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Note

Determination of Complete Genome Sequence of Korean Isolate of *Potato virus X*

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The complete nucleotide sequences of a Korean isolate of *Potato virus X* (PVX-Kr) has been determined. Full-length cDNA of PVX-Kr has been directly amplified by long template reverse transcription and polymerase chain reaction (RT-PCR) using virus specific 5'-end primer and 3'-end primer, and then constructed in a plasmid vector. Consecutive subclones of a full-length cDNA clone were constructed to identify whole genome sequence of the virus. Total nucleotide sequences of genome of PVX-Kr were 6,435 excluding one adenine at poly A tail, and genome organization was identical with that of typical PVX species. Comparison of whole genome sequence of PVX-Kr with those of European and South American isolates showed 95.4-96.8% and 77.4-77.9%, in nucleotide similarity, respectively. Sequenced PVX-Kr in this study and twelve isolates already reported could be divided into two subgroups in phylogeny based on their complete nucleotide sequences. Phylogenetic tree analysis demonstrated that PVX-Kr was clustered with European and Asian isolates (Taiwan, os, bs, Kr, S, X3, UK3, ROTH1, Tula) in the same subgroup and South American isolates (CP, CP2, CP4, HB) were clustered in the other subgroup.

Keywords : complete genome sequence, phylogeny, *Potato virus X*

PVX causing viral disease in potatoes is a type species of the *Potexvirus* genus. PVX derived gene expression vector has been used widely for various purposes. The complete nucleotide sequences of twelve isolates of PVX including European (S, X3, UK3, ROTH1 and Tula), South American (CP, CP2, CP4 and HB) and Asian isolates (Taiwan, os, bs) have been determined so far (Huisman et al., 1988; Kagiwada et al., 2002; Kagiwada et al., 2005; Kavanagh et al., 1992; Malcuit et al., 2000; Orman et al., 1990; Querci et al., 1993; Skryabin et al., 1988). Genomic RNA of PVX was characterized as five open reading frames coding for proteins of 165 kDa (viral replicase), 25 kDa (triple gene block 1, TGB1), 12 kDa (TGB2), 8 kDa (TGB3) and 25 kDa (coat protein, CP) from the 5' to 3' end. PVX could be classified into four groups according to their reactions with the genes for hyper-sensitivity (*Nb*, *Nx*) in potato (Cockerham, 1955; Cockerham, 1970). Also the *Rx* gene was reported as another type of resistant gene which was not associated with hypersensitive response. Among all the reported PVX isolates, only PVX-HB was reported to be able to break the *Rx*-derived resistance in potato (Moreira et al., 1980). Querci et al. (1995) also confirmed resistance-breaking capacity of PVX-HB in the mutagenesis study of CP4 and HB isolates of PVX. The 121st and 127th codon in the CP revealed major determinants for resistance breaking in potato (Goulden and Baulcombe, 1993; Goulden et al., 1993). As mutations in CP were revealed as an important factor to render resistance-breaking ability (Kavanagh et al., 1992), there have been several phylogenetic reports on comparison of CP nucleotide sequences (Santa Cruz and Baulcombe, 1995; Jung et al., 2000). Although two isolates of PVX (PVX-KO1 and KO2) in Korea have been analyzed for their CP sequences (Jung et al., 2000), the complete genome sequences of a Korean isolate of PVX has not been reported yet. Therefore, we determined the complete nucleotide sequence of Korean isolate of PVX (PVX-Kr) and analyzed its genomic organization. Phylogenic analyses based on sequence alignments of the five coding regions and 5' and 3' nontranslated regions (NTR) of PVX-Kr with other known isolates were carried out to clarify the taxonomic status of PVX-Kr.

