Detection of Eight Different Tospovirus Species by a Monoclonal Antibody against the Common Epitope of NSs Protein

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Abstract

Rabbit antisera against the nucleocapsid protein (NP) have been commonly used for detection of tospoviruses and classification into serogroups or serotypes. Mouse monoclonal antibodies (MAbs) with high specificity to the NPs have also been widely used to identify tospovirus species. Recently, a serogroup-specific MAb against the NSs protein of *Watermelon silver mottle virus* (WSMoV) was produced by our laboratory to react with five members of WSMoV serogroup, i.e., WSMoV, *Capsicum chlorosis virus* (CaCV), *Calla lily chlorotic spot virus* (CCSV), *Peanut bud necrosis virus* (PBNV) and *Watermelon bud necrosis virus* (WBNV). The epitope recognized by the NSs MAb was determined and the comparison with the reported sequences of tospoviral NSs proteins revealed that the epitope is highly conserved at the N-terminal region of NSs proteins among members of WSMoV and *Iris yellow spot virus* (IYSV) serogroups, and *Melon yellow spot virus* (MYSV) serotype. When the NSs MAb was further used to react with the crude antigens of MYSV serotype, IYSV and *Tomato yellow ring virus* (TYRV) of IYSV serogroup, strong serological reactions, both in ELISA and western blotting, were observed. Thus, our results indicated that the NSs MAb is a useful and convenient tool for detection of the eight tospovirus species. It is also suggested that these eight Asian-type tospoviruses, i.e., WSMoV, CaCV, CCSV, PBNV, WBNV, MYSV, IYSV and TYRV, may share a common evolutionary ancestor.

INTRODUCTION

Tospovirus is the only plant-infecting genus among the five genera of the family *Bunyaviridae*. An enveloped quasi-spherical particle of *Tospovirus* contains three single-stranded (ss) nucleic acid segments, denoted as L, M and S RNAs (Fauquet et al., 2005). The L RNA is negative sense, encoding an RNA-dependent RNA polymerase in the viral complementary (vc) strand for replication (de Haan et al., 1991). Both M and S RNAs are ambisense. The viral (v) strand of M RNA encodes an NSm protein for movement by forming tubule structures (Kormelink et al., 1994; Lewandowski and Adkins, 2005); and its vc strand encodes Gn and Gc glycoproteins composing spikes on the envelope (Kormelink et al., 1992). The S RNA encodes a nucleocapsid protein (NP) in the vc strand that encapsidates viral RNAs (de Haan et al., 1990); and its v strand encodes an NSs protein that forms filamentous inclusion bodies (Kormelink et al., 1991) and is a gene-silencing suppressor (Bucher et al., 2003; Takeda et al., 2002).

Sequence homology of NPs is the most important descriptor for establishing a distinct species and classifying tospoviruses into serogroups and serotypes (Fauquet et al., 2005). Serological and phylogenetic relationships of NPs indicate that the current 16 tospovirus species are clustered into three serogroups and four distinct serotypes (Lin et al., 2005). Viruses serologically related to *Tomato spotted wilt virus* (TSWV) are grouped into the TSWV serogroup. Viruses having serological relationships with *Watermelon silver mottle virus* (WSMoV) are clustered in the WSMoV serogroup. *Iris yellow spot virus* (IYSV) and *Tomato yellow ring virus* (TYRV) share a high degree of homology are

grouped in the IYSV serogroup. In addition, *Impatiens necrotic spot virus* (INSV), *Peanut yellow spot virus* (PYSV), *Peanut chlorotic fan-spot virus* (PCFV), and *Melon yellow spot virus* (MYSV) having no serological relationships with any other tospoviruses are classified as monospecies serotypes.

