Susceptibility of Several Citrus Relatives to Satsuma Dwarf Virus

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ABSTRACT. The ability of satsuma dwarf virus (SDV) to infect several citrus relatives was investigated. The plants examined were: *Fortunella polyandra, Clymenia polyandra, Microcitrus australis, Eremocitrus glauca, Atalantia monophylla, Severinia buxifolia, Feroniella lucida, Swinglea glutinosa, and Aegle marmelos.* The scions of these plants were grafted on rough lemon rootstocks which were infected with SDV by graft-inoculation. ELISA and indexing on sesame showed that all inoculated plants were infected with SDV. However, when the plants were maintained at a low temperature (22 C day, 18 C night), SDV was not detected from *S. glutinosa* by ELISA. Furthermore, the titer of citrus tristeza virus was lower in *S. glutinosa* than in rough lemon at low and high (30 C day, 24 C night) temperatures. The low virus titer of citrus tristeza virus (CTV) in *S. glutinosa* under these conditions was confirmed also by dot-immunobinding assay. These results suggest that *S. glutinosa* is resistant to SDV at a low temperature and to CTV at both low and high temperatures.

Since the discovery of satsuma dwarf, efforts have been made to find a cultivar resistant to this formidable virus disease. It was shown in early studies that many citrus cultivars other than satsuma mandarin do not show typical satsuma dwarf symptoms (20). These cultivars are now recognized to be infected with satsuma dwarf virus (SDV) without showing typical symptoms, rather than being resistant to infection. Some strains of SDV induce severe symptoms on sweet orange (5). Miyakawa (13) reported that some citrus relatives were infected with SDV. Furthermore, China laurestine (*Viburnum odoratissium* Ker.), which is commonly planted as a windbreak in Japan, was shown to be infected with SDV (11) and this suggested a relatively wide host range of SDV among woody plants.

In this study, we investigated on the ability of SDV to infect several more citrus relatives which were previously unexamined, hoping that we could find some plants that are immune to SDV, just as *Poncirus trifoliata* is immune to citrus tristeza virus (CTV)(19).

MATERIALS AND METHODS

Plant materials and growing conditions. Tests were conducted in an air-conditioned greenhouse with ambient temperatures of 22-30 C/18-24 C (day/night). Rough lemon rootstocks were used throughout the tests. Scions of citrus relatives (see Table 1) were obtained from the collection of the gene bank of MAFF, Japan. Two replicates were prepared per species. Plants were grown following the methods described by Koizumi and Kano (11).

Virus and inoculation. SDV and a closely related virus, citrus mosaic virus (CiMV, 18) were used as inoculum. The source of SDV, S-58 was isolated from satsuma mandarin showing typical satsuma dwarf symptoms. The source of CiMV, Ci-968 was isolated from satsuma mandarin with mosaic symptoms on fruit. We consider both to be strains of SDV. For comparative study, CTV isolate 1595, which causes severe stem pitting (7,8) was used. Blind buds of rough lemon which contains one of these viruses were side-grafted onto the rootstocks.

Virus indexing. Double sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and dot-immunobinding assay (DIBA) using polyclonal antibodies were conducted for the detection of SDV and CTV. Virus was extracted from the young tender leaves and the midrib of the young leaves for SDV and CTV respectively. Polyclonal antibodies against S-58, Ci-968, and 1595 had been raised in rabbits previously in our laboratory. DAS-ELISA were conducted essentially as described earlier (1, 15). For DIBA, the

TABLE 1
ASSAY BY DAS-ELISA AND BY INOCULATION TO SESAME ON SEVERAL CITRUS RELATIVES GRAFT-INOCULATED WITH SATSUMA DWARF VIRUS