PVX-Kr was originally isolated from potato showing typical PVX symptom in Kangwon province, Korea. PVX-Kr was propagated in *Nicotiana tabacum* cv. Xanthi-nc for purification of the virus. Systemically infected leaf materials were homogenized in extraction buffer solution containing 0.1 M Tris-citric acid, 2 mM $MgCl_2$ pH 9.0, 0.1% thioglycolic acid. The supernatant was treated with half volume of pre-chilled chloroform. Virus particles were precipitated twice from aqueous phase by addition of 4% polyethylene glycol and 0.1 M NaCl. After centrifugation the pellet was resuspended in 0.1 M Tris-citric acid (pH 9.0). This suspension was centrifuged to save supernatant containing PVX virion (Huisman et al., 1988). Viral genomic RNA was extracted from purified virion particles by SDS, proteinase-K/phenol extraction followed by ethanol precipitation, and dissolved in H_2O and stored at −80°C. Synthesis of first-

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strand cDNA was primed by oligonucleotide which is complimentary to the 3'-terminal nucleotides of viral RNA. The cDNA was used as templates for full-length cDNA amplification with upstream primer containing SP6 promoter site (5'-GGAGCTCTATTTAGGTGACACTATAGGAAA-ACTAAACCAAACACCA-3') and downstream primer (5'-GGCATGCTTTTATTTATATTATTCATACAATC-3') by PCR using *Taq* DNA polymerase (Roche). Double-stranded DNA molecules were cloned into *SacI*/*SphI* site of pUC19 vector. Recombinant plasmids were propagated in *E. coli* JM109. Obtained full-length PVX clone was treated with restriction enzyme *PstI* for consecutive subcloning. Subclones containing each restricted DNA fragments were sequenced in both orientations by the dideoxynucleotide chain termination method (Sambrook et al., 2001). Gaps were determined by designed primers which were made according to previously determined sequence information. Obtained genome sequences were stored and analyzed with EditSeq program (Lasergene ver. 6, DNASTAR Inc.). PVX genome sequences for sequence comparison were retrieved from the NCBI database. Multiple alignments were performed by clustal W method of MegAlign (Lasergene ver. 6 DNASTAR Inc.). Bootstrap percentages based on 1000 resamplings were calculated for each internal branches of the tree. Sequence similarity percentage and phylogeny were acquired and investigated.

Full-length cDNA of PVX-Kr was amplified by long template RT-PCR techniques and cloned. Full-length RT-PCR product and its recombinant cDNA clone were validated by RFLP analysis (data not shown). Sequences of other known twelve isolates of PVX retrieved from the GenBank were compared at nucleotide and amino acid levels (Table 1). The PVX-Kr genome was 6,435 nucleotides long excluding one adenine at the 3' terminal poly A tail and contained five ORFs coding for proteins of replicase (1,456 amino acid; aa), TGB-1 (226 aa), TGB-2 (115 aa), TGB-3 (70 aa) and viral coat protein (237 aa) from the 5' to 3' end (Table 1). The total numbers of nucleotides in the 5' and 3' nontranslated regions (NTRs) were 84 and 72, respectively. The length of the PVX-Kr genome was identical to those of other eight known isolates (S, X3, UK3, ROTH1, Tula, Taiwan, os, bs). South American isolates of PVX including HB, CP, CP2 and CP4 were 6,432 in length and their CP size were 236 amino acids which were one codon shorter than that of PVX-Kr (Table 1). Comparison of whole genome sequence between PVX-Kr and twelve PVX isolates in this study revealed that sequence similarity showed 95.4-96.8% with European isolates and 77.4-77.9% with South American isolates, respectively. Five ORFs of PVX-Kr were compared to those of other isolates. According to replicase sequence alignment between PVX-Kr and other isolates, sequence similarity was ranged from 76.6% with three South American isolates (CP, CP2, CP4) to 96.5% with Taiwan isolate at nucleotide level, and from 89.1% (CP, CP2) to 98.9% with Tula isolate at amino acid level. Sequence similarity of TGB1 between PVX-Kr and other isolates showed 77.2% (HB) - 97.2% (Taiwan) and 89.4% (CP4) - 99.1% (X3, ROTH1), at nucleotide level and amino acid level, respectively. Sequence alignment of TGB2 brought that sequence similarity between PVX-Kr and other isolates showed 80.5% (CP, CP2) - 98.6% (ROTH1) and 88.7% (HB, CP, CP2) - 100% (S, X3, UK3, ROTH1, os), at nucleotide level and amino acid level, respectively. TGB3 sequence similarity between PVX-Kr and other isolates showed 71.8% (CP4) - 98.6% (S) and 71.4% (CP4) - 100% (UK3, ROTH1, Tula, os), at nucleotide level and amino acid level, respectively. Between PVX-Kr and other isolates, coat protein sequence

