JARQ 44 (1), 1 - 6 (2010) http://www.jircas.affrc.go.jp

REVIEW
Properties and Control of Satsuma Dwarf Virus

Toru IWANAMI*

Citrus Greening Research Team, National Institute of Fruit Tree Science, National Agriculture and Food Research Organization (Tsukuba, Ibaraki 305-8605, Japan)

Abstract

Satsuma dwarf virus (SDV), Citrus mosaic virus (CiMV), Natsudaidai dwarf virus (NDV), Navel orange infectious mottling virus (NIMV), and Hyuganatsu virus (HV) are widely spread and cause serious damage to citrus production in Japan. SDV also occurs in some parts of China, Korea and Turkey. Biological, serological and molecular characterization of these viruses revealed both inter-relationships among these viruses and relationships with other plant viruses. SDV is a definite virus species in the genus *Sadwavirus*, and CiMV and other related viruses are classified as strains of SDV. Based on the serological and molecular biological information, useful detection methods utilizing DAS-ELISA, immunochromatographic assay (ICA), and RT-PCR have been developed. These detection methods will promote distribution of SDV-free nursery plants, which is a key control measure. Transgenic rootstocks which harbor coat protein gene and are tolerant to CiMV have also been developed.

Discipline: Plant disease
Additional key words: citrus, immunochromatographic assay, RT-PCR, Sadwavirus, virus disease

Introduction

The dwarfing problem of Satsuma mandarin (*Citrus unshiu*) was first noted in the early 1930s in Shizuoka Prefecture. The disease was shown to be graft-transmissible in 1952, and designated "dwarf disease of Satsuma orange", and later popularized as satsuma dwarf[30, 31]. The disease is spread in most of the citrus production areas in Japan and in some parts of China, Korea and Turkey[1,3,18,32]. The affected trees are stunted, develop typically boat-, or spoon-shaped leaves (Fig. 1), and the quality and yields of fruit are reduced[30]. The causal agent of satsuma dwarf is a spherical virus approximately 26 nm in diameter, and designated *Satsuma dwarf virus* (SDV)[23,30]. SDV is readily graft-transmitted between citrus plants and can be mechanically transmitted between citrus and herbaceous plants[27,29]. Local natural spread, apparently through soil, has been observed in many areas of Japan[20]. Citrus mosaic virus (CiMV), Natsudaidai dwarf virus (NDV), Navel orange infectious mottling virus (NIMV), and Hyuganatsu virus (HV) are related to SDV, and they also occur in some areas of Japan[6,7,26]. Historically, these viruses were recognized as causal agents of dapples of fruit rind of Satsuma mandarin (CiMV), of mottling and curling of new leaves of *C. natsudaidai* (NDV), of chlorotic spots of navel orange (NIMV), and of brown growth rings of Hyuganatsu (HV)[7,26]. CiMV, NDV and NIMV induce similar symptoms on Satsuma mandarin and on herbaceous plants[26].

SDV and related viruses have been extensively studied in Japan for many years. In the 1970's and 80's, the main research topics were biological and serological characterizations[6,28,29]. In the early 90's, molecular characterization

Fig. 1. Boat-shaped leaves appearing on spring shoot of Satsuma mandarin infected with Satsuma dwarf virus

*Corresponding author: e-mail tiwsw37@affrc.go.jp
Received 10 November 2008 ; accepted 26 February 2009.

of SDV and related viruses have been extensively conducted[7-16,19]. This review summarizes the properties and control of SDV, with emphasis on the recent advances on molecular characterization and development of sensitive and simple detection methods of SDV and related viruses, which might contribute to culling of contaminated budwoods.

