Virus Certification of Fruit Trees in Japan

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As fruit trees have been propagated vegetatively, it is common to harbor more than one virus within one tree. Principal fruit tree viruses found in Japan are shown in Table 1. Most commercial fruit trees consist of a rootstock variety, a scion variety, and sometimes an interstock variety. All of them can differ greatly in their sensitivity to virus infection. Sometimes, a symptomless infection occurs or a symptomless infection follows shock symptoms, but sometimes, an infection causes destructive damage. Recently, the spread of these viruses has been accelerating in its extent and speed as a result of the

Table 1. Fruit tree viruses found in Japan

Citrus	**Peach** (*continued*)
tristeza	star mosaic
satsuma dwarf	oil blotch
citrus mosaic	yellow leaf
navel orange infectious mottling	apple chlorotic leafspot
natsudaidai dwarf	Prunus S
tatter leaf (=citrange stunt)	**Plum**
leaf rugose	plum line pattern
exocortis (viroid)	Prunus necrotic ringspot
vein enation	cucumber mosaic
yellow mottle	**Apricot**
Apple	Prunus necrotic ringspot
chlorotic leafspot	**Sweet Cherry**
stem pitting	prune dwarf
stem grooving	green ring mottle
mosaic	**Grapevine**
scar skin (=dapple apple)	ajinashika (tasteless)
green crinkle	corky bark
russet ring	fanleaf
Platycarpa scaly bark	fleck
necrosis	leaf roll
Japanese pear	stunt
necrotic spot	Arabis mosaic
vein yellows	**Fig**
ring pattern mosaic	fig S
ringspot	**Passion fruits**
apple stem grooving	cucumber mosaic
Peach	**Papaya**
Prunus necrotic ringspot	watermelon mosaic
yellow mosaic	papaya ringspot
enation	
dwarf	

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world-wide development of the means of transportation and the top-grafting techniques for the renewal of cultivars.

How fruit trees suffer from virus disease

1) Apple topworking disease

Topworking of the new cultivar of Delicious type onto old cultivars, such as Ralls Janett or McIntosh on maruba-kaido (*Malus prunifolia* var. *ringo*) rootstocks, was performed extensively during 1930's. After a few years, topworked apple trees began to decline and ultimately died. It was elucidated by Yanase et al.[9,10,11] that apple chlorotic leafspot virus (CLSV) type strain which is latent in Delicious type caused stem pitting and necrosis in the rootstock. Later, it was confirmed that apple stem pitting virus (SPV) and apple stem grooving virus (SGV) also caused similar decline of apple tree on mitsuba-kaido (*Malus Sieboldii*) or kobano-zumi (*Malus Sieboldii* var. *arborescens*) rootstock.

2) Citrus stem pitting disease

Although satsuma mandarin (*Citrus unshiu*) on trifoliate orange (*Poncilus trifoliata*) which cover nearly 80% of citrus acreage in the country is highly tolerant to citrus tristeza virus (CTV), the virus is harboring in satsuma mandarin without symptoms, because CTV and its vector, brown aphid (*Toxoptera citricidus*) are common in citrus orchards in Japan. In order to rectify the oversupply of satsuma mandarin, the mandarin is now changing to middle or late maturing cultivars such as sweet oranges (*C. sinensis*), hassaku (*C. hassaku*), and iyo (*C. iyo*) which are all very susceptible to CTV. Although the change is done by the replanting of nursery trees and/or topworking of other citrus, these citrus cultivars always suffer from damage by the infection of CTV. Stem pitting occurs not only on twigs but also on the wood of limbs. As a result tree growth is poor and fruit size becomes small. To prevent the damage, it is considered desirable to use the interference phenomena induced by preimmunization of mild strain of CTV. In case of hassaku, Sasaki[5,6] has succeeded in the use of mild strain, HM–55, to prevent hassaku dwarf (a stem pitting disease of hassaku).

3) Spread of Citrus mosaic virus

Citrus mosaic virus (CiMV), a member of satsuma dwarf virus group, had been localized in satsuma mandarin orchards in Wakayama Prefecture for over 40 years. Distribution of scions of Miyamoto-wase, which is a newly found early ripening cultivar with excellent quality, resulted in the extensive spread of the virus[8]. This virus causes mosaic symptom on fruit rind and commercial value of fruit becomes nil. In 1980, the campaign for the indexing CiMV in all Miyamoto-wase nursery trees was performed. More than 50,000 nursery trees have been indexed by ELISA (Enzyme-Linked Immunosorbent Assay) and infested trees have been discarded[1].