Table 1. Comparison of genome size, 5'-, 3'-NTR size, and amino acid sequences of ORFs between PVX-Kr and other isolates

Isolate	Genome size	5'NTR (n.t.)	Replicase (a.a.)	TGB1 (a.a.)	TGB2 (a.a.)	TGB3 (a.a.)	CP (a.a.)	3'NTR (n.t.)	Accession No.
Kr	**6435**	**84**	**1456**	**226**	**115**	**70**	**237**	**72**	AF373782
S	6435	84	1456	226	115	70	237	72	NC_ 001455
X3	6435	84	1456	226	115	70	237	72	D00344
UK3	6435	84	1456	226	115	70	237	72	M95516
ROTH1	6435	84	1456	226	115	70	237	72	AF111193
Tula	6435	84	1456	226	115	70	237	72	EU021215
Taiwan	6435	84	1456	226	115	70	237	72	AF272736
os	6435	84	1456	226	115	70	237	72	AB056718
bs	6435	84	1456	226	115	70	237	72	AB056719
HB	6432	84	1456	226	115	71	236	72	X72214
CP	6432	84	1456	226	115	70	236	72	M31541
CP2	6432	84	1456	226	115	70	236	72	X55802
CP4	6432	84	1456	226	115	70	236	72	AF172259

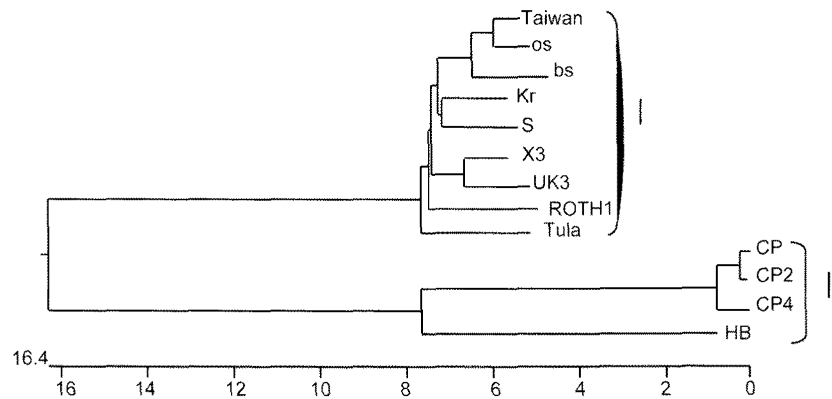

Fig. 1. The Phylogenetic tree based on the complete genome sequence from thirteen PVX isolates. Horizontal lengths indicate degree of genetic distance.

similarity showed 79.1% (HB) - 97.6% (Taiwan) and 87.3% (CP) - 100% (X3, Taiwan), at nucleotide level and amino acid level, respectively. Alignments from determined nucleotide or amino acid sequences and their phylogenetic analyses of the coding regions from PVX-Kr and other PVX isolates were performed to address its relationship with the known PVX isolates. Aligned thirteen PVX isolates could be divided into mainly two groups which could be distinguished from one another based on complete nucleotide sequences of genome (Fig. 1). There were PVX-Taiwan, os, bs, Kr, S, X3, UK3, ROTH1 and Tula in one group (subgroup I), and CP, CP2, CP4 and HB were in the other group (subgroup II). In subgroup I, PVX-Taiwan and two Japanese isolates (os and bs) were grouped together in a small subgroup. PVX-Kr was paired with PVX-S originated from Russia, and the rest of subgroup I were other European isolates. Separate analyses of the nucleotide or amino acid sequences of the coding regions or non-coding regions confirmed that PVX-Kr was grouped with the same isolates. Aligned CP region sequences produced a phylogeny having typical two groupings at nucleotide level as well as amino acid level (Fig. 2). In this dendrogram, PVX-Kr was grouped with Asian or Japanese isolates, and South American isolates such as HB, CP, CP2 and CP4 were categorized as another group. Results from phylogeny of other coding regions and both NTRs showed that PVX-Kr could be clustered in European or Asian isolates and thirteen isolates were clustered mainly into two groups like as phylogeny from complete genome sequences despite minor position changes within groups (data not shown).