General properties

SDV has icosahedral virions, approximately 26 nm in diameter, and they consist of three components, named according to the relative rates of sedimentation of purified preparations (Fig. 2). Top component (T) particles are empty shells. Middle component (M) and bottom component (B) particles contain genomic RNA. The buoyant densities in CsCl are about 1.43 and 1.46 g/cm^3, respectively[29,30]. All virions have two species of coat proteins with molecular weights of about 42,000 and 22,000[9]. They encapsidate separately two single-stranded, positive-sense RNAs, RNA-1 and RNA-2[13]. Both RNAs are polyadenylated at the 3'-termini, and they encode a single polyprotein, that is processed to yield mature proteins[15]. SDV infects nearly all of the citrus and citrus relatives[10,24]. The most sensitive citrus variety is mandarin including Satsuma. SDV affects oranges, lemon, pummelo, and other hybrids to a lesser degree, but tree vigor is still drastically decreased. Among citrus relatives, SDV infects *Aegle marmelos*, *Aeglopsis chevalieri*, *Atalantia monophylla*, *Clymenia polyandra*, *Fortunella polyandra*, *Poncirus trifoliate*, and *Swinglea glutinoa*[10,24]. SDV is readily transmitted to herbaceous plants by mechanical inoculation. Sesame is very sensitive to SDV, and had been widely used as an indexing plant. A good host plant for propagating SDV is *Physalis floridana*[27]. Field observation suggests that transmission through soil occurs in some citrus orchards. The vector is still unknown.

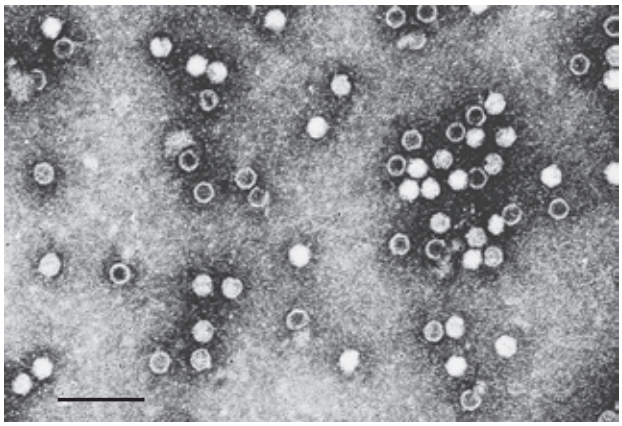

Fig. 2. Electron micrograph of negatively stained virus particles of Satsuma dwarf virus
The bar represents 100 nm.

Occurrence of Satsuma dwarf outside of Japan

Outside of Japan, satsuma dwarf occurs on Satsuma mandarin grown in China, Korea and Turkey[1,3,18,32]. On Jeju Island, Korea, the incidence of SDV was 8.5% during 1995-1997[18]. The disease has been apparently introduced to these countries by the movement of budwoods from Japan.

Complete nucleotide sequence and gene organization

The sequences of SDV RNA-1 and RNA-2 of SDV are 6,795 nucleotides and 5,345 nucleotides in length, respectively, excluding the 3' poly(A) sequence[15]. Both RNA-1 and RNA-2 have a single large open reading frame (Fig. 3). The general genome organization is similar to that of como-, and nepoviruses[15]. The polyprotein encoded by RNA-1 contains domains for helicase, protease and RNA dependent RNA polymerase (RdRp), whereas the polyprotein encoded by RNA-2 has movement protein and coat proteins (Fig. 3). The amino acid sequences of the N-termini of polyproteins encoded by RNA-1 and RNA-2 of SDV are highly conserved and tentatively designated 5' protein (Fig. 3).

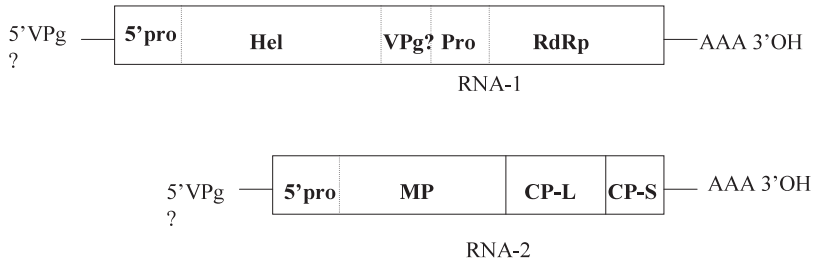

Fig. 3. Genome organization of *Satsuma dwarf virus*
The boxes represent polyproteins. The vertical solid lines within the box show sites where cleavages occur in the polyprotein and the dashed lines indicate sites where cleavages are presumed to occur.
Presence of VPg at 5' termini of both RNAs and its gene in RNA-2 is assumed but not experimentally proved. Abbreviations: VPg, genome-linked viral protein; 5' pro, 5' protein; Hel, helicase; Pro, protease; RdRp, RNA dependent RNA polymerase; MP, movement protein; CP-L and CP-S, large and small components of coat protein; AAA, poly (A).