Plants	Inoculum	DAS-ELISA	Sesame indexing
Atalantia monophylla-1	S-58	1.223	25[y]/25[x]
Atalantia monophylla-2	S-58	1.262	25/25
Fortunella polyandra-1	S-58	1.343	28/28
Fortunella polyandra-2	S-58	1.299	20/21
Clymenia polyandra-1	S-58	1.320	16/28
Clymenia polyandra-2	S-58	1.311	18/21
Microcitrus australis-1	Ci-968	0.960	19/28
Microcitrus australis-2	Ci-968	0.982	21/28
Severinia buxifolia-1	Ci-968	0.920	17/28
Severinia buxifolia-2	Ci-968	0.954	20/18
Eremocitrus glauca-2	Ci-968	0.952	12/21
Eremocitrus glauca-2	Ci-968	0.960	14/21
Swinglea glutinosa-1	S-58	0.668	2/21
Swinglea glutinosa-2	Ci-968	0.748	2/21
Feroniella lucida-1	Ci-968	0.962	10/21
Feroniella lucida-2	Ci-968	0.986	15/21
Aegle marmelos-1	S-58	1.176	10/21
Aegle marmelos-2	S-58	1.124	12/21
Rough lemon-1	S-58	1.324	25/25
Rough lemon-2	Ci-968	0.980	15/21
Healthy rough lemon		0.002	0/21

[z]Absorbance at 415 nm after 2 hr of incubation in the presence of substrate. DAS-ELISA was conducted using polyclonal antibodies to SDV isolate S-58 for the detection of S-58 and polyclonal antibodies to CiMV isolate Ci-968 for the detection of Ci-968. Values are the average of four wells.
[y]Number of sesame plants which expressed symptoms.
[x]Number of sesame plants inoculated.

Immun-Blot Assay Kit (BIO-RAD, goat anti-rabbit IgG HRP conjugate) was used. Biological indexing on sesame (10) was also conducted for SDV. All these virus tests were conducted at least six months after graft-inoculation.

RESULTS

Screening of plants for resistance to SDV. The initial screening of the citrus relatives revealed that none of the plants tested was immune to SDV. The results of DAS-ELISA and sesame indexing are summarized in Table 1. Although it became clear that all the tested plants were infected with SDV, the relatively low ELISA values and lower number of positive plants in sesame indexing of *Swinglea glutinosa* were noteworthy. Further investigation was conducted to examine virus titer of SDV in *S. glutinosa*.

Virus titer of SDV in *Swinglea glutinosa* at different ambient temperatures. ELISA values of SDV in *S. glutinosa* were significantly lower than those in SDV-infected rough lemon and *Poncirus trifoliata* at three temperature conditions (30 C/24 C, 28 C/22 C, 22 C/18 C, day/night). The ELISA values of *S. glutinosa* were very low at a low temperature condition (22 C/18 C), while *P. trifoliata* and rough lemon showed high ELISA values (Table 2).

Virus titer of CTV in *Swinglea glutinosa* at different ambient temperatures. ELISA values of CTV in *S. glutinosa* were also significantly lower than those of CTV-infected rough lemon at the three examined temperature conditions. At low (22 C/ 18 C) and high (30 C/ 24 C) temperatures, the ELISA values of CTV-infected *S. glutinosa* were as low as those of healthy controls and of *P. trifoliata*, which is immune to CTV, while rough lemon showed high ELISA values (Table 2). The ELISA of *S. glutinosa* was significantly higher than those of the healthy control, but lower than those of rough

TABLE 2
DAS-ELISA OF SDV AND CTV-INOCULATED PLANTS KEPT AT DIFFERENT AMBIENT TEMPERATURES

Plants	SDV inoculation			CTV inoculation		
	Ambient temperatures			Ambient temperatures		
	30/24C	28/22C	22/18C	30/24C	28/22C	22/18C
Poncirus trifoliata	1.148(0.018)	1.173(0.037)	1.020(0.039)	0.010(0.009)	0.004(0.001)	0.058(0.005)
Rough lemon	1.112(0.001)	1.150(0.026)	0.846(0.069)	1.205(0.005)	1.246(0.006)	1.138(0.030)
Swinglea glutinosa	0.590(0.060)	0.688(0.019)	0.193(0.007)	0.037(0.009)	0.492(0.098)	0.043(0.020)
Healthy rough lemon	0.009(0.002)	0.007(0.002)	0.017(0.003)	0.001(0.001)	0.004(0.001)	0.018(0.006)

