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Complementary DNA Cloning and Sequencing of the Coat Protein Gene of Potato Virus Y-Ordinary Korean Strain

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감자바이러스 Y의 OK계통에 대한 외피단백질 유전자 cDNA 클로닝 및 염기서열 분석

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ABSTRACT : Complementary DNAs (cDNAs) to the coat protein gene of an ordinary Korean strain of potato virus Y (PVY-OK) isolated from potato (cv. Superior) were synthesized and cloned into a plasmid pUC119 and sequenced. The RNA of the virus propagated in tobacco (*Nicotiana sylvestris*) was extracted by the method of phenol extraction. The first strand of cDNAs to the coat protein genomic RNA of the virus was made by Moloney murine leukemia virus reverse transcriptase. The cDNAs were synthesized and amplified by the method of polymerase chain reaction (PCR) using a pair of oligonucleotide primers, PVYCP3P and PVYCP3M. The size of cDNAs inserted in pUC119 plasmid was estimated as about 840 bp upon agarose gel electrophoresis. Double stranded cDNAs were transformed into the competent cell of *E. coli* JM109. Sequence analysis of cDNAs was conducted by the dideoxynucleotide chain termination method. Homology of cDNAs of the PVY-OK coat protein genomic RNA with those of PVY-O (Japan), PVY-T (Japan), PVY-T_H (Japan), PVY^N (The Netherlands), and PVY^N (France) was represented as 97.3%, 88.9%, 89.3%, 89.6 and 98.5%, respectively. Homology at the amino acid level turned out to be 97.4%, 92.5%, 92.9%, 92.9% and 98.5%, respectively.

Key words **: potato virus Y, cDNA cloning, sequencing, homology comparison.**

Potato virus Y (PVY) is the type member of the potyvirus group which is one of the largest, most-widely-distributed and economically-important groups of of plant viruses. Potyviruses have numerous strains or pathotypes which differ mainly in biological properties such as host range, pathogenicity, and so on (4, 12). The particles of potyviruses are flexuous thread, and their genomes consist of a single positive-sense RNA, approximately 10 Kb nucleotides long, with a covalently-attached, genome-linked protein (VPg) at the 5′ terminus and a poly(A) tract at the 3′ terminus (7, 8). The genome of potyviruses is translated as a single, large polyprotein precursor, which is subsequently processed into smaller, functional proteins (5). Recently, the genetic map of PVY was established from the complete nucleotide sequence of PVY^N (15) and the partial nucleotide sequences of PVY^N (19), PVY-T_H (9) and PVY-T (13). The PVY genome encodes eight proteins from the 5′ terminus to the 3′ terminus: 31 KD protein, helper component (HC), cytoplasmic inclusion protein (CI), VPg, two nuclear inclusion proteins <NIa (protease) and NIb(putative polymerase)>, and coat protein (15). The coat protein (CP) gene of potyviruses is located about 259 bp upstream of the 3′ untranslated region. The introduction of viral CP genes into plant genome decreased the susceptibility of the plants to infections with corresponding viru-

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Table 1. Synthetic oligodeoxynucleotide primers used in PCR reaction.

Primer Name	Nucleotide sequence
Plus strand (PVYCP3P)	5′CTTATGAAGTGCACCATCAA3′
Minus strand (PVYCP3M)	3′ACAGAAAGGCCTGCTATATA5′

ses (1, 2, 10, 11, 14, 18). As a first step toward genetically engineered protection against PVY, we report the cloning and nucleotide sequence determination of the CP gene of an ordinary PVY strain isolated in Korea, which has been designated as PVY-OK.

MATERIALS AND METHODS

RNA extraction. The virus, which was isolated from potato plant cv. Superior in Korea, identified as PVY-O strain and further designated as PVY-OK (3) was propagated in tobacco plants (*Nicotiana sylvestris*). The viral RNA was extracted from purified PVY-OK by the method of ethanol precipitation with 2% SDS, proteinase K and phenol/chloroform solution (6).