The 5' protein is apparently encoded redundantly both by RNA-1 and RNA-2. Redundant gene products are very unusual in plant viruses. Similar redundancy is observed in the polyproteins of *Tomato ringspot virus* in the genus *Nepovirus*. The function of these regions is unknown.

The structure of virions and genome organization of SDV suggested that SDV is one of the plant picorna-like viruses. To reveal relationships among SDV and como-, and nepoviruses, the RdRp region, which is the most conserved sequence among sadwa-, como-, and nepoviruses, has been used for phylogenetic comparisons[19]. Alignment of the most conserved regions of the RdRp region shows that SDV is distinct from como-, and nepoviruses (Fig. 4). By comparison with these viruses, both RNA-1 and RNA-2 apparently have genome-linked viral protein (VPg) at the 5' terminus, and its gene is located between the helicase domain and the RdRp region. Recently, *Strawberry latent ringspot virus, Strawberry mottle virus* and Black raspberry necrosis virus (BRNV) have been shown to be closely related to SDV in phylogenetic analysis[23].

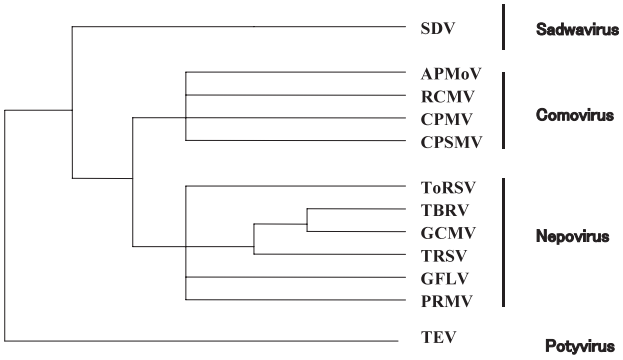

Fig. 4. Phylogenetic tree derived from the alignment of the amino acid sequences of the four most conserved domains of SDV and como-, and nepoviruses

Acronyms of viruses: *Andean potato mottle virus* (APMoV), *Cowpea mosaic virus* (CPMV), *Cowpea severe mosaic virus* (CPSMV), *Grapevine chrome mosaic virus* (GCMV), *Grapevine fanleaf virus* (GFLV), *Peach rosette mosaic virus* (PRMV), *Red clover mottle virus* (RCMV), *Tobacco etch virus* (TEV), *Tobacco ringspot virus* (TRSV), *Tomato black ring virus* (TBRV), *Tomato ringspot virus* (ToRSV).

Relationship among SDV-related viruses

CiMV, NDV, NIMV, and HV have the same biological properties as SDV. These viruses have similar virion structure and genome organization[6,9,16]. SDV is serologically related to CiMV, NDV and HV[6,7,9]. NIMV is serologically somewhat distantly related to SDV more than CiMV and NDV[9]. The CP genes of SDV (isolate S-58 and MIE-88), CiMV (Ci-968, LB-1, AZ-1), NDV (ND-1), NIMV (NI-1), and HV (KNO2f) were determined[7,16]. They share 77-99% amino acid sequence identity with S-58, the type isolate of SDV (Table 1).

Taxonomy

Based on the biological, physicochemical and molecular properties, SDV was assigned as a type species of the genus *Sadwavirus* in the 8th report of ICTV[22]. The genus is not assigned to any higher taxon, and it is sometimes called a floating genera of plant viruses.

