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Note

Nucleotide Sequence Analysis of Movement Protein Gene from Tobacco Mosaic Virus Korean Pepper (TMV-KP) Strain

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담배 모자이크 바이러스 한국고추계통에서 분리한 이동 단백질 유전자의 염기서열 분석

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ABSTRACT : Complementary DNA of the movement protein (MP) gene of tobacco mosaic virus Korean pepper strain (TMV-KP) was synthesized from purified TMV-KP RNA by using the reverse transcription and polymerase chain reaction (PCR) system. The synthesized double stranded cDNA was cloned into the plasmid pUC9 and transformed into *Escherichia coli* JM110. The movement protein gene of TMV-KP of the selected clones was subjected to sequence analysis by Sanger's dideoxy chain termination method. The complete sequence of viral MP gene from TMV-KP strain was 807 nucleotides long. The nucleotide of MP gene from TMV-KP has thirteen and two nucleotide differences from TMV vulgarae (TMV-OM) and Korean (TMV-K) strains, respectively. Thus, the nucleotide sequence of TMV-KP MP gene showed higher homology of 99% with that of TMV-K MP gene.

Key words **: movement protein (MP) gene, TMV-KP RNA, cDNA cloning, nucleotide sequence analysis.**

Tobacco mosaic virus (TMV) genome consists of single stranded RNA with 6,395 nucleotides (5). Genomic structure and nucleotide sequence of the viral RNAs were analyzed in several TMV strains including ordinary (OM) and tomato (T) strains (4, 6, 10). Tobacco mosaic virus korean pepper strain (TMV-KP) was isolated from pepper plants cultivated in Korea (1, 2). The TMV-KP strain showed several differences from OM and T strains of TMV in the host range and serological tests. Here we suggest the strain name of TMV isolated from korean pepper plants as TMV-KP changing from TMV-P (8). The strain name of TMV-P was already proposed by Nagai *et al.* (9) in Japan. The strain name of TMV-K was also reported by Koh *et al.* (7) in Korea.

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The TMV-RNA genome carries informations to make four different proteins of 183 K, 126 K, 30 K and coat protein (6). The 183 K and 126 K proteins are directly translated from the genomic RNA and responsible for TMV replication. The 183 K protein is synthesized by suppression of amber termination codon of the 126 K protein gene. The 30 K protein is a nonstructural protein and is required for cell-to-cell movement during the TMV replication. The movement and coat proteins are encoded by internal open reading frames (ORFs) and are translated from unique 3′ coterminal subgenomic RNA (3).

For the genomic studies and viral protein analysis, we cloned and sequenced the cDNA of movement protein (MP) gene from the TMV-KP strain. The first strand of cDNA was synthesized from the purified TMV-KP RNA by the reverse transcriptase of Moloney Murine Leukemia Virus (MMLV) with

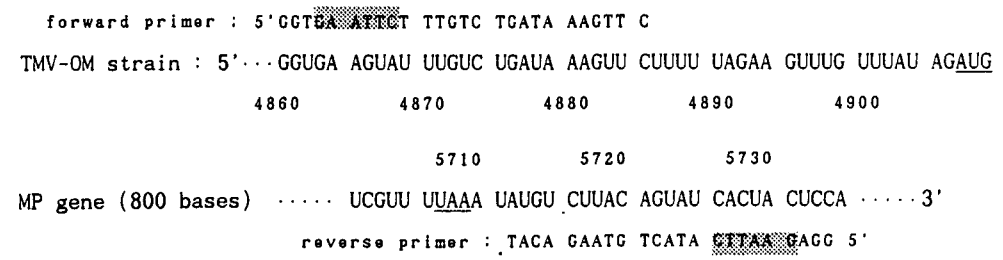

Fig. 1. Schematic construction system of the reverse and forward primers based on the MP gene of TMV-OM strain for the cDNA synthesis of TMV-KP movement protein gene. Underlines and shadows show the start and end codon of movement protein gene and restriction site of *Eco*RI, respectively.