[z]Plants were graft-inoculated with either SDV (S-58) or CTV (1595) (see text) and grown in air-conditioned greenhouses with controlled ambient temperatures (day/night). DAS-ELISA were carried out using anti-S-58 or anti-CTV-1595 polyclonal antibodies. Values are the average of OD415 readings of four wells X three repetitions. Standard deviation is indicated in parentheses.

lemon at 28 C/ 22 C. The low virus titer shown by DAS-ELISA was further confirmed by DIBA (Fig. 1).

DISCUSSION

The initial screening of citrus relatives did not reveal plants which were immune to SDV or to the closely related CiMV. The plants tested in this study, in addition to the ones tested in an earlier study (13) include most of the citrus relatives which are graft-compatible with citrus. These results indicate that it seems unlikely that graft-compatible citrus relatives which are immune to SDV will be found.

The results of DAS-ELISA showed that *S. glutinosa* is resistant to SDV at a low temperature (22 C/ 18 C, day/night). As shown previously, temperature is a significant factor which influences the expression of genes for virus resistance (3, 4, 9). It is probable that *S. glutinosa* has temperature-dependent resistant gene(s) to SDV.

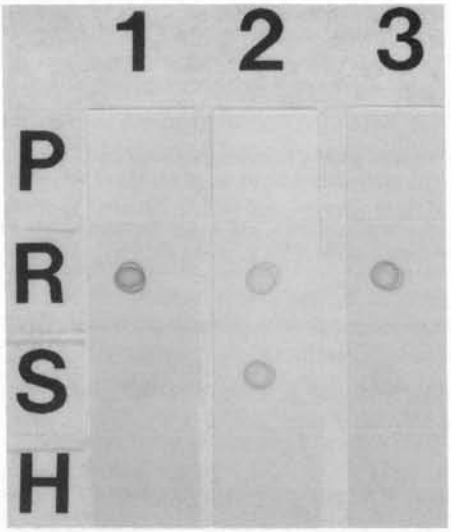

Fig. 1. Reaction of CTV-infected plants grown at different temperatures to CTV polyclonal antibodies in dot-immunobinding assay. Lane 1: 30 C/ 24 C, Lane 2: 28 C/ 22 C, Lane 3: 22 C/ 18 C (day/night). P: *Poncirus trifoliata*, R: rough lemon, S: *Swinglea glutinosa*, H: healthy rough lemon.

S. glutinosa makes a good rootstock for citrus if planted in tropical and subtropical areas (2, 6, 16). This means that *S. glutinosa* is not suitable as a rootstock in Japan, where soil temperature in winter is relatively low. However, it now seems possible to obtain a somatic hybrid between *S. glutinosa* and cold-resistant citrus or *Poncirus*. Recently, the regeneration of intergeneric somatic hybrids between citrus and *S. glutinosa* utilizing electrical fusion was reported (17).

S. glutinosa was also resistant to CTV at low and high temperatures. Müller and Garnsey (14) reported that CTV was not detected in *S. glutinosa* by DAS-ELISA after graft-inoculation. We speculate that the tissue used for DAS-ELISA in their study was collected from *S. glutinosa* plants which were grown in the green-house with relative low or high ambient temperatures.

It is noteworthy that *S. glutinosa* was resistant to both SDV and CTV at low temperatures. It is probable that *S. glutinosa* is resistant to other citrus viruses, such as citrus tatter leaf virus and citrus vein enation virus, at a low temperature. Investigations are now in progress in our laboratory to confirm this hypothesis.

It is interesting that CTV does not replicate well both at low and high temperatures in *S. glutinosa*. The mechanism for CTV resistance in *S. glutinosa* might be different from that of SDV, where virus titer is very low only at a low temperature.

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