Complementary DNA(cDNA) synthesis and cloning. The primers were synthesized as reported by Hataya *et al.* (1990), and the nucleotide sequences of the primers are shown in Table 1. The 1st strand of the cDNA was synthesized with Moloney murine leukemia virus (M-MLV) reverse transcriptase, and the double-stranded-cDNA (Ds-cDNA) was synthesized by polymerase chain reaction (PCR) with PVYCP3P and PVYCP3M primers. The PCR mixture consisted of 2 μl of the above reverse-transcription sample, 2 μl of PVYCP3P, 2 μl of PVYCP3M, 49.5 μl of distilled water (DW), 10 μl of 10× buffer, 16 μl of 1.25 mM dNTPs and 0.5 μl of Taq polymerase, and the conditions of PCR reaction were 94℃ for 1 min, 51℃ for 2 min and 72℃ for 3 min for a total of 25 cycles. Both ends of the PCR-amplified cDNAs were blunted by using T4 DNA polymerase, and the blunt-ended cDNAs were ligated into the *Sma* I site of pUC119 plasmid. The recombinant plasmids were introduced into competent cells of *E. coli* JM109, and the cells harboring the recombinant plasmids were selected on the LB medium containing ampicillin and isopropylthio-D-galactoside (IPTG).

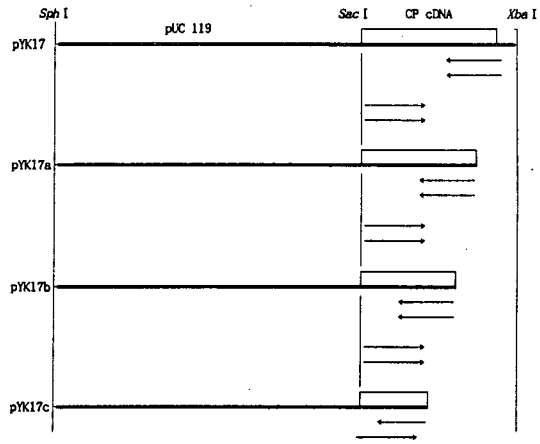

Fig. 1. Strategy for sequencing the cDNA of PVY-OK coat protein gene. Arrows indicate the direction of sequencing.

Nucleotide sequencing. Strategy for sequencing the cDNA was as shown in Fig. 1. The Erase-a-Base System (Promega) was used for sequence analysis. The cDNA-inserted plasmid (pYK17) DNA was digested with *Xba* I and *Sph* I and was incubated with exonuclease III at 37℃ for 30 sec, 1 min and 1.5 min. The digested cDNA was treated with S1 nuclease and Klenow enzyme to remove 5′ and 3′ overhangs and was analyzed with 0.7% agarose gel electrophoresis. The DNA eluted from the electrophoresis was ligated into pUC119 with DNA polymerase I, dNTPs and T4 DNA ligase, and was subcloned into *E. coli* JM109. Nucleotide sequencing was done by dideoxy chain termination method using dATP [α-^{35}S].

RESULTS AND DISCUSSION

RNA extraction and cDNA cloning. The UV absorbance of the genomic RNA extracted from the purified PVY-OK showed a maximum peak at 260 nm and a minimum peak at 232 nm as shown in Fig. 2, and it was formerly identified as a typical PVY-OK strain. The size of the cDNA for the CP gene of PVY-OK genomic RNA synthesized by RT-PCR reaction was about 840 bp (Fig. 3), which was almost the same compared with that of PVY viruses of previous reports (9, 15, 19). The cDNA was blunted at both ends and ligated in *Sma* I site of pUC 119. The recombinant plasmid was introduced into the competent cell of *E. coli* JM109. Clones contai-

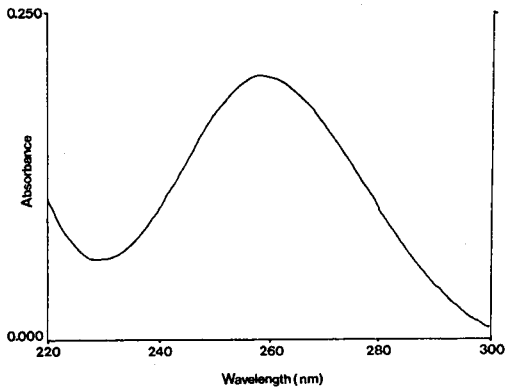

Fig. 2. UV absorption spectrum of PVY-OK RNA extracted from the purified virus by SDS/phenol/proteinase K method.

