Cloning and sequencing of the 3' half of a potato virus Y (O strain) genome encoding the 5k protein, protease, polymerase and coat protein

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Submitted May 22, 1992

DDBJ accession no. D01241

Potato virus Y (PVY) is the type member of the potyvirus group that is the largest of the plant virus groups. Among several PVY strains, PVYO strains are spread worldwide (1) and PVY-O is one of Japanese PVYO strains. We propagated PVY-O on Nicotiana tabacum cv. Samsun NN and isolated its genomic RNA. The synthesized double-stranded cDNA was inserted into the EcoKL site of pBR322 using EcoRI linker after the modification of internal EcoJU sites with EcoRI methylase. A 5.0-kb cDNA was cloned and its nucleotide sequence was determined.

A poly(dA) tract was found at one end of the cDNA, implying that the cDNA corresponds to the 3'-half PVY-O genomic RNA. The sequence around the distal EcoRI end does not agree with the linker sequence, suggesting that it is an intrinsic EcoRI site which escaped from the methylation. The region spanning from the 5' end to the 4667th nucleotide of the cDNA, which consists of 4998 nucleotides and the poly(dA) tract, forms a part of a long open reading frame (ORF). Four consensus sequences of the potyviral protease cleavage site, i.e., three pieces of VXXQ/A and one VXXQ/G, were found in the deduced amino acid sequence of the ORF. The structure of C-terminal half of PVY-O polyprotein was presumed as shown in Figure 1 according to the proposed structure of the potyviral polyprotein (2).

The nucleotide sequence of PVY-O RNA shows 95% homology with that of the 3'-half PVYN genomic RNA (3). The two nonstructural proteins, i.e., 5k protein and protease, and the coat protein of PVY-O are 98%, 97% and 97% identical with the corresponding proteins of PVYN, respectively. However, the homology between the polymerases of PVY-O and PVYN is lower, being 94%. This difference is mainly due to the presence of a 6-bp deletion and a short-range frameshift in the PVY-O RNA (Figure 2). Moreover, polymerases of PVY-O and PVYN exhibit only 92% and 89% homology with that of PVY strain GO16 (4), respectively. It is interesting whether the difference of the symptoms caused by these PVY strains depends on the variation of polymerases.

ACKNOWLEDGEMENT

We thank Dr Tadanori Goto (Hokkaido Nogyoshikenjo, Japan) for supplying PVY-O.

REFERENCES