OTHER VIRUS DISEASES

The Occurrence of Satsuma Dwarf Virus in China

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ABSTRACT. From 1985 to 1991, 84 samples from satsuma mandarins, mandarin hybrids and navel oranges, imported from Japan in recent years, were collected from eight provinces, and indexed for satsuma dwarf virus (SDV) by using the indicator plants, White sesame, Blackeye cowpea and Satisfaction kidney bean, and by ELISA. Miyamoto satsuma samples collected trom Huangyan, Zhejiang Province and from Fengjie, Sichuan Province were infected with SDV, while all the others were SDV-free. Nine cultivars of extreme early satsuma, especially those collected from Huangyan and Huaning, Yunnan Province, were infected with citrus tatter leaf virus (CTLV). Other than satsuma, 25 important citrus cultivars collected from the National Citrus Genetic Resources Collection at Chongqing were found to be SDV-free by ELISA. The results suggest that the occurrence of SDV in China is limited to a few cultivars in a few locations.

Satsuma dwarf is an economically important virus disease in Japan. The typical symptom, the so-called "boat-shaped leaf," appears on the spring flush. Since the first record in 1950 (5), it has become widespread in almost all the citrus areas of Japan (3). SDV has also been reported from Turkey (1) and Korea (4). Satsuma mandarin was imported into China from Japan, and in recent years, some cultivars of extreme early satsuma have also been introduced. In many satsuma plantings in China, boat-shaped leaves have been found. In order to determine whether SDV is present in China or not, samples were collected from eight provinces and indexed by using herbaceous indicator plants, and ELISA from 1985 to 1991. Additional indexing was done to determine if citrus tatterleaf (CTLV) was present in these samples.

MATERIALS AND METHODS

From 1985 to 1991, 84 samples were collected from eight provinces, namely Sichuan, Zhejiang, Hunan, Hubei, Guangxi, Jiangxi, Yunnan Provinces and Shanghai Municipality. Forty-four cultivars originally introduced from Japan were involved, including extreme early satsuma (Guchi No. 4, Hashimoto, Ichifumi, Imada, Inaba, Ishitsuka, Kitaguchi, Mitake, Miyamoto, Oomura, Ooura, Tanimoto, Takabayashi, Toku mori, Ueno, Wakiyama and Yamakawa No. 3), early satsuma (Kamei, Matsuyama, Miyagawa, Okitsu and Tachima), middle satsuma (Aoe, Dobashibeni, Hayashi, Kunou, Morita, Mukaiyama, Nankan No. 4, Nankan No. 20, Owari, Seto, Suruga, Tomono and Yonezawa), late satsuma (Aoshima, Ikeda and Ishikama), navel orange (Shirayanagi and Suzuki) and mandarin hybrid (Amanatsu, Hassaku and Miyauchi iyokan).

In the autumn of 1991, an additional 25 important citrus cultivars were collected from the National Citrus Genetic Resources Collection at Chongqing for ELISA indexing for SDV, including sweet orange (Anliucheng, Xinhuicheng, Jingcheng, Edangan No. 8, Taoyecheng, Bintangcheng, Xuegan, Robertson navel, Washington navel, Valencia, Hamlin, Tarocco blood, Ruby blood and Washington Sanguine), mandarins (Dahongpao, Ponkan, Jiaogan, Nanfengmiju, Shiju, Manju, Zaoju, Fuju, Bendizao and Nianju) and Eureka lemon.

Ordinary sap-inoculation techniques (1 g young leaf tissue in 5 ml

0.05M K_2HPO_4 solution) was used for indexing SDV on the herbaceous indicators, White sesame, Blackeye cowpea and Satisfaction kidney bean. Five to 20 seedlings of each indicator were used for indexing each sample, and observed for symptoms, and every sample has been identified at least twice. The positive control seedlings were sap-inoculated with SDV from an SDV-infected plant, and negative control seedlings were rubbed with the sap of the young shoots of a healthy satsuma seedling. Test plants were held in the laboratory at 20-24 C for about 20 days.