In Korea, two isolates of PVX (PVX-KO1, KO2) were reported already by Jung et al. (2000). Their sequence similarity percentage showed 95.9 between Kr and KO1, 95.7 between Kr and KO2 in coat protein nucleotide sequences, and 99.6 between Kr and KO1, 99.2 between Kr and KO2 in coat protein amino acid sequences. CP sequences were most commonly determined in many PVX isolates and there have been several reports on phylogeny based on CP sequences. Santa Cruz and Baulcombe (1995) showed that PVX CPs could be classified into two major types, X and B, and type B were further divided into Bi and Bii subtypes based on CP sequences. CP type X and subtype Bi were derived from Europe and Bii subtype was from America. According to this classification, PVX-Kr

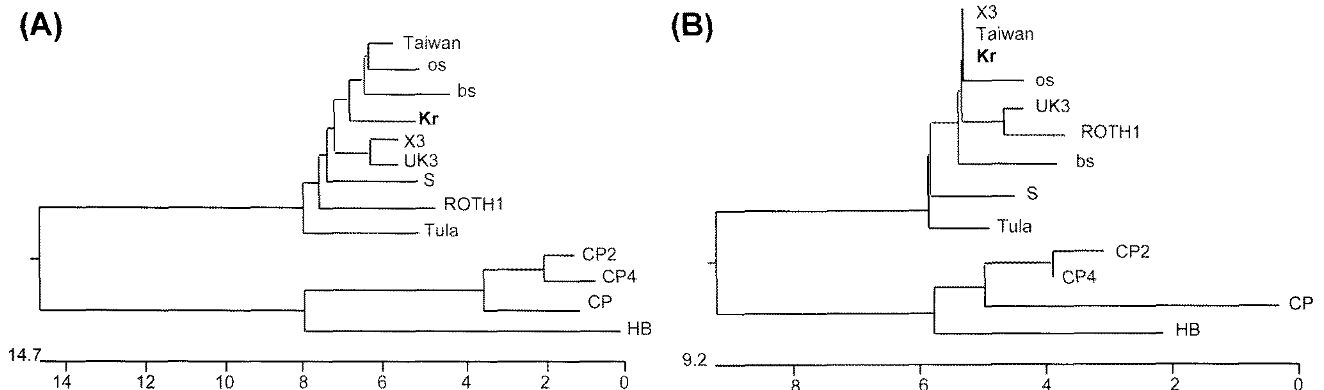

Fig. 2. The Phylogenetic tree based on coat protein nucleotide sequences (A) or amino acid sequences (B) from thirteen PVX isolates. Horizontal lengths indicate degree of genetic distance.

seemed to be categorized into X type of CP together with KO1 and KO2. The initiation nucleotides for 5'NTR in most cases of PVX including PVX-Kr started with Cytosine except for Thymine from four South American isolates, whereas TGB3 ended with TGA in most cases except for TAA in PVX-Tula and PVX-HB. And 3'NTR started with Cytosine in most isolates including PVX-Kr except for Thymine in PVX-ROTH1. Both NTRs showed relatively higher similarity than among ORFs (data not shown), which indicates that 5'NTR is the region displaying the motif associated with the initiation of encapsidation in potexvirus. And high similarity in 3'NTR showed that the presence of a common functional domain at this region, which is usually implicated in the replication of the negative strand. According to phylogenetic groupings in this study, we could find out a peculiar pattern that South American isolates (HB, CP, CP2 and CP4) were grouped together and PVX-Kr was included in subgroup I which contained all the isolates from Europe and Asia in all alignment analyses. Several Japanese isolates of PVX were classified into two groups according to the response against Mexican wild potato (*Solanum demissum*) (Kagiwada et al., 2005). PVX-os and -bs compared their sequences in this study were isolated from Sapporo, but PVX-os was included in common group and PVX-bs was in necrotic group by this classification (Kagiwada et al., 2005). Two Japanese isolates were distinguishable from PVX-Kr in our phylogeny, but they seemed to be clustered with PVX-Kr in the same group based on phylogenies. Conclusively, we could confirm this grouping practice in our phylogeny based on whole genome sequence as well as other ORFs or NTRs. Here, we determined complete genome sequence of PVX-Kr to better understand its molecular and genetic characteristics. And we could offer the opportunity to evaluate PVX-Kr among other PVX isolates worldwide through sequence comparison and we might get the information of the origin of PVX-Kr.

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