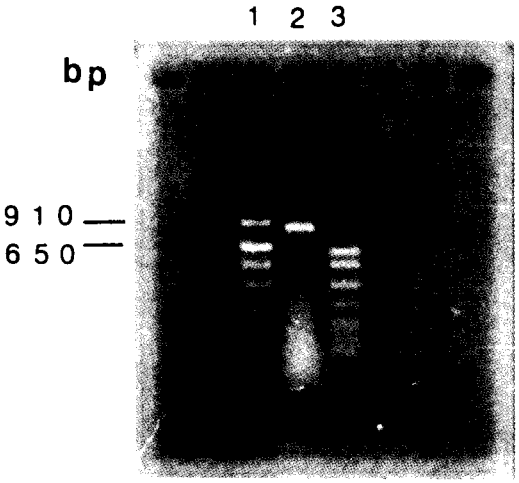

Fig. 3. Agarose gel electrophoresis of PVY-OK CP gene cDNA synthesized by PCR. Lane 1 : Molecular size marker, pBR322 digested with *Alu* I; Lane 2 : cDNA of PVY-OK CP gene; Lane 3 : Molecular size marker, pBR322 digested with *Hpa* II.

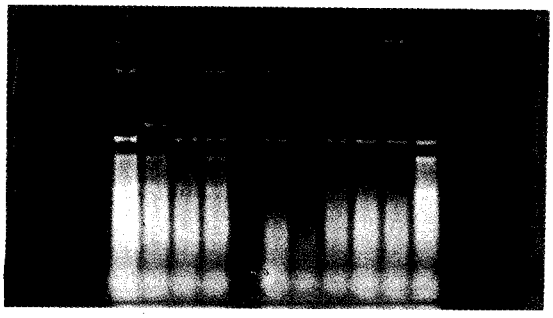

Fig. 4. Agarose gel electrophoresis of recombinant plasmids isolated from transformed *E. coli* JM109.

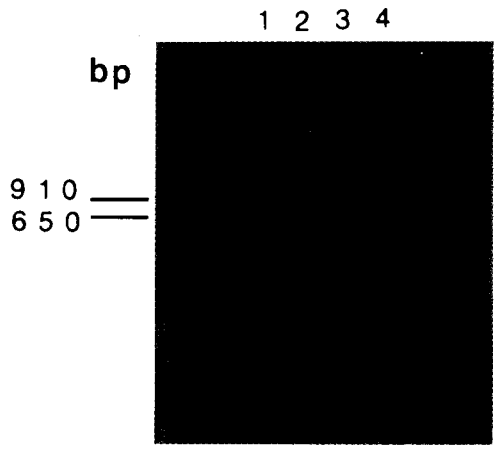

Fig. 5. Agarose gel electrophoresis of the recombinant plasmids digested with *Xba* I and *Sac* I. Lanes 1 and 4 : Molecular size marker, pBR322 digested with *Alu* I and *Hpa* II, respectively; Lanes 2 and 3 : Recombinant plasmid containing cDNAs isolated from pYK6 and pYK17, respectively.

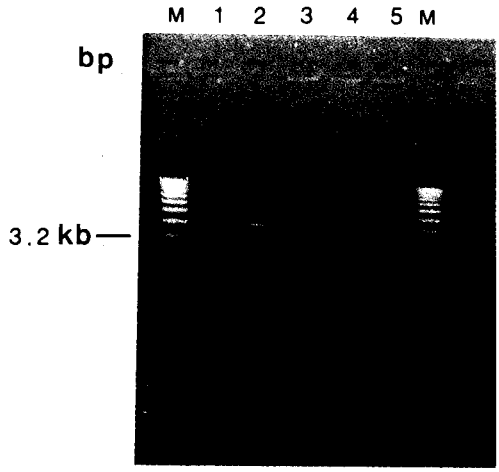

Fig. 6. Agarose gel electrophoresis of cDNA fragment deleted with exonuclease III (Exo III). Lane 1 : Linearized plasmid vector pUC119 without Exo III treatment; Lanes 2~5 : Recombinant plasmids digested with Exo III for 0.5, 1, 1.5 and 2 min, respectively. M : Size marker, 1 kb ladder.

