Molecular Characterization of Satsuma dwarf virus Strains Collected in Japan

Kenta Tomimura[1*] and Toru Iwanami[2]

[1]Kuchinotsu Citrus Research Station, National Institute of Fruit Tree Science (NIFTS), National Agriculture and Food Research Organization (NARO), Minami-shimabara, Nagasaki, 859-2501, Japan

[2]NIFTS, NARO, Tsukuba, Ibaraki, 305-8605, Japan

*Corresponding author: E-mail: tomimura@affrc.go.jp

ABSTRACT. *Satsuma dwarf virus* (SDV), Citrus mosaic virus (CiMV), Natsudaidai dwarf virus (NDV), Navel orange infectious mottling virus (NIMV), and Hyuganatsu virus (HV) are strains of *Satsuma dwarf virus* in the genus *Sadwavirus* that are widely spread in citrus orchards in Japan. The aim of this study was to investigate the population structure of these strains and the geographical distribution in Japan. The nucleotide sequences of two coat protein genes (CPL and CPS) and the 3' non-coding region in RNA2 were determined. Of six isolates sequenced in this study, four isolates (N3, T4, MIE09-3-1 and TZ31) were SDV, and the other two isolates (SIYO and JATA) were CiMV. The phylogenetic analysis based on amino acid sequences of CPL and CPS showed that SDV isolates clustered one tight-knit group and CiMV isolates divided in two definite groups, which was supported a high bootstrap values. These results suggested that the genetic diversity of CiMV was larger than that of SDV. The inter-strain relationships between SDV, CiMV, NDV, NIMV, and HV are discussed.

Leaf samples were collected from infected citrus trees in different orchards throughout Japan (Fig. 1). Total RNA was extracted from infected citrus leaves using ISOGEN (Nippon Gene, Japan). The sensitive detection among *Satsuma dwarf virus* (SDV), Citrus mosaic virus (CiMV), Natsudaidai dwarf virus (NDV) and Navel orange infectious mottling virus (NIMV) was reported by Iwanami (7). To determine the nucleotide sequences of two CPL and CPS and 3'non-coding region in RNA2, we designed the specific primers for each SDV strain. First-strand cDNA synthesis was performed using PrimeScript™ reverse transcriptase (Takara, Japan), according to the manufacturers' instructions. Polymerase chain reaction (PCR) amplifications were done using Ex Taq DNA polymerase Hot Start Version (Takara, Japan). The amplified PCR products were separated by electrophoresis in an agarose gel and purified using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. Each purified PCR product was sequenced by primer walking in both directions using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an Applied Biosystems Genetic Analyzer DNA Model 310. Sequence data were assembled using BioEdit version 5.0.9 (6). The amino acid sequences of the 14 strains of SDV were first aligned using Clustal X (8). The phylogenetic relationships of the sequences were determined by use of the neighbor-joining algorithm of PHYLIP (version 3.5) (5). For neighbor-joining analysis, a distance

matrix was calculated by PROTDIST with the Dayhoff PAM250 matrix (3). The tree was constructed from this matrix by use of the neighbor-joining method (11). A bootstrap value for each internal node of the neighbor-joining tree was calculated using 1,000 random resamplings with SEQBOOT (4). The calculated tree was displayed by use of TREEVIEW (10).

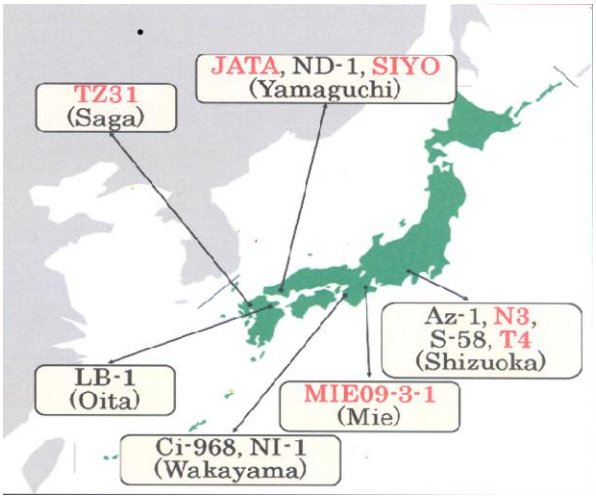

Fig. 1. Geographical locations of the collected SDV strainsin Japan. The strains sequenced in this study were shown in red

Phylogenetic trees represent the evolutionary relationships among populations. A phylogenetic tree was constructed based on the deduced amino acid sequences of the 14 strains of SDV. The relationships among SDV strains were investigated by neighbor-joining analysis. The obtained tree was separated into four groups (SDV, CiMV, NIMV and Hyugantsu virus (HV)) (Fig. 2). Of six isolates sequenced in this study, four isolates (N3, T4, MIE09-3-1 and TZ31) were SDV, and the other two isolates (SIYO and JATA) were CiMV. The phylogenetic analysis based on amino acid sequences of CPL and CPS showed that SDV isolates clustered one tight-knit group and CiMV isolates divided in two definite groups, which was supported a high bootstrap values (Fig. 2). In particularly, the relationship among SDV group showed the topology of star-like phylogeny, suggesting the founder effect. The genetic diversity of CiMV was larger than that of SDV, and CiMV isolates divided in two definite groups. No isolate belongs to the NIMV and HV groups in this study.

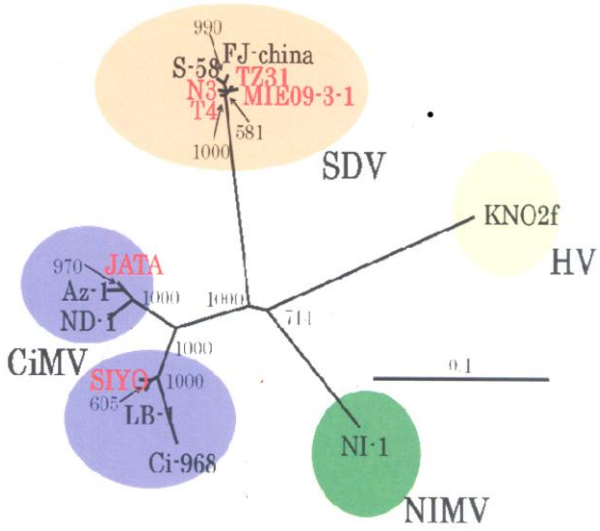

Fig. 2. A neighbor-joining tree calculated from 14 strains of CPL and CPS amino acid sequences of SDV. Numbers at each node indicate the percentage of supporting bootstrap samples (only values >500 are shown). Branch length is drawn to scale with the bar indicating 0.1 nucleotide replacements per site.

Outside of Japan, satsuma dwarf occurs on Satsuma mandarin grown in China, Korea and Turkey (1, 2, 9, 12). Although we analyzed the geographical distribution of several SDV strains in Japan, the fine population structure of them was not clear-cut. A more detailed genetic study of the population is required to identify the

evolutionary relationships among SDV, CiMV, NIMV and HV. The determination of dominant strain in each citrus growing region is important for the utilization of a simple detection kit, and will promote an expansion of virus-free citrus orchards in the near future. The knowledge obtained in this study should be considered when designing strategies for controlling satsuma dwarf disease.

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