LETTER TO THE EDITOR

P22 of tomato chlorosis virus, an RNA silencing suppressor, is naturally expressed in the infected plant

R. ZHAO1†, N. WANG1†, S. LIU1, K. LING2, Z. FAN1, T. ZHOU1*

1State Key Laboratory of Agrobiotechnology and Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China; 2U.S. Department of Agriculture, Agricultural Research Service, U.S. Vegetable Laboratory, Charleston, SC 29414, USA

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Since its first discovery nearly two decades ago (1), tomato chlorosis virus (ToCV) (the genus Crinivirus of the family Closteroviridae) has emerged as a serious disease problem in field and greenhouse tomato production worldwide (2–9). The infected tomato plants exhibit symptoms including interveinal chlorosis, leaf brittleness, and limited necrotic flecking or leaf bronzing (10). ToCV has a bipartite genome of single-stranded positive-sense RNA (1). RNA 1 encodes four open reading frames (ORFs) and RNA 2 encodes up to nine ORFs. The proteins are not well studied and their putative functions are associated with virus replication, encapsidation, movement, and whitefly transmission (11–13). The p22 protein encoded in the RNA1, as well as the major (CP) and minor (CPm) coat proteins encoded in the RNA2 of ToCV are implicated as RNA silencing suppressors (RSS) (14). Until now, the CP is the only viral protein being detected in ToCV-infected tomato plants (15). In vitro assays demonstrated that the p22 protein is bound preferentially to the long dsRNAs, thus protecting these dsRNAs from dicer cleavage (16). Since an antibody is not yet available for p22, until now, there is no direct evidence showing p22 is expressed naturally in the ToCV-infected plants. The objective of the present study was to provide a direct physical evidence for the presence of p22 protein in a ToCV-infected plant. By developing an infectious clone of ToCV with a triple-FLAG tag fused to the C-terminus of p22, using an anti-FLAG monoclonal antibody, we present here, for the first time, a physical evidence for the natural expression of p22 in ToCV-infected Nicotiana benthamiana plant.

To develop an infectious cDNA clone of ToCV Beijing isolate (ToCV-BJ) (17), reverse transcription and polymerase chain reactions (RT-PCR) were used to synthesize and amplify full-length cDNA of ToCV-BJ RNA 1 and 2. Viral genomic RNAs were prepared from purified virions. Specific primers were designed based on the complete genome sequence of ToCV-BJ (accession number KC887998 and KC887999) (17). First strand cDNA was synthesized with moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, USA) according to the manufacturer’s protocol. PCR amplifications were performed using Phusion® High-fidelity DNA Polymerase (New England Biolabs, Kallang, Singapore). The full-length cDNAs of ToCV RNA1 and 2 were cloned under a double 35S promoter in the binary vector pCass-RZ (18) to produce the two constructs pCa-ToCR1 and pCa-ToCR2 for ToCV RNA1 and RNA2, respectively. Biological activity of the constructs pCa-ToCR1 and pCa-ToCR2 was demonstrated through agroinfection of Nicotiana benthamiana plants according to reported protocols (15).

To detect the natural expression of p22 in vivo, triple FLAG-tag (DYKDDDDK) sequences were fused in frame to the C-terminus of ORF(p22) in pCa-ToCR1 to obtain...
Detection of p22 in ToCV-BJ-infected plants

(a) A triple FLAG-tag coding sequence was fused to the C-terminus of ORF (p22) in RNA1. (b) Western blot analyses with anti-FLAG monoclonal antibody detected the accumulation of p22 in the inoculated (IL, upper panel) and upper systemic leaves (SL, lower panel). The arrows indicate the bands with molecular weight 25 kDa.

References