ning the cDNA were selected and designated as pYK6 and pYK17 among 11 clones from agarose gel electrophoresis analysis (Fig. 4). Recombinant plasmid eluted from lysates of *E. coli* JM109 was digested with *Xba* I and *Sac* I and confirmed to contain about 840 bp which is approximately the same size of PCR amplification product on agarose gel

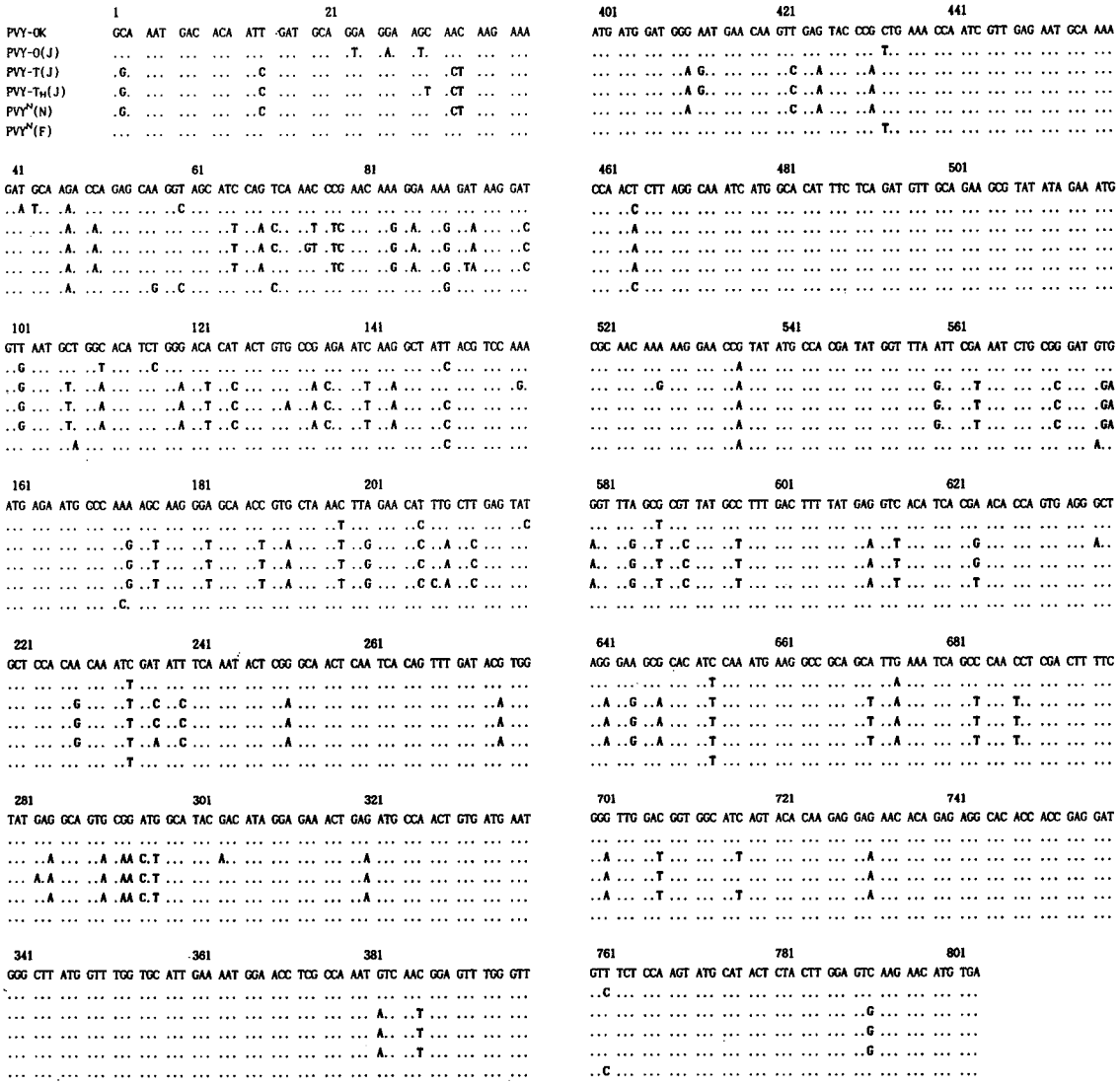

Fig. 7. Nucleotide sequence of PVY-OK coat protein (CP) gene. Different nucleotide sequences of CP of PVY-OK gene compared to those of Japan (J), the Netherlands (N) and France (F) isolates are described by bold letters.

electrophoresis (Fig. 5).

Nucleotide and amino acid analysis. To compare nucleotide and amino acid with other PVY strains, nucleotide sequencing and homology comparison analysis of PVY-OK genomic RNA, were conducted with clone pYK17. Purified plasmids from pYK17 were cut with *Sph* I and *Xba* I. *Sph* I makes 3′ overhangs not digested by exonuclease III and *Xba* I