Due to instability of tospoviruses, the purification of sufficient amounts of their individual proteins directly from the infected plant tissues for investigations is difficult. Recently, the various tospoviral NPs and the NSs protein of WSMoV were successfully expressed by a *Zucchini yellow mosaic virus* (ZYMV) vector for production of polyclonal antibodies (PAbs) and monoclonal antibodies (MAbs) (Chen et al., 2005b, 2006). In our previous study, the MAb against the NSs protein of WSMoV, denoted MAb-WNSs, exhibits serogroup specificity by reacting with the members of WSMoV serogroup. Epitope scanning revealed that the MAb-WNSs-recognized epitope is conserved among all members of WSMoV serogroup (Chen et al., 2006).

In this investigation, the MAb-WNSs was further used to react with the crude antigens of MYSV, IYSV and TYRV. Our results indicated that the MAb-WNSs is a useful and convenient tool for detecting eight different Asian tospovirus species.

MATERIALS AND METHODS

Virus Sources

WSMoV isolated from watermelon (Yeh et al., 1992) and CCSV isolated from calla lily (Chen et al., 2005a), both originated in Chinese Taipei. A high temperature-recovered gloxinia isolate (HT-1) of CaCV from the United States (Hsu et al., 2000). IYSV (Cortes et al., 1998) and TYRV (Hassani-Mehraban et al., 2005) were kindly provided by R. Kormelink. TSWV isolated from tomato in New York (TSWV-NY) was provided by R. Provvidenti. PCFV isolated from peanut (Chu et al., 2001) and MYSV isolated from watermelon (Chen et al., 2008) were also originated in Chinese Taipei. All tospoviruses were mechanically maintained in *Nicotiana benthamiana* and *Chenopodium quinoa*.

Amino Acid Alignment of the NSs Proteins

Amino acid sequences of the reported NSs proteins of the tospoviruses, CaCV, CCSV, IYSV, MYSV, PBNV, TYRV and WSMoV, for analysis were obtained from databases under the following accession numbers: CaCV HT-1, AAC15500; CCSV, AAW58114; IYSV, AF001387; MYSV, AB038343; PBNV, AAB04144; TYRV, AY686718; and WSMoV, AAB36955. Multiple sequence alignments were carried out using the ClustalW program version 1.82 (Thompson et al., 1994).

Western Blotting

Crude antigens from leaves of tospovirus-infected *N. benthamiana* plants were extracted with a 50-fold dilution in dissociation buffer (100 mM Tris-HCl, pH 7.2, 2% β-mercaptoethanol, 10% sucrose, 0.005% bromophenol blue, and 10 mM EDTA). The MAb-WNSs were used at a 1.0 × 10-4 dilution to react with all tested tospoviruses. The alkaline phosphatase (AP)-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was used at a dilution of 1:5000 as the secondary antibody for detection of mouse antibodies. Reactions were visualized by the addition of chromogenic substrate (nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate paratoluidine salt in 100 mM NaCl, 5 mM $MgCl_2$, and 100 mM Tris-HCl, pH 9.5).

Indirect ELISA

Crude extracts of tospovirus-infected *N. benthamiana*, diluted 50-fold in coating buffer (50 mM sodium carbonate, pH 9.6, containing 0.01% sodium azide), were used as coating antigens. The MAb-WNSs were used at a 1.0 × 10-4 dilution to react with the tested samples. The AP-conjugated goat anti-mouse IgG was used at a 1:5000 dilution as the secondary antibody. The absorbance at 405 nm (A405) was determined using an

ELx800 universal microplate reader (Bio-Tek instrument, Winooski, VT) 30 to 60 min after the addition of ρ-nitrophenyl phosphate substrate (Sigma-Aldrich, St. Louis, MO) dissolved in substrate buffer (9.7% diethanolamine and 0.02% sodium azide, pH 9.8).

RESULTS

The MAb-WNSs-Targeted Region is Conserved among the NSs Proteins of Asian-Type Tospoviruses

Our previous report described that the MAb-WNSs reacts with the members of WSMoV serogroup, including WSMoV, CaCV, CCSV, PBNV and WBNV; and confirmed the serogroup specificity of MAb-WNSs (Chen et al., 2006). In this investigation, we compared the sequences of NSs proteins of other Asian tospoviruses with those of WSMoV serogroup. The conserved WNSscon was also found to be present in the NSs proteins of MYSV, IYSV and TYRV (Fig. 1). Our results of sequence analysis implied that the MAb-WNSs may react with the NSs proteins of MYSV, IYSV and TYRV.

Western Blotting

To test the hypothesis from sequence comparison, the MAb-WNSs was used to react with the crude extracts from *N. benthamiana* plants infected with different tospoviruses, including WSMoV, CaCV, CCSV, MYSV, IYSV, TYRV, TSWV, and PCFV, by serological reactions. In western blotting, the deduced molecular sizes of approximately 50 kDa corresponding to the NSs protein were detected from 6 of 8 tested samples, i.e., WSMoV, CaCV, CCSV, MYSV, IYSV and TYRV (Fig. 2). No signals were detected from other samples of TSWV and PCFV. Each tospovirus was identified by the individual MAbs or PAbs against the NP to avoid contamination (data not shown).

Indirect ELISA

In addition, serological comparison was also analyzed by indirect ELISA. The MAb-WNSs positively reacted with six crude antigens of WSMoV, CaCV, CCSV, IYSV, TYRV and MYSV, but did not react with samples of TSWV and PCFV (Fig. 3). Combined with our previous report (Chen et al., 2006), the results indicated that the MAb-WNSs can be used to detect eight different tospovirus species of two serogroup and one serotype, including WSMoV, CaCV, CCSV, PBNV and WBNV belonging to the WSMoV serogroup; IYSV and TYRV belonging to the IYSV serogroup; and MYSV that is a distinct serotype.

DISCUSSION

For the detection of NSs protein, the antibody specifically against the NSs protein of TSWV has been described earlier (Kormelink et al., 1991). Subsequently, an oligopeptide of 24 aa, YFLSKTLEVLPKNLQTMSYLDSIQC, developed from a conserved sequence at the C-terminal region of the NSs proteins of serogroup I to IV of tospoviruses was synthesized for production of PAbs. The PAbs were able to broadly react with NSs proteins of TSWV (serogroup I), GRSV, TCSV (serogroup II) and INSV (serogroup III), but unfortunately, not with WSMoV (serogroup IV), in western blotting and ELISA (Heinze et al., 2000). As our understanding now, TSWV, GRSV and TCSV are grouped as the TSWV serogroup, and INSV is a monospecies serotype, but phylogenetically related to TSWV serogroup (Lin et al., 2005). Also, they are classified as the European-American type. Thus, the produced PAbs were assumed as a tool for detection of the European-American type of tospoviruses.

As previously reported, the NSs protein of WSMoV was expressed by a ZYMV vector for production of PAbs and MAbs (Chen et al., 2006). The produced PAbs specifically and strongly reacted with the NSs protein of WSMoV and weakly reacted with that of CaCV; however, the produced MAbs (MAb-WNSs) broadly reacts with those of CaCV, PBNV, WBNV, CCSV and WSMoV. The MAb-WNSs-recognized epitope is

conserved among those of tospoviruses belonging to the WSMoV serogroup. Therefore, the MAb-WNSs was considered as a serogroup-specific antibody (Chen et al., 2006).

The tospoviruses of WSMoV serogroup distributed in Asian areas are typical Asian-type viruses. Five additional tospoviruses, MYSV, IYSV, TYRV, PCFV and PYSV, were also found in Asian areas. All of them are considered as Asian-type tospoviruses. In this study, we compared the sequences of NSs proteins among the Asian-type tospoviruses, the consensus WNSscon sequence were also found within the N-terminal region of NSs proteins of MYSV, IYSV and TYRV (Fig. 1). Subsequently, our results showed that the MAb-WNSs serologically reacted with the NSs proteins of MYSV, IYSV and TYRV in western blotting and ELISA as well (Figs. 2 and 3). The results matched well with the comparison of NSs sequences. Our results indicate that the MAb-WNSs is a useful and convenient tool for detection of the eight Asian-type tospoviruses, WSMoV, CaCV, CCSV, PBNV, WBNV, MYSV, IYSV and TYRV. The presence of the highly conserved WNSscon in the NSs proteins of these tospovirus species suggested that all these Asian tospoviruses may share a common evolutionary ancestor.

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Figures

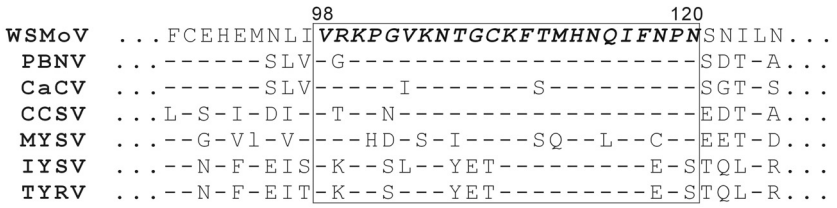

Fig. 1. Comparison of the MAb-WNSs-targeted region of the NSs proteins of Asian-type tospoviruses. Sequences of the aa 89-125 of NSs proteins of WSMoV, PBNV, CaCV, CCSV, MYSV, IYSV and TYRV are shown. A consensus sequence VRKPGVKNTGCKFTMHNQIFNPN (denoted WNSscon) is boxed in bold and italic. The position of WNSscon corresponding to the aa 98-120 of NSs protein is indicated.

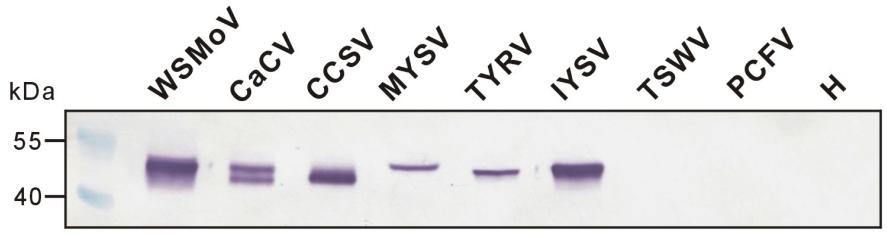

Fig. 2. Serological reactions of the MAb-WNSs with the NSs proteins of different tospoviruses in western blotting. A protein with molecular size about 50 kDa was detected from the crude extracts of *N. benthamiana* inoculated with WSMoV, CaCV, CCSV, MYSV, IYSV and TYRV, whereas no signals were detected from the samples of TSWV and PCFV. The crude extract from a healthy plant of *N. benthamiana* (H) was used as a negative control.

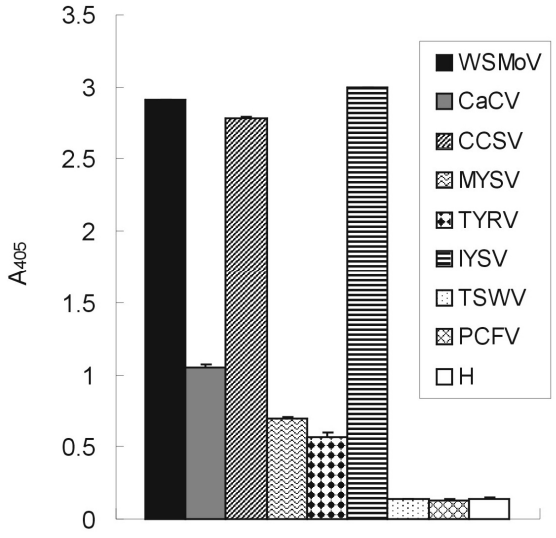

Fig. 3. Indirect ELISA using monoclonal antibody MAb-WNSs to react with the NSs proteins of different tospoviruses. Positive reactions were obtained from the samples of *N. benthamiana* inoculated with WSMoV, CaCV, CCSV, MYSV, IYSV and TYRV. No signals were recorded in the samples of *N. benthamiana* inoculated with TSWV and PCFV. A healthy plant of *N. benthamiana* (H) was used as a negative control.