4) Grapevine ajinashi-ka disease

A misterious malady called 'ajinashi-ka' (tasteless) grape was noticed in Yamanashi Prefecture in the mid 1960's. Grapes on affected trees lose sugar content and become tasteless. Researchers in Yamanashi Fruit Tree Experiment Station[7] confirmed the graft-transmissibility of the disease and concluded that causal agent might be the combination of grapevine leafroll virus and grapevine fleck virus. On the other hand, Namba et al.[2] found virus-like spherical particles within sieve tubes of fruit and named them 'grapevine ajinashi-ka virus (GAV)'. These particles have been isolated but not yet back-transmitted to grapevines. It is considered that the infestation of rootstocks with the virus resulted in the infestation of many grapevine cultivars.

5) Spread of fruit viruses

The renewal of cultivars by topworking is also in progress for apple cultivars. Those fruit viruses such as dapple apple, scar skin and green crinkle are spreading by human hands. Virus-free materials are needed urgently.

Control measures of fruit tree viruses

No chemical therapeutants are yet available for controlling the virus diseases of fruit trees. Therefore, control measures have to correspond to the mode of transmission of each virus for breaking the infection chain.

Insect transmission: Citrus tristeza virus, which causes stem pitting disease or tristeza decline, is transmitted by brown aphid (*Toxoptera citricidus*). Spraying insecticides, preimmunization by mild-strain and/or use of tolerant cultivars are recommended.

Pollen transmission: Prunus necrotic ringspot virus and Prune dwarf virus can be transmitted by pollen. Removing affected trees is recommended.

Contact transmission: Citrus exocortis viroid can be transmitted by tools such as knives or scissors. Disinfection of the tools and removing affected trees are recommended.

Soil transmission: Satsuma dwarf group viruses (Satsuma dwarf virus, Citrus mosaic virus, Navel orange infectious mottling virus, and Natsudaidai dwarf virus) are believed to be transmitted through soil, while no vector was elucidated. For these viruses, use of soil-disinfectant is recommended.

Graft transmission: Although each virus can be transmitted by the peculiar way stated above, some viruses are known to be transmitted only by grafting and all viruses are

Table 2. Indicator plants for indexing viruses of mother trees currently used by Plant Protection Station

Fruit tree	Disease or virus	Indicator plants
Citrus	Stem pitting (CTV)	Mexican lime (*Citrus aurantifolia*) ELISA
	Satsuma dwarf (SDV)	*Sesanum indicum* ELISA
	Exocortis viroid (CEV)	Etrog citron (*Citrus medica*)
	Bud-union disorder (CTLV)	Rusk citrange *Chenopodium quinoa*
Apple	Apple topworking (CLSV)	*Malus scheidekeri* Maruba-kaido, MO-84 (*Malus prunifolia* var. *ringo*) ELISA
	Apple topworking (SGV)	*Malus scheidekeri*
	Apple topworking (SPV)	Mitsuba-kaido, MO-65 (*Malus sieboldii*)
Prunus	PRSV and PDV	Shirofugen (*Prunus serrulata*) *Cucumis sativus* *Cucurbita maxima* ELISA
	GRMV	Kwanzan (*Prunus serrulata*)
Grapevine	GFV	St. George (*Vitis rupestris*) *Chenopodium quinoa* *Gomphrena globosa* ELISA
	GLRV	Mission Pino Noir Cabernet Flanc

CTV: Citrus tristeza virus; SDV: Satsuma dwarf virus; CEV: Citrus exocortis viroid; CTLV: Citrus tatter leaf virus; CLSV: Apple chlorotic leafspot virus; SGV: Stem grooving virus; SPV: Stem pitting virus; PRSV: Prunus necrotic ringspot virus; PDV: Prune dwarf virus; GRMV: Cherry green ring mottle virus; GFV: Grapevine fanleaf virus; GLRV: Grapevine leafroll virus; ELISA: Enzyme-linked immunosorbent assay.

easily transmissible by grafting. The use of virus-free or certified scions and/or nursery trees is the most important and the best way to prevent fruit tree virus diseases.

