MAJOR VIRUS AND VIRUS-LIKE DISEASES OF CITRUS IN INDIA, THEIR DIAGNOSIS AND MANAGEMENT

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ABSTRACT

Increasing emphasis on increased production along with improved fruit quality compatible at international standards and subsequently keeping an eye on global markets for exports in coming years warrants a thorough stock taking of constraints associated with citrus production in India. Among virus diseases, tristeza, ringspot, mosaic, and citrus yellow vein clearing virus are important in India along with witch’s broom of lime and rubbery wood, phytoplasmal diseases and citrus exocortis and yellow corky vein viroid diseases. In addition to these, citrus greening disease is also very important which is caused by a bacterium, Liberobacter asiaticum. Citrus tristeza virus (CTV), a member of the closterovirus group has flexuous particles of 10–12 x 2000 nm with single stranded, positive sense RNA of about 19,256 nucleotides which encodes 12 open reading frames that potentially code at least 17 proteins. Virus coat protein is about 25 kDa. Diagnostic reagents based on CTV coat protein and genomic RNA have been developed for quick and reliable indexing of CTV. Indian citrus ringspot virus (ICRSV) had filamentous particles measuring 650 x 15 nm and a ssRNA of about 7.5 Kb with a coat protein of 34 kDa. Short amino acid motifs in the CP sequence revealed limited similarities with the genera Potex, Carla, Fovea and Aleexiviruses but no strong similarity to any one of these. Citrus mosaic disease is transmitted by a mealy bug, Planococcus citri, and has bacilliform virions measuring 130 x 30 nm. Based on its bacilliform morphology and
serological reactivity, the citrus mosaic bacilliform virus was identified as a member of badnavirus group. Radiolabelled probes have been used to detect the virus in field trees by dot–blot hybridization. Yellow vein clearing disease of citrus is newly discovered virus and had flexuous particles measuring 685 x 14 nm with a coat protein of 32 kDa. The virus was detected in DAS–ELISA, DAC ELISA and ISEM using virus–specific antibodies. Presence of citrus greening was confirmed by electron microscopy or immunofluorescence or DNA–DNA hybridization in leaf samples collected from field trees from different parts of India. Yellow corky vein disease of citrus in India is known to cause yield losses up to 89.7% in kagzi lime in Assam and has been established as a viroid disease. Methods to manage these diseases have been suggested.

INTRODUCTION

Citrus is grown in 49 countries of the world and is one of the choicest fruits having high consumer’s preference both as fresh fruit as well as its refreshing processed juice. In India, it is the largest fruit industry after banana and mango. Citrus fruits are grown all over India but commercially it is grown in Maharashtra, Andhra Pradesh, West Bengal, Sikkim, Punjab and Assam. The major species grown in India are sweet orange (Citrus sinensis L. Osbeck), mandarin (C. reticulata Blanco), and acid lime (C. aurantifolia, Swingle). The estimated production of citrus fruits is 3.79 m tons from an area cover of 0.045 m ