCh07A, CaCV, CCSV, GBNV, groundnut chlorotic fan-spot virus (GCFSV), GRSV, INSV, IYSV, MYSV, SVNaV, TCSV, TSWV, TYRV, WBNV and WSMoV were used to test the response of RAs-CSNV-N and MAb-CSNV-N. All tospoviruses were isolated from single lesions and identified by RT-PCR using individual species-specific primer pairs to confirm the serological results (data not shown). RAs-CSNV-N positively reacted with ANSV, GRSV, TCSV and TSWV as well as CSNV-TcCh07A in indirect ELISA and western blotting (Fig. 2a). Similarly, RAs-TSWV-N [3] positively reacted with ANSV, CSNV-TcCh07A, GRSV, INSV and TCSV as well as TSWV in western blotting (Fig. 2c). Noteworthily, MAb-CSNV-N positively reacted not only with CSNV-TcCh07A but also with INSV in both indirect ELISA and western blotting (Fig. 2b). Sequence alignment revealed that a consensus sequence "YANILKAC" is present in the N proteins of CSNV, ZLCV and INSV. Whether this is the epitope recognized by MAb-CSNV-N will be investigated further.

CSNV seriously damages chrysanthemum, which is one of the most economically important ornamentals worldwide. Development of serological tools for virus diagnosis and detection allows researchers and farmers to inspect plants and prevent the disease caused by CSNV. Both RAs-CSNV-N and MAb-CSNV-N were used to detect CSNV infection of diseased chrysanthemum samples collected from the field in Japan. The results showed that positive reactions can be obtained when both RAs-CSNV-N and MAb-CSNV-N are tested against chlorotic tissues, but not asymptomatic tissues. Virus particles present in a 100-fold dilution of the sap from symptomatic chrysanthemum tissue can be easily detected by RAs-CSNV-N. The use of MAb-CSNV-N increases the detection sensitivity up to a 1000-fold dilution of the sap (Fig. S3). Thus, sampling from chlorotic tissues coupled with detection using MAb-CSNV-N is recommended for CSNV detection. RAs-CSNV-N and MAb-CSNV-N were also used for a field survey of the chrysanthemum-growing farms of central Taiwan, and no CSNV-infected samples were detected. Although CSNV has not yet been found in Taiwan, one of its insect vectors, *F. schultzei*, is present. In addition, Taiwan neighbors with Japan and China, where CSNV

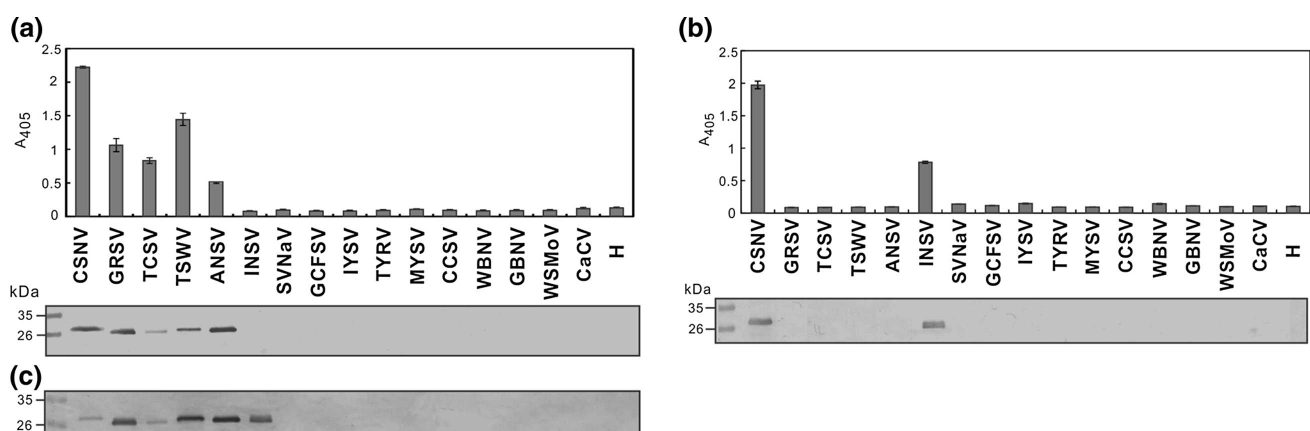


Fig. 2 Serological reactions of rabbit polyclonal antiserum RAs-CSNV-N (**a**) and monoclonal antibody MAb-CSNV-N (**b**) with different tospoviruses. Crude leaf extracts of *Nicotiana benthamiana* plants infected with individual tospoviruses (CSNV-TcCh07A, GRSV, TCSV, TSWV, ANSV, INSV, SVNaV, GCFSV, IYSV, TYRV, MYSV, CCSV, WBNV, GBNV, WSMoV and CaCV; full virus names are listed in Table S2) were used for indirect enzyme-linked immunosorbent assays (top panel) and western blotting (lower panel). (**c**) Antiserum against the N protein of TSWV (RAs-TSWV-N) [3] was used for comparison in western blotting. An uninfected *N. benthamiana* plant (H) was used as a negative control

occurs, and this implies a high risk of introduction. Since chrysanthemum is an important cut flower that is exported from Taiwan to Japan, prevention of CSNV occurrence is highly relevant.

Here, we report for the first time the complete genomic sequence of a CSNV isolate and propose that tospoviruses can be divided into five evolutionary groups. Moreover, our findings confirm previous results showing that CSNV antibodies cross-react with TSWV, TCSV and MeSMV [7], and this extends the serological relationship of CSNV with GRSV, ANSV and INSV, as reciprocally verified using RAs-TSWV-N [3] (Fig. 2). Serological relationships among tospoviruses generally match with phylogenetic groupings. Verification of serological relatedness among tospoviruses is helpful for prompt detection of tospovirus infections in field.

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