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Full Length Research Paper

Molecular characterization of capsid protein gene of potato virus X from Pakistan

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Potato (*Solanum tuberosum* L.) is one of the most economically important vegetable crops in Pakistan. Chlorotic thickness veins spots intermingled with a dark green area, mosaic and decrease in size of the leaves were observed in the Lahore during a survey in 2009. Reverse transcriptase polymerase chain reaction (RT-PCR) based detection conditions were optimized for potato virus X using specific primers 5'-GGCGCAACTCCTGCCACAGC -3' and 5'- TTGTTGTTCCAGTGATACGA -3'. 613 bp amplicon of capsid protein (CP) gene was amplified, cloned and sequenced (Accession number HE577130). Comparisons as well as phylogenetic reconstructions of CP sequence with PVX sequences retrieved from Genebank showed that the Pakistani PVX isolates (HE577130) has close relationship with USSR isolate. This is the first report on the molecular characterization of full length PVX coat protein sequence infecting potato from Pakistan. Homology of the sequenced gene of PVX with reported genes in Gene Data Bank was observed within the range of 90 and 99.7%. Maximum homology was observed to be 99.7% with the gene (Genebank accession No. M38480 and M72416).

Key words: Potato virus X, capsid protein.

INTRODUCTION

The genus *Solanum*, to which cultivated potato belongs, is large consisting of about 1000 species. More than 200 species of potato have been found, but just eight are cultivated (Fukuoka et al., 2009). This genus is found all over the world, except for the far south and north. The cultivated potato, *Solanum tuberosum* originated from the South American Andes. Potatoes have been cultivated for the last 2000 years (Zhang et al., 2009). Potato ranks fourth among the most important food crops in the world, after wheat, rice and maize (Anonymous, 1995). One billion people eat potato worldwide, and is the part of diet of a billion consumers in the developing countries. During the 1960s, 11% of the global potato was produced by developing countries but by the 1990s, this has reached 30% (Anonymous, 1995).

More than 80% of plant-infecting viruses are RNA viruses and unfortunately Pakistan, is home to members of many taxonomic groups of plant-infecting viruses (Verma et al., 2005; Mubin et al., 2009) which is one of the reasons for low agriculture productivity in the country and their control requires the development of appropriate, sensitive and reliable detection methods (Salazar, 1994). Potato virus X (PVX) was first found in *S. tuberosum* in the U.K. (Smith, 1931). The disease prevails in all potato growing areas of the world (Petrzik, 2009; Choi and Ryu, 2008). In Pakistan, PVX is prevalent in all potato growing areas, and Mughal et al. (1988) and Khalid et al. (2000) mentioned that PVX is among the most important viruses in the Punjab. The importance of viruses present in planting material has been recognized by potato producers since the beginning of the 20th century and measure taken to eliminate such viruses have met with

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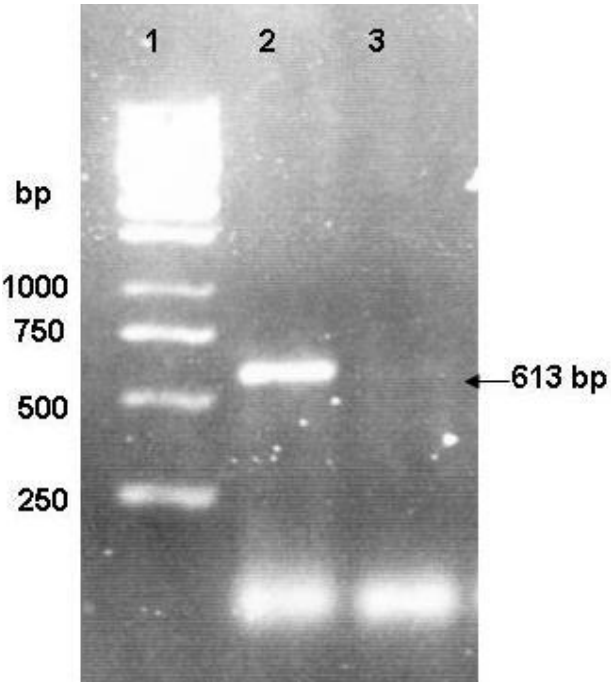

Figure 1. Agarose gel electrophoresis of RT-PCR product of PVX. Lane 1, 1 kb marker; lane 2, amplified fragment of PVX; lane 3, negative control.

limited success (Webber, 1908). Currently, post-harvest methods, such as the grow-out of seed tubers samples in southern climates during winter months supplemented by laboratory tests including enzyme linked immunosorbent assay (ELISA), have been employed to assess the virus level in seed potatoes (Cepica et al., 1990). However, both of these methods are time consuming and cannot be performed on dormant tubers for all viruses (Singh and Singh, 1996). Some plant organs contains very little virus, which cannot be detected by ELISA. So, reverse transcription polymerase chain reaction (RT-PCR) was developed for the detection of three major potato viruses, PVX, Potato virus S (PVS) and Potato leaf roll virus (PLRV) by Peiman and Xie (2006) for Chinese isolates. The goal of this study was to detect and characterize PVX through RT-PCR amplification, cloning and sequencing of CP gene for determination of local genotypes and strains of these viruses. The sequence which we reported on the basis of DNA sequencing and homology studies has not been reported before. Through this study, we developed a reliable RT-PCR based molecular detection method for local strains of PVX.

MATERIALS AND METHODS

Potato virus X was maintained *in vitro* in regeneration medium (MS–Medium 4.43 g/l, sucrose 3 g/l, NAA 0.1 mg/l, BA 2 mg/l, phytagel 1.25 g/l). Total RNA was extracted using Gentra RNA Isolation Kit (QIAGEN) from fresh leaves showing mild mosaic symptom and infected with PVX. Leaf and stem extract containing PVX RNA was used in reverse transcription reaction according to the supplier instructions (Pharmacia-Amersham). After cDNA synthesis, PCR was performed using 5'-GGCGCAACTCCTGCCACAGC -3' and 5'-TTGTTGTTCCAGTGATACGA -3' primers for amplification of PVX coat protein (CP) gene fragment (nucleotide position in PVX genome 8717 to 9196). The PCR conditions were as follows: 1) 94°C for 5 min (1 cycle) 94 for 1 min, 58°C for 1 min, 72°C for 1 min (35 cycles) and final extension at 72°C for 10 min. The purified DNA fragment of coat protein was cloned into pGEM-T (Promega Corporation, Madison, WI) according to the supplier's instructions for carrying out the restriction mapping and sequence analysis of the coat protein. The ligated DNA was then transformed into *Escherichia coli* strain *DH5α* for amplification. The verification of the clones was carried out by isolating plasmid from the transconjugants and performing the restriction enzyme *Eco* RI reaction on the isolated plasmid.

Automated DNA sequencing system (ABI 3700) from applied Biosystems together with ABI PRISM Ready Reaction Dideoxy Terminator Cycle Sequencing Kit was used according to the manufacturer's instructions for sequencing PVX CP gene cloned in pGEM-T (Promega Corporation, Madison, WI). Sequences were assembled and analyzed with the aid of Lasergene Software (DNAStar Inc., Madison, WI, USA), and multiple sequences alignments were performed using DNAMAN Version 4.0 for Windows (Lynnon Biosoft, Quebec, Canada). Sequence similarity searches were performed using BLAST (Basic Local Alignment Search Tool) software available at NCBI web site (http://www.ncbi.nlm.nih.gov/genbank/). A phylogenetic tree and homology matrix was constructed using DNAMAN. All the sequences used for the comparison were obtained from the GenBank.

RESULTS AND DISCUSSION

Viral RNA was extracted from potato virus X infected leaves of potato. Then, 613 bp RT-PCR (Figure 1) product was cloned in pGEM-T (Promega Corporation, Madison, WI). The PVX clone was used as a template with oligonucleotide primers for the full-length sequencing of the gene through automated DNA sequencing system. Comparisons as well as phylogenetic reconstructions of PVX-CP gene nucleotide sequence with PVX sequences retrieved from gene bank showed that the Pakistani PVX isolates (HE577130) has 99.7% homology with PVX gene sequence present in the gene data bank (Genebank accession No. M38480 and M72416) (Figure 2 and Table 1).

ELISA is the most common method for the detection of plant viruses (Al-Mrabeh et al., 2009) because it is robust, specific and rapid. Limited availability of antisera and questions regarding the specificity of antisera produced from preparations containing virus mixtures has the drawbacks of the serological methods. RT-PCR has become an important method for potato pathogen detection (Agindotan et al., 2007; Petrunak et al., 1991; Esfandiari et al., 2006). In contrast, a diagnostic technique using RT-PCR can be rapidly implemented in independent laboratories after the basic protocol and primer sequences are made available (Agindotan et al.,

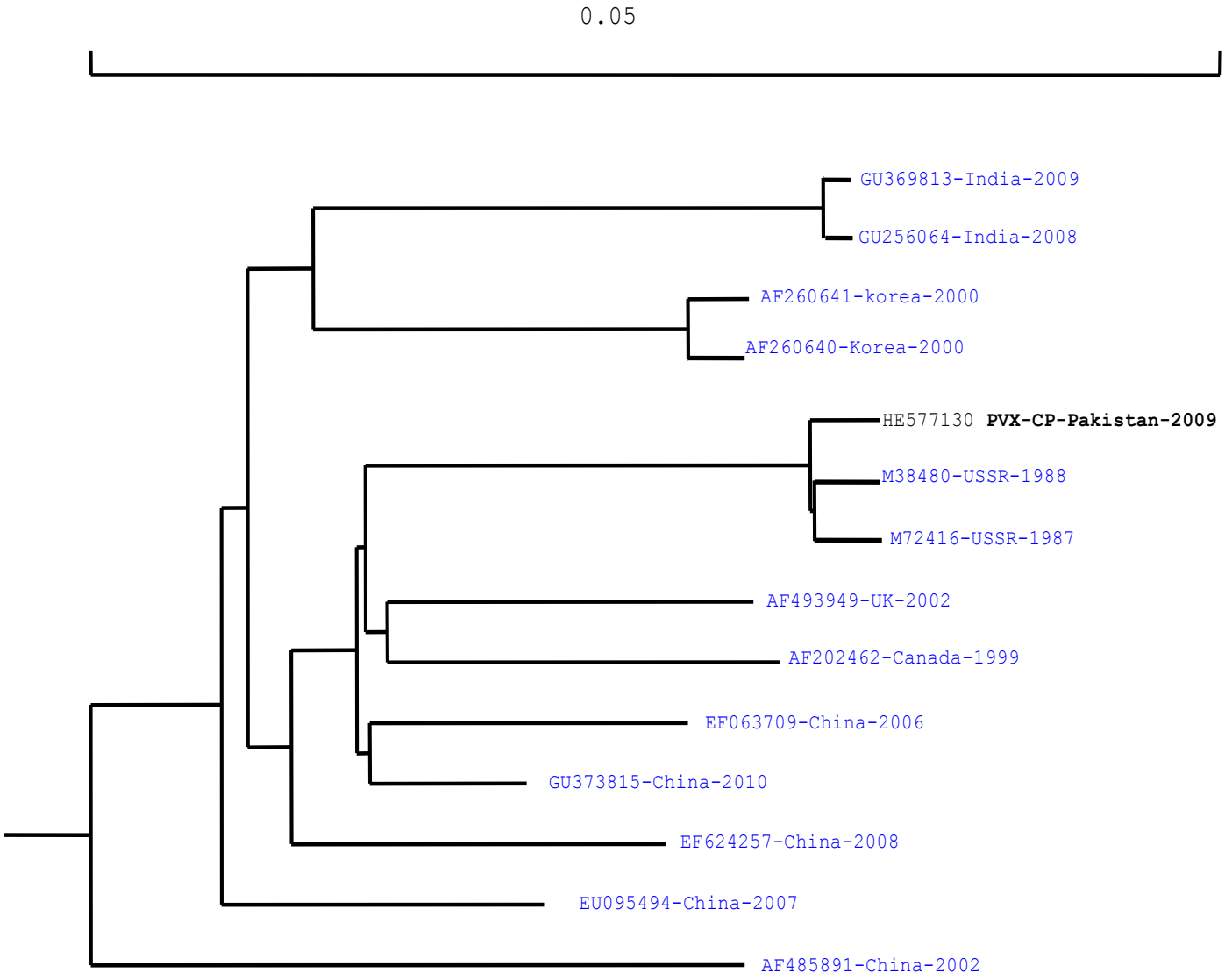

Figure 2. Phylogenetic tree of PVX isolate constructed with the coat protein (CP) genes. Isolates are indicated in the tree by accession number, country of origin and year of collection.

2007).

The availability of nucleotide sequences of many plant pathogens has made possible the development of PCR assays for the detection and diagnosis of several viroids, viruses and other pathogens. Peiman and Xie (2006) and Haididi (1990) reported the detection of viroids in apple scar skin group by RT-PCR amplification. Because of its great sensitivity, the PCR provides good alternative to other diagnostic methods and can speed diagnosis.

The objective of the present study was to develop RT-PCR based detection protocol of PVX for commercial purposes. So, primer was designed on the highly conserved coat protein region of PVX from the available gene sequence in the database. In the present study, 613 bp of the total capsid protein gene size of PVX was sequenced and homology of the sequenced genes was studied with the already present sequences in the gene data bank through BLAST (Basic Local Alignment Search Tool) software and it showed least homology (99.7%) with the USSR isolates (Gene bank Accession No. M38480 and M72416) (Figure 2 and Table 1). The production of virus-free seed potatoes is one of the important steps in potato production; therefore, the diagnostic assays to detect plant viruses are needed to assist in the generation of high health planting material. In this study, RT-PCR condition for the detection of PVX in *in vitro* potato leaves was optimized. Our research demonstrates that the coat protein gene of PVX is reliable regions of the virus genome for the detection of these viruses using RT-PCR. This is also the first report

Table 1. Homology matrix of Pakistani PVX CP with 14 sequences from the database.

HE577130-Pakistan-2009	**100%**													
GU369813-India-2009	94.4%	100%												
AF493949-UK-2002	95.9%	95.1%	100%											
AF485891-China-2002	93.3%	94.1%	94.2%	100%										
EU095494-China-2007	95.3%	96.1%	96.1%	95.1%	100%									
EF624257-China-2008	95.9%	95.8%	96.1%	94.2%	96.2%	100%								
EF063709-China-2006	96.1%	95.4%	96.9%	94.7%	96.8%	96.6%	100%							
AF260641-korea-2000	95.1%	95.8%	95.5%	93.8%	96.1%	96.3%	95.7%	100%						
AF260640-Korea-2000	95.4%	96.1%	95.8%	94.1%	96.4%	96.6%	95.9%	99.7%	100%					
AF202462-Canada-1999	95.9%	94.9%	96.6%	94.4%	96.4%	95.9%	96.5%	95.2%	95.5%	100%				
GU373815-China-2010	96.9%	95.8%	97.5%	94.8%	97.2%	97.3%	97.9%	96.3%	96.6%	97.5%	100%			
AF260640-Korea-2000	94.5%	100.0%	95.1%	94.1%	96.1%	95.8%	95.4%	95.8%	96.1%	95.0%	95.8%	100%		
M38480-USSR-1988	99.7%	94.8%	96.2%	93.6%	95.6%	96.2%	96.4%	95.4%	95.8%	96.2%	97.2%	94.8%	100%	
M72416-USSR-1987	99.7%	94.8%	96.2%	93.5%	95.5%	96.2%	96.3%	95.3%	95.7%	96.2%	97.2%	94.8%	100.0%	100%

on the PVX coat protein sequence from Pakistan.

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