SDV, and two strawberry viruses (*Strawberry latent ringspot virus* and *Strawberry mottle virus*) are definite species of the genus *Sadwavirus*[22]. Recently, BRNV was reported as another member of the genus *Sadwavirus*[4,23]. Virus species in the genus *Sadwavirus* are demarcated on the basis of type of biological vector, if known, host range, absence of serological cross-reaction, absence of cross-protection, and difference in amino acid sequence (less than 75% in the large coat protein and the proteinase-polymerase region). Due to the homology of over 75% at the amino acid level, CiMV, NDV, NIMV, and HV are classified as distantly related strains of SDV, and thus, they are no longer definite virus species[22].

Table 1. Comparison of combined amino acid sequences of large and small components of coat protein among SDV and related viruses

Isolates	S-58	MIE-88	Ci-968	LB-1	Az-1	ND-1	NI-1	KNO2f
S-58(SDV)		99	81	81	81	81	80	77
MIE-88(SDV)			82	81	81	81	80	77
Ci-968(CiMV)				98	91	91	83	78
LB-1(CiMV)					92	91	84	78
Az-1(CiMV)						97	84	79
ND-1(NDV)							84	79
NI-1(NIMV)								81
KNO2f(HV)								

Control measures

Use of virus-free budwoods is a primary control measure. To remove contaminated budwoods, several detection methods have been developed. Sensitive detection methods (DAS-ELISA and RT-PCR) are applied for certification of mother-trees, whereas a simple detection kit utilizing immunochromatographic assay (ICA) is helpful in rapid detection in fields. Development of a resistant or tolerant transformant has been tried, but it is still at the experimental stage.

1. Development of serological detection methods

Good polyclonal and monoclonal antibodies have been prepared to detect SDV[25,29] and DAS-ELISA has been widely used in Japan, China and Korea for SDV, CiMV and NDV indexing[18,20,33,34]. Most of the NIMV isolates, and some of the SDV, CiMV and NDV react poorly in ELISA using SDV antisera, and DAS-ELISA should not be used as the only method for the indexing of mother trees. Biological indexing using sesame seedlings and sensitive detection utilizing RT-PCR should also be performed for certification of virus-free mother trees. For field diagnosis, a simple and rapid detection kit utilizing ICA has been developed and is commercially available (Fig. 5)[21]. However, it should be noted that some isolates of CiMV and NiMV are not detected by the ICA kit using SDV antiserum, due to weak serological cross reaction.

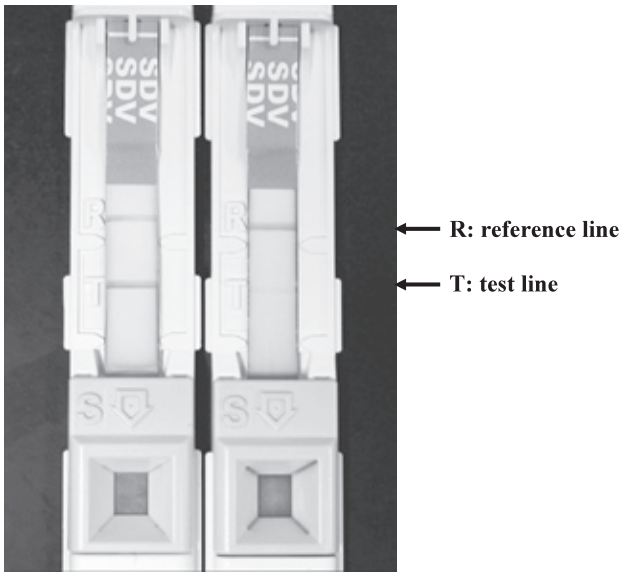

Fig. 5. Detection of SDV by immunochromatographic assay (ICA)
SDV positive samples produce two bands at the positions of "R" and "T", whereas negative ones develop only one band at the position of "R".