the 3′ end reverse primer which was designated as 23 nucleotides with *Eco*RI restriction site -GAA-TTC- (Fig. 1). The second DNA strand of the movement protein gene was synthesized by the active polymerase chain reaction (PCR) using 25 nucleotides long forward primer containing *Eco*RI restriction site (Fig. 1). The successive reaction temperatures and times were 30 cycles of 96℃, 62℃, 70℃ for 1 minute at each step. The synthesized double stranded cDNA was digested with *Eco*RI restriction enzyme, ligated into the *Eco*RI restriction site of plasmid pUC9 with T4 DNA ligase and transformed into *Escherichia coli* JM 110 (8).

Transformed cells were cultured on the LB plates containing Ampicillin and X-gal for overnight. White colonies grown on LB media supplemented with Ampicillin and X-gal were selected. Plasmid DNAs were isolated from the 12~24 hr grown culture in LB medium by the plasmid DNA purification method using Qiagen columns (11). The isolated plasmid DNAs were digested with *Eco*RI restriction enzyme to analyze the inserted DNA fragments in the recombinant plasmids. One of five selected clones showed an additional band with about 800 bp in size (Fig. 2), which is similar to the TMV movement protein gene.

Nucleotide sequence of the cloned fragment in Fig. 2 was determined by Sanger's dideoxy chain termination method (12). The 807 nucleotide sequence of MP gene of TMV-KP strain is three nucleotides smaller than that of TMV-OM strain, but has the same size as that of TMV-K strain. Nucleotide sequence analysis of the MP gene from TMV-KP strain showed thirteen and two nucleotide differences from those of TMV vulgarae (TMV-OM) and Korean (TMV-K) strains, respectively. Thus, the MP gene of TMV-KP showed very similar homologies

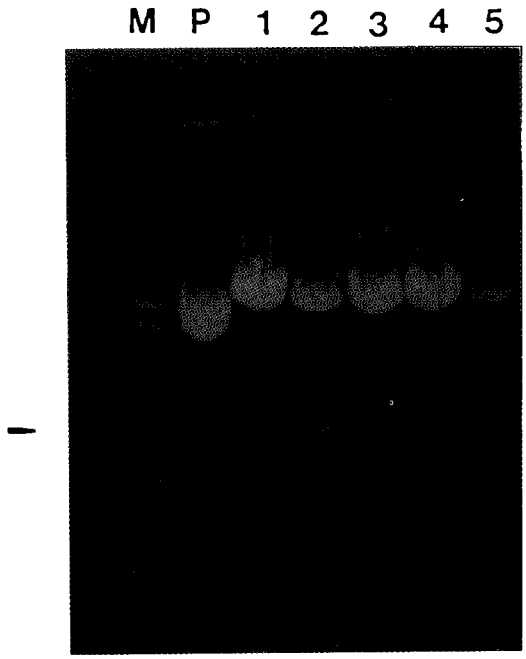

Fig. 2. Agarose gel electrophoresis of purified plasmid containing the cDNA insert of TMV-KP movement protein gene. Lane P indicates plasmid DNA not digested with EcoRI. Lane 1, 2, 3, 4, and 5 show the plasmid DNAs of pTM 201, 202, 203, 204 and 205 digested with *Eco*RI. The molecular size marker (lane M) is pBR 328 DNA digested with *Bgl*I and *Hin*fI. The bands of size marker represent 2167, 1766, 1230, 1033, 653, 517, 453, 394, 298, 234 base pairs from upper, respectively. The arrow indicates about 800 bp position.

of 98.4 and 99.8 % to those of TMV-OM and TMV-K strains. The two nucleotides of 645th and 686th in TMV-KP showed the changes of A→G and T →A compared with TMV-K strain. The change of the 645th nucleotide presents the same Gly→Gly composition of amino acids with the TMV-K strain.