The double-antibody sandwich ELISA technique (2) was used for SDV indexing, and the absorbance values were measured at 410nm in a DG-3022 ELISA Reader. The SDV-antiserum and alkaline phosphatase-linked antibody used were made in October 1990 by Plant Protection Assoc. Res. Inst. of Japan and kindly supplied by Dr. A. Sasaki from Hirosima Fruit Tree Exp. Sta., Japan. Each sample was tested by ELISA at least twice. Positive and negative control extracts were prepared by grinding young shoots of SDV-infected satsuma and of healthy satsuma, respectively.

During the indexing, some samples were found to react positively on Satisfaction kidney bean and Blackeye cowpea, but did not react on White sesame or in ELISA tests. In order to determine the cause, indexing for CTLV was done on Rusk citrange, in the spring and autumn of 1991. Each sample mentioned above was grafted onto four Rusk citrange plants.

RESULTS

Indexing for SDV by herbaceous indicators. In general, symptoms appeared 1-2 weeks after inoculation. Positive control seedlings of the three indicators produced positive results in all tests. Symptoms on White sesame were local lesions on inoculated leaves followed by systemic infection; the systemically infected leaves showed vein necrosis and mottled, malformed leaves. Symptoms on Blackeye cowpea and Satisfaction kidney bean were local lesions on the inoculated leaves, and mottling, vein-clearing and local lesions on the upper leaves, and necrotic streaks on the petioles and stems. Control seedlings produced negative results in each test. The indexing results of the different samples were as follows:

(a) Miyamoto satsuma collected from Huangyan, Zhejiang Province and from Fengjie, Sichuan Province, which were imported from Japan in early 1980s, reacted positively on the three indicators.

(b) Nine cultivars of extreme early satsuma, including Wakiyama, Ichifumi, Hashimoto, Ishitsuka, Takabayashi, Toku mori, Oomura, Kitaguchi, and Inaba collected from Huangyan, Zhejiang, Wakiyama and Ichifumi from Huaning, Yunnan and Wakiyama and Toku mori collected from Chongqing, produced positive reactions on Satisfaction kidney bean and Blackeye cowpea, but no reaction on White sesame. These results indicate that these extreme early satsumas are infected by other viruses, but not by SDV.

Detection of SDV by ELISA. In 1990-1991, 109 samples collected from eight provinces were indexed for SDV by ELISA. Both Miyamoto satsumas collected from Huangyan and Fengjie were infected with SDV, but all the others were free of the virus.

Indicator indexing of CTLV. Typical symptoms of CTLV on Rusk citrange were observed in the autumn of 1991 and the spring of 1992. All of the extreme early satsuma samples, which caused positive reactions on cowpea and kidney bean but negative reactions on sesame, were infected with CTLV.

DISCUSSION

In this study, two samples of Miyamoto satsuma imported from

Japan in the early 1980s were found to be infected with SDV, while 76 other satsuma samples collected in eight provinces were SDV-free. Mandarin hybrids (Hassaku, Miyauchi iyokan and Amanatsu), and navel oranges (Shirayanagi and Suzuki) imported from Japan were also found to be SDV-free. Other than satsuma, mandarin hybrids and navel oranges introduced from Japan, 25 important citrus cultivars collected in Chongqing, were also found to be SDV-free. The results suggest that the history of SDV occurrence in China is recent, and its distribution is limited in a few cultivars and in a few locations.

Typical symptoms of satsuma dwarf, such as the boat-shaped leaf, have been found in many satsuma producing areas. Most samples tested were collected from the trees which showed the boat-shaped leaf symptoms. However, the ELISA and indicator tests indicate that SDV is not present. The boat-shaped leaf is therefore caused by factors other than SDV infection. Zhou *et al.* (6) have shown previously that this boat-shaped leaf symptom is graft-transmissible. The diagnosis of satsuma dwarf in the field should therefore be confirmed using the sesame test and/or by ELISA.

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