2. Development of sensitive detection utilizing RT-PCR

One drawback of DAS-ELISA using anti-SDV serum is that some isolates of CiMV and NIMV do not test positive due to poor serological cross reaction. To overcome this problem, other detection methods utilizing molecular techniques have been extensively explored. One solution was RT-PCR detection based on the common sequences among many isolates. To do this, the complete nucleotide sequences of RNA-2 of SDV, CiMV, NDV, and NIMV were determined, and the accession numbers of the sequences are AB009959 for SDV, AB465581 for CiMV, AB465582 for NDV, and AB465583 for NIMV. Specific primers for RT-PCR detection were designed based on the common sequence among SDV, CiMV, NDV, and NIMV (FW146,5'-ACTAGGGATAGCGCCCTAG-3'; RV488, 5'-GGACCGATATTGGGCCAT-3'). A conserved sequence at the 5' terminal region of genome RNA is commonly observed among picronaviruses, which have many common properties with SDV. The conserved sequences are utilized to detect as many samples in some clinical tests. For example, an assay utilizing a short amplicon in the conserved 5' terminal region was found to be highly sensitive in the case of human rhinovirus infection[5]. Based on these observations, it is expected that RT-PCR used to produce an amplicon with FW146 and RV488 from the 5' terminal region might be applicable for detecting most isolates of SDV, CiMV, NDV, and NIMV.

By utilizing the primer pair of FW146 and RV488, RT-PCR was performed. Total RNA from 0.2 g of infected young fully-expanded leaves was extracted with an RNA extraction kit (ISOGEN, Nippon Gene, Toyama, Japan). The first strand cDNA was synthesized using the primer RV488 and the reverse transcriptase from avian myeloblastosis virus (Takara, Shiga, Japan) for 30 min at 42℃. PCR was conducted to amplify the cDNA product using the primer set of FW146 and RV488. The thermal cycling conditions consisted of the initial denature of 2 min at 94℃, 35 cycles of 30 sec at 94℃, 30 sec at 55℃, and 30 sec at 72℃. The RT-PCR product was separated by electrophoresis in agarose

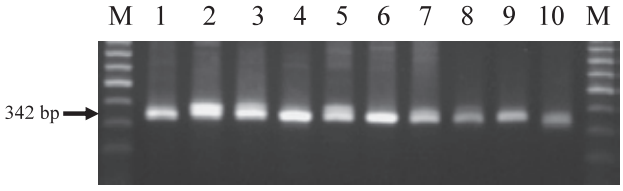

Fig. 6. Detection of isolates of SDV, CiMV, NDV, NIMV, and HV by RT-PCR and agarose gel electrophoresis
1, S-58(SDV); 2, Ci-968 (CiMV); 3, ND-1(NDV); 4, NI-1 (NIMV); 5, Az-1(CiMV); 6, LB-1(CiMV); 7, KNO2f (HV); 8, JATA(CiMV); 9, SIYO(CiMV); 10, SAN(SDV).

gel and visualized under UV illumination after staining with ethidium bromide. The results showed that many isolates of SDV, CiMV, NDV, and NIMV are constantly detected from infected leaves (Fig. 6). The results suggested that the RT-PCR detection using the primers FW146 and RV 488 is applicable to detecting most, if not all isolates of SDV, CiMV, NDV, and NIMV, as well as HV.

3. CP mediated resistance

The CP-mediated resistance had been initially reported in transgenic tobacco that accumulated CP of *Tobacco mosaic virus*[2]. It has been demonstrated in many other plants. The efficiency of transformation in citrus is low and few agriculturally important genes have been transferred. In our laboratory, attempts were made to induce CP mediated resistance against CiMV. Resistance against viruses like comoviruses, which have two capsid proteins, had been achieved by expressing the polyprotein that contains the two capsid proteins. The same strategy was used for CiMV. Like SDV, field observation suggested that CiMV is soil-borne, and trifoliate orange plants (*Poncirus trifoliata*), major rootstocks in Japan, were chosen as hosts for transgenes. The epicotyl segments of trifoliate orange plants were transformed with a binary vector containing the capsid polyprotein (pCP) gene of CiMV via *Rhizobium tumefaciens*. Southern blot hybridization analysis showed that the transgenes were stable in the transgenic lines after regeneration and propagation by grafting. Transgenic lines were screened for resistance to CiMV by mechanical inoculation. Infection was monitored for up to 120 days after inoculation by RT-PCR. The transgenic line 24 had the lowest infection rate (7.1%) at 60 days after inoculation, in contrast to that of non-transgenic plants (65.1%). The response of other lines to inoculation ranged from susceptibility to moderate resistance. The results suggested that some transgenic lines might be used as rootstock to prevent the spread of CiMV through soil[17]. However, full evaluation of the resistance under field conditions and safety of the transformants to the environment are needed before introducing these rootstocks in infested fields.