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1    ATGGCTCTAG  TTGTTAAAGG  AAAAGTGAAT  ATCAATGAGT  TTATCGACCT
51   GACAAAAATG  GAGAAGATCT  TACCGTCGAT  GTTTACCCCT  GTAAAGAGTG
101  TCATGTGTTC  AAAAGTTGAT  AAAATAATGG  TTCATGAGAA  TGAGTCATTG
151  TCAGAGGTGA  ACCTTCTTAA  AGGAGTTAAG  CTTATTGATA  GTGGATACGT
201  CTGTCTAGCC  GGTTTGGTCG  TCACGGGCGA  GTGGAACTTG  CCTGACAATT
251  GCAGAGGAGG  TGTGAGCGTG  TGTCTGGTGG  ACAAAAGGAT  GGAAAGAGCC
301  GACGAGGCCA  CCCTCGGATC  TTACTACACA  GCAGCTGCAA  AGAAAGAGCC
351  TCAGTTCAAG  GTCGTTCCCA  ATTATGCTAT  AACCACCCAG  GACGCGATGA
401  AAAACGTCTG  GCAAGTTTTA  GTTAATATTA  GAAATGTAAA  GATGTCAGCG
451  GGTTTCTGTC  CGCTTTCTCT  GGAGTTTGTG  TCGGTGTGTA  TTGTTTATAG
501  AAATAATATA  AAATTAGGTT  TGAGAGAGAA  GATTACAAAC  GTGAGAGACG
551  GAGGGCCCAT  GGAACTTACA  GAAGAAGTCG  TTGATGAGTT  CATGGAAGAT
601  GTCCCTATGT  CAATCAGGCT  TGCAAAGTTT  CGATCTCGAA  CCGGAAAAAA
651  GAGTGATGTC  CGTAAAGGGA  AAAATAGTAG  TAGTGATCGG  TCAGTGCCGA
701  ACAAGAACTA  TAGAAATGTC  AAGGATTTTG  GAGGAATGAG  TTTTAAAAAG
751  AATAATTTAA  TCGATGATGA  TTCGGAGGCT  ACTGTCGCCG  AATCGGATTC
801  GTTTTAA
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Fig. 3. Nucleotide sequence of the cDNA clone of movement protein gene from tobacco mosaic virus Korean pepper strain (TMV-KP) RNA genome. The shadowed sites are two nucleotides different from those of TMV-K strain.

But the change of the 686th nucleotide shows the different Val→Asp composition of amino acids. Interestingly the amino acid change of Val→Asp in the TMV-KP shows the same amino acid with the TMV-OM strain.

The genomes of many plant viruses encode specific movement protein which facilitates viral movement or transport through plasmodesmata in their host plants (13). The nucleotide change of movement protein gene may affect the transport of virus particles through plasmodesmata and to the symptomatic expression ultimately. But the relationship between nucleotide changes of movement protein gene and their expression into viral symptoms is still a question. It needs to be further studied about the molecular mechanism of the movement protein gene and any relationship with viral symptoms.

要 約

담배 모자이크 바이러스 한국고추계통(TMV-KP)의 이동 단백질의 상보적 DNA(cDNA)는 TMV-KP RNA를 분리하고 이로부터 역전사방법과 *Taq* 중합효소를 사용한 PCR 방법을 이용하여 합성하였다. 합성된 cDNA는 pUC 9 plasmid vector에 삽입한 후, *E. coli* JM110에 형질전환시켜서 클로닝하였다. 선발된 클론의 이동 단백질 유전자에 대한 염기서열을 분석하였다. TMV-KP의 이동 단백질 유전자에 대한 염기배열 분석실험의 결과에 의하면 전체 807개의 염기배열 가운데 이미 발표된 TMV-OM의 염기배열과는 13 개의 차이를 보이며, 또한 이것은 한국에서 발표된 TMV-K 계통에서의 염기배열과 마찬가지의 숫적인 변화를 보인다. 그리고 TMV-KP 이동 단백질의 염기배열에서의 상동성은 TMV-K와 아주 높은 상동성을 보이고 있다.

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