makes 5′ overhangs digested by exonuclease III. The deduced sizes of the exonuclease-treated cDNAs were 3915 bp, 3790 bp, 3665 bp, and 3540 bp, respectively, upon 0.5, 1, 1.5 and 2 min treatments (Fig. 6). Results from the sequence analysis showed that the PVY-OK CP gene was comprised of 801 nucleotides when counted from the cleavage site of CAG(Gln)-GCA(Ala) to the stop codon of TGA, encoding 267 amino acids (Figs. 7 and 8). The molecular weight of the encoded polypeptides was calculated to be 34,630 daltons. The base composition of the CP gene was 33.3% of adenine, 25.2% of guanine, 20.1%

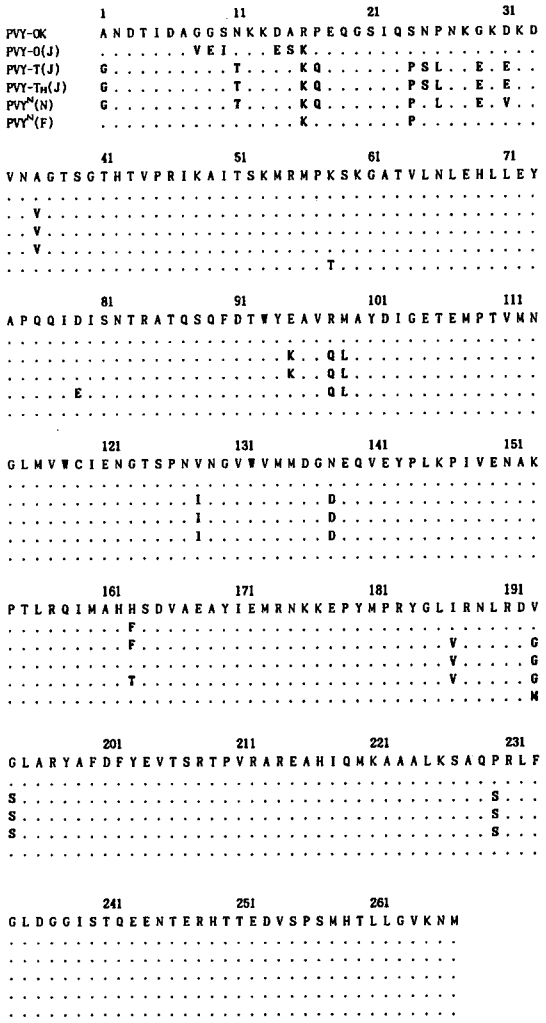

Fig. 8. Predicted amino acid sequence of PVY-OK CP and its comparison with those of other PVY isolates.

Table 2. Base composition of coat protein gene of PVY-OK.

Base	No. of base (%)
Adenine	267 (33.3)
Guanine	202 (25.2)
Cytosine	161 (20.1)
Uracil	171 (21.4)
Total	801

Table 3. Amino acid composition of coat protein gene of PVY-OK.

Amino acid	Amino acid Residue
Alanine (A)	22
Arginine (R)	16
Asparagine (N)	17
Aspartic acid (D)	14
Cysteine (C)	1
Glutamine (Q)	11
Glutamic acid (E)	19
Glycine (G)	18
Histidine (H)	7
Isoleucine (I)	14
Leucine (L)	15
Lysine (K)	16
Methionine (M)	14
Phenylalanine (F)	4
Proline (P)	15
Serine (S)	15
Threonine (T)	20
Tryptophan (W)	3
Tyrosine (Y)	9
Valine (V)	17
Total	267

of cytosine and 21.4% of uracil (Table 2). The polypeptide encoded by PVY-OK CP gene was comprised of 22 alanines, 20 threonines, 19 glutamic acids and 18 glycines in order (Table 3). The homology of the nucleotide sequence of PVY-OK CP gene with those of PVY-O (Japan), PVY-T (Japan), PVY-T_H (Japan), PVY^N (the Netherlands), and PVY^N (France) was represented as 97.3%, 88.9%, 89.3%, 89.6%, and 98.5%, respectively. The amino acid sequence homolgy of the polypeptide encoded by PVY-OK CP gene with those encoded by PVY-O (Japan), PVY-T (Japan), PVY-T_H (Japan), PVY^N (the Netherlands), and PVY^N (France) was represented as 97.4 %, 92.5%, 92.9%, 92.9%, and 98.5%, respectively. Considering the nucleotide sequence homolgy of PVY-OK CP gene with those of PVY-O(J), PVY-T(J), PVY-T_H(J), PVY^N(N), and PVY^N(F), the strain of PVY^N(F) seems to be grouped in PVY-O. This is because the sequence homology of CP of PVY-OK has about 89% similarity with PVY-T(J), PVY-T_H(J), and PVY^N(N) strains which are grouped in PVY^N strains, but the sequence homology with PVY^N(F) and PVY-O(J) is 98.5% and 97.3%. The amino acid sequence homology shows the same declined differences. Therefore, we think that the sequence homolgy is one of tools which can be used to identify

virus strains. In addition, Sukla and Ward (16, 17) reported that amino acid sequence homology of viruses was the criteria for the identification of viruses and virus strains.

요 약

우리나라의 감자에 발생하는 감자 Y 바이러스(potato virus Y, PVY) 중에서 PVY-O 계통에 속하는 PVY-OK를 공시하여 coat protein gene에 대한 cDNA 합성과 클로닝 및 염기서열 분석을 실시하였다. PVY-OK를 담배(*Nicotiana sylvestris*)에 증식하여 순화한 후 phenol 추출법으로 RNA를 정제하였다. PVY-OK RNA의 coat protein gene에 대한 cDNA 합성은 바이러스로부터 추출된 RNA로부터 M-MLV 역전사효소를 이용하여 cDNA의 1st strand를 합성하고 한쌍의 primer(PVYCP3P와 PVYCP3M)를 이용하여 polymerase chain reaction(PCR)법으로 2nd strand를 합성하였다. 여기에서 얻어진 cDNA는 pUC119 플라스미드에 삽입한 후 *E. coli* JM109에 형질전환시켜 선발한 결과 클론 pYK6 및 pYK17로부터 비교적 길이가 긴 cDNA가 확인되었고, pUC 119에 삽입된 cDNA를 *Sac* I과 *Sph* I으로 절단한 후 exonuclease III로 deletion시켜 dATP[α^{35}S]를 이용하여 dideoxy chain termination 방법으로 염기서열을 분석하여 이미 보고된 PVY-O(J), PVY-T(J), PVY-T_H(J), PVY^N(N) 및 PVY^N(F) 등의 CP gene과 유사정도를 비교한 결과, 염기서열은 97.3%, 88.9%, 89.3%, 89.6와 98.5%의 유사성을 보였으며, 아미노산 배열은 97.4%, 92.5%, 92.9%, 92.9% 및 98.5%의 유사성을 나타내었다.

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