Concluding remarks

Molecular and serological characterizations lead to elucidation of relationships between SDV and other plant and insect viruses, and has contributed significantly to the taxonomy of SDV. Comparison of nucleotide sequences also revealed the relationships among SDV, CiMV, NDV, NIMV, and HV. These viruses are classified as strains of SDV. Basic knowledge on the nucleotide sequence and serological relationships was applied for developing useful molecular and serological detection methods as well as promising resistant transformants.

References

1. Azeri, T. (1973) First report of satsuma dwarf virus disease on Satsuma mandarin in Turkey. *Plant Dis. Reptr.,* **57**, 149-153.
2. Bendahmane, M. & Beachy, R. N. (1999) Control of tobamovirus infections via pathogen-derived resistance. *Adv. Virus Res.,* **53**, 369-386.
3. Chi, P. F., Gu, C. F. & Roistacher, C. N. (1991) Occurrence of satsuma dwarf virus in Zhejiang Province, China. *Plant Dis.,* **75**, 242-244.
4. Halgren, A., Tzanetakis, I. E. & Martin, R. R. (2007) Identification, characterization, and detection of Black raspberry necrosis virus. *Phytopathology*, **97**, 44-50.
5. Hyypia, T. et al. (1998) Molecular diagnosis of human rhinovirus infections: comparison with virus isolation. *J. Clin. Microbiol.,* **36**, 2081-2083.
6. Imada, J. (1984) Purification and some properties of natsudaidai dwarf virus. *Bull. Fruit Tree Res. Stn*. **E5**, 55-59.
7. Ito, T., et al. (2004) A new virus related to Satsuma dwarf virus: the nucleotide sequence of the 3'-terminal regions of Hyuganastsu virus RNAs 1 and 2. *Arch. Virol.,* **149**, 1459-1465.
8. Iwanami, T. et al. (1991) Leaf blotch and stem mosaic symptoms in sweet orange (*Citrus sinensis* Osbeck) caused by a strain of satsuma dwarf virus (SDV). *Bull. Fruit Tree Res. Stn.,* **21**, 75-83.
9. Iwanami, T., Koizumi, M. & Ieki, H. (1993) Diversity of properties among Satsuma dwarf virus and related viruses. *Ann. Phytopathol. Soc. Jpn.,* **59**, 642-650.
10. Iwanami, T., Omura, M. & Ieki, H. (1993) Susceptibility of several citrus relatives to Satsuma dwarf virus. *In* Proc. 12th conference of IOCV, eds. Moreno, P. et al., IOCV, Riverside, California, 352-356.
11. Iwanami, T. & Ieki, H. (1996a) Nucleotide sequence of the coat protein genes of citrus mosaic virus. *Virus Res.,* **42**, 181-186.
12. Iwanami, T. & Ieki, H. (1996b) Nucleotide sequence of 3'-terminal region of RNA-1 of citrus mosaic virus. *In* Proc. 13th conference of IOCV, eds. Timmer, L.W. et al., IOCV, Riverside, California, 201-207.
13. Iwanami, T. et al. (1996) Nucleotide sequence of the 3'-terminal region of RNA1 of satsuma dwarf virus. *Ann. Phytopathol. Soc. Jpn.,* **62**, 4-10.
14. Iwanami, T. et al. (1998) The nucleotide sequence of the coat protein genes of satsuma dwarf virus and navel infectious mottling virus. *Arch. Virol.,* **143**, 405-412.
15. Iwanami, T., Kondo, Y. & Karasev, A. V. (1999) Nucleotide sequences and taxonomy of satsuma dwarf virus. *J. Gen. Virol.,* **80**, 793-797.
16. Iwanami, T. et al. (2001) Sequence diversity and interrelationships among isolates of satsuma dwarf-related viruses. *Arch. Virol.,* **146**, 807-813.
17. Iwanami, T., et al. (2004) Resistance to citrus mosaic virus in transgenic trifoliate orange lines harboring capsid polyprotein gene. *Plant Dis.,* **88**, 865-868.
18. Kim, D. H. et al. (2001) Study on the virus infection