Virus certification in Japan

In 1961, the Japanese government settled on a mother orchard system to fulfil farmer's need for a continuous supply of healthy and true-to-type fruit cultivars. The legally controlled inspection of citrus and apple virus diseases began by the government plant inspectors. Since 1968, this system has been extended to stone fruits and grapevines. Mother trees are designated by prefectural government in orchards of farmers or of prefectural horticultural experiment station after inspection for known viruses by prefectural researchers. Government plant inspectors carefully observe all mother trees annually for the presence of any virus symptoms on leaves or stems. All trees bearing no visible symptoms are indexed for viruses by biological or serological means. Recently revised indicator plants used by the Plant Protection Station are shown in Table 2.

Horticultural association or some similar organizations ask commercial nurseries to grow nursery trees using scions from the inspected mother trees. Thus farmers can get certified scions for topworking or nursery trees made with scions from the mother trees. Although it seems difficult to estimate with accuracy the exact ratio of the number of certified scions to all scions demanded in our country, designated mother trees of each cultivar of each fruit tree may not be sufficient to meet whole demand of farmers.

According to the success of heat treatment for fruit tree viruses and the application of ELISA to detection of viruses, the Japanese government has introduced heat treatment facilities to principal fruit-producing prefectures since 1978. Soon virus-free scionwood may be supplied to growers.

How to obtain virus-free materials?

Some virus-free cultivars can be found by indexing a number of trees for virus content. In the case of apples, it may take over 5 years to detect all known viruses. However, once some cultivars are certified to be free from all known viruses, those cultivars are very valuable and have to be maintained with care to prevent infection. Mother tree blocks of these cultivars will be propagated and scions for topworking will be supplied from them to commercial nurseries.

If all trees of some cultivars or clones of a variety are infected with viruses, it is necessary to produce virus-free materials by heat treatment or shoot tip culture or shoot tip grafting. Sometimes, heat treatment is combined with shoot tip grafting.

Heat treatment: Heat treatment of virus infected plants has been attempted by exposing whole plants or budded plants to high air temperature. Exposure periods and the temperatures depend upon the kinds of tree fruit and the virus (Table 3).

Table 3. Heat treatment to obtain virus-free materials

Viruses	Temperature, °C (day time/night time)	Weeks
Citrus		
tristeza virus	40/30	4~5
satsuma dwarf virus	40/30	4~5
tatter leaf virus	40/30	>12
Apple		
chlorotic leafspot virus	37 or 40/30	3
stem pitting virus	37 or 40/30	4
Japanese Pear		
necrotic spot virus	35 or 40/30	2~3
Prunus		
necrotic ringspot virus	38 or 40/30	3~4
prune dwarf virus	38 or 40/30	3~4
Grapevines		
leaf roll virus	40/30	> 8

Shoot tip culture or grafting: The use of meristem or shoot tip culture for obtaining virus-free materials has been tried successfully with many herbaceous and bulbous plants. Attempts to apply this method to fruit trees have just begun. Trial for *Vitis* (Hirabayashi et al., personal communication) has potential value in mass production of virus-free materials.

Navarro et al.[3] developed a procedure consisting of placing a 0.15 mm long shoot tip excized from an infected citrus plant onto a decapitated rootstock seedling under aseptic conditions, and obtained cultivars free from tristeza virus and exocortis viroid.

Okudai et al.[4] combined shoot tip grafting technique with short time heat treatment. After heat treatment of 2~3 weeks, a 0.5 mm long shoot tip was placed on a decapitated hypocotyl of trifoliate orange seedling, and virus-free citrus plants were obtained.

Conclusion

Horticulturists have faced many troubles such as weakness of trees, incompatibility of grafting, early or late ripening of fruits and so on. Recently, some of these troubles are shown to be due to virus infection. Breeding of fruit trees usually takes a long time. Sometimes valuable hybrid clones suffer from infection with viruses by pollination or insect transmission. Many of these troubles can be avoided with caution and some knowledge of how individual viruses spread. Replacing all fruit trees with virus-free trees may eventually contribute greatly to increased production of good quality fruits.

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