Generation of Infectious Transcripts from Korean Strain and Mild Mottle Strain of Potato Virus X

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Full-length cDNAs of two different strains of Potato virus X (PVX-Kr and PVX-Mo) have been directly amplified by long template reverse transcription polymerase chain reaction (RT-PCR) using the 5'-end primer containing a SP6 or T7 RNA promoter sequence and the virus-specific 3'-end primer, and then constructed in plasmid vectors. Capped in vitro transcripts from cloned full-length cDNAs as well as those RT-PCR amplicons proved to be infectious systemically on tobacco plants. Symptom expression on tobacco plants from PVX-Mo transcripts was faster and severer than that from PVX-Kr. In replication stability test of transcripts derived from PVX clones, progeny viruses showed stable replication according to sequencing through passages. This highly infectious transcript system from the full-length cDNA clones for PVX can be useful for recombinant molecules for functional analysis of viral proteins in plant-virus interaction study as well as for expression of foreign protein in planta.

Keywords: Potato virus X, in vitro transcription, infectious cDNA clone, plant virus, mild mottle strain, Korean strain

Potato virus X (PVX) having many strains or isolates reported throughout the world is a type species of the genus Potexvirus which is well described and characterized biologically and molecularly. Especially, PVX has been used widely as a vector for foreign gene expression (Chapman et al., 1992; Baulcombe et al., 1995), and also has been used for RNA silencing mechanism (Hamilton and Baulcombe, 1999; Hamilton et al., 2002). As many reports indicated, PVX has been used as a virus-induced gene silencing vector to down regulate specific gene (Baulcombe et al., 1995; Verchot-Lubicz et al., 2007). Genomic RNA from PVX was characterized as containing five open reading frames coding for proteins of 165 kDa (viral replicase), 25 kDa [triple gene block (TGB)-1], 12 kDa (TGB-2), 8 kDa (TGB-3), and 25 kDa (coat protein) from the 5’ to 3’ end. Especially, among TGB proteins, 25 kDa protein (p25) is known to be responsible for silencing suppressor (Vincent et al., 2000). We analyzed coat protein (CP) sequences of Korean strain (Kr) and mild mottle strain (Mo) of PVX to compare between them before synthesizing infectious transcripts from cDNA of these strains. In addition we analyzed those transcripts from PVX cDNAs whether similar or different in their symptomatic reaction and infection mechanism compared to wild type.

For functional study of the virus genome in plant-pathogen interactions and for developing a viral vector such as PVX vector from UK3 strain (Kavanagh et al., 1992), we have constructed full-length cDNA clones from which infectious transcripts of the two kinds of strains could be synthesized.

Materials and Methods

Virus source and RNA extraction
PVX Korean strain (PVX-Kr) and mild mottle strain (PVX-Mo) were propagated in Nicotiana tabacum cv. Xanthi-nc and used as sources of viruses. Kr strain was originally obtained from potato showing typical PVX symptom in Kangwon province, Korea (Jung et al., 2000), and Mo strain was obtained from American Type Culture Collection (ATCC) (PV-197, USA). Those were purified by polyethylene glycol precipitation and differential centrifugation methods (Jung et al., 2000). Viral genomic RNA was extracted from purified virus particles by SDS, proteinase-K/phenol extraction followed by ethanol precipitation (Ryu and Park, 1995).

Full-length cDNA amplification and cloning
Synthesis of first-strand cDNA of Kr was primed by oligonucleotide PKS3 which is complimentary to the 3’-terminal nucleotides of RNA of PVX-Kr (Fig. 1) based on nucleotide information obtained from GenBank (NCBI). This reverse primer contained four thymine at the end of complementary viral RNA sequences as four adenines (Fig. 1). The cDNA was used as templates for full-length cDNA amplification with forward primer PKS5 containing SP6 promoter site and reverse primer PKS3 by PCR using Taq DNA polymerase (Roche) (Choi et al., 2007). Double-stranded DNA molecules were cloned into SacI/SpH site of pUC19 vector. For PVX-Mo full-length cloning, we carried out PCR with PMT5 forward primer containing T7 promoter site and PKS3 reverse primer, and then full-length PCR product was inserted into pUC19 at the site of SalI/SpH (Fig. 1). Recombinant plasmids were propagated in Escherichia coli (strain JM109, Promega). Obtained PVX clones as well as
RT-PCR amplicons were confirmed by their digestion site using various endonucleases.

Comparison of coat protein sequences between Kr and Mo strains
Sequences of CP and 3’ nontranslatable region (NTR) were determined to compare Kr strain with Mo strain. Phylogenetic analysis was performed with other reported sequences from potato host in Korea to know the genetic relationship among them.

Transcription and inoculation of systemic host plants
RT-PCR amplicons and full-length cDNA clones were directly used as template DNA for in vitro transcription. The full length clones of Kr and Mo were linearized with SphI, and digested DNAs were purified by phenol/chloroform extraction. In vitro transcription was done in the presence of cap analogue using SP6 RNA polymerase for PVX-Kr or T7 RNA polymerase for PVX-Mo. Transcripts were from 50 ng template of each strain in a final volume of 50 µl. Poly adenine (A) tail was synthesized to 3’-end of each transcript from Kr and Mo clone as well as PCR products by E. coli poly A polymerase (Roche). They were inoculated onto Nicotiana tabacum cv. Samsun or cv. Xanthi-ne and N. benthamiana plants with same transcript concentration of each strain. Plants at four leaf stage were inoculated with 20 µl in vitro transcripts. Tobacco plants were grown in growth chamber at 25°C/15°C (daytime/nighttime). Virus detection was performed by western blot analysis and RT-PCR to confirm their infectivity.

RT-PCR and immunoblotting for infectivity of in vitro transcripts of PVX-Kr and PVX-Mo
Virus infection was assessed by RT-PCR and Western blotting at 10 days post inoculation (dpi). Samplings for detection were done in both inoculated and systemic leaves of tobacco plants showing symptom. Total nucleic acids were obtained from five leaf disks (1 cm diameter) and purified from the supernatant by phenol/chloroform extraction and ethanol precipitation. PCR amplification was performed in a reaction tube with total RNA and CP specific primers (PVXCP5; 5’-GTTTCCAGTGATAATTGAAAG-3’ and PVXCP3; 5’-GTCGGTTATGTGGACGTAG-3’) to generate 750 bp product containing BstXI restriction site which could be used for confirmation (Lee et al., 2007).

Total proteins for western blotting were obtained from the inter-phase in the phenol/chloroform extracted solution. Western blotting was performed with prepared total proteins from inoculated or systemic leaves inoculated with transcripts of PVX-Kr or -Mo. Hybridizations of nitrocellulose (NC) membrane binding coat proteins with anti-serum specific for CP were carried out to detect CP accumulation level and to compare with RT-PCR data. NC membrane was probed with antibody (1:2,500 dilutions) against PVX CP, and then membrane was incubated with an alkaline phosphatase (AP)-conjugated secondary antibody (1:7,500 dilution; Promega, USA). To visualize antibody-specific proteins, membrane was reacted with AP-substrate solution (Western Blue Stabilized Substrate Solution, Promega, USA).