state of Cheju-do area and the culture of virus-free stock. *Hortscience*, **36**, 601.
19. Karasev, A. V., Han, S. S. & Iwanami, T. (2001) Satsuma dwarf and related viruses belong to a new lineage of plant picorna-like viruses. *Virus Genes*, **23**, 45-52.
20. Koizumi, M. et al. (1988) China laurestine: a symptomless carrier of satsuma dwarf virus which accelerate natural transmission in the field. *In* Proc. 10th conference IOCV, eds. Timmer et al., IOCV, Riverside, California, 348-352.
21. Kusano, N. et al. (2007) Immunochromatographic assay for simple and rapid detection of Satsuma dwarf virus and related viruses using monoclonal antibodies. *J. Gen. Plant Pathol.,* **73**, 66-71.
22. Le Gall, O., Iwanami, T. & Karasev, A. (2005) Genus *Sadwavirus*. *In* Virus taxonomy, Eighth report of the International Committee on Taxonomy of Viruses, eds. Fauquet, C. M. et al., Elsevier Academic Press, San Diego, 799-802.
23. Le Gall, O. et al. (2007) *Cheravirus* and *Sadwavirus*: two unassigned genera of plant positive-sense single-stranded RNA viruses formerly considered atypical members of the genus *Nepovirus* (family *Comoviridae*). *Arch. Virol.,* **152**, 1767-1774.
24. Miyakwawa, T. (1969) Susceptibility of *Citrus* spp. and the other related plants to the satsuma dwarf virus (SDV). *Ann. Phytopath. Soc. Jpn.,* **35**, 224-233.
25. Nozu, Y., Usugi, T. & Nishihori, K. (1985) Production of monoclonal antibodies to satsuma dwarf virus. *Ann. Phytopath. Soc. Jpn.,* **52**, 86-89.
26. Tanaka, H. & Yamada, S. (1972) Evidence for a relationship among the viruses of satsuma dwarf, citrus mosaic, navel infectious mottling, natsudaidai dwarf, citrus variagation and citrus crinkley leaf. *In* Proc. 5th conference IOCV, ed. Price, W. C., University of Florida Press, Florida, 71-76
27. Tanaka, H. & Imada, J. (1974) *Physalis floridana*, a good host for satsuma dwarf virus. *Plant Dis. Reptr.,* **58**, 603-605.
28. Tanaka, H. & Imada, J. (1976) Purification of viruses of citrus mosaic and navel orange infectious mottling. *In* Proc. 7th conference IOCV, ed. Calavan, E. C., IOCV, Riverside, Calif., 116-118.
29. Usugi, T. & Saito, Y. (1977) Some properties of satsuma dwarf virus. *Ann Phytopathol Soc. Jpn.,* **43**, 137-144.
30. Usugi, T. & Saito, Y. (1979) Satsuma dwarf virus. CMI/AAB Descriptions of Plant Viruses, no. 208.
31. Yamada, S. & Sawamura, K. (1952) Studies on the dwarf disease of satsuma orange, *Citrus unshiu* Marcovitch. Preliminary report. *Hort. Div., Tokai-Kinki Agr. Exp. Sta. Bull.,* **1**, 67-71.
32. Zhou, C. Y., Zhao, X. Y. & Jian, Y. H. (1994) The distribution of satsuma dwarf virus (SDV) in China. *Acta Phytophylacica*, **20**, 123-127.
33. Zhou, C. Y., Zhao, X. Y. & Jian, Y. H. (1994) Annual change of ELISA values of satsuma dwarf virus budding and citrus tristeza virus in different parts of sweet orange seedling. *Virologia Sinica*, **9**, 239-244.
34. Zhou, C. Y., Zhao, X. Y. & Jian, Y. H. (1996) Purification of satsuma dwarf virus (SDV), production of SDV antiserum and its application. *Chinese Journal of Virology*, **12**, 55-61.