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 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 (Petzlik, 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
The PVX clone was used as a template for DNA sequencing and amplification of PVX according to the manufacturer's instructions for sequencing PVX CP gene cloned in 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). Multiple sequence 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/genebank/). 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., 2006).
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

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.
on the PVX coat protein sequence from Pakistan.

REFERENCES


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

<table>
<thead>
<tr>
<th>HE577130-Pakistan-2009</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GU396913-India-2009</td>
<td>94.4% 100%</td>
</tr>
<tr>
<td>AF493949-UK-2002</td>
<td>95.9% 95.1% 100%</td>
</tr>
<tr>
<td>AF485981-China-2002</td>
<td>93.3% 94.1% 94.2% 100%</td>
</tr>
<tr>
<td>EU095494-China-2007</td>
<td>95.3% 96.1% 96.1% 95.1% 100%</td>
</tr>
<tr>
<td>EF624257-China-2008</td>
<td>95.9% 95.8% 96.1% 94.2% 96.2% 100%</td>
</tr>
<tr>
<td>EF063709-China-2006</td>
<td>96.1% 95.4% 96.9% 94.7% 96.8% 96.6% 100%</td>
</tr>
<tr>
<td>AF260641-Korea-2000</td>
<td>95.1% 95.8% 95.5% 93.8% 96.1% 96.3% 95.7% 100%</td>
</tr>
<tr>
<td>AF260640-Korea-2000</td>
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</tr>
<tr>
<td>AF202462-Canada-1999</td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>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%</td>
</tr>
<tr>
<td>M72416-USSR-1987</td>
<td>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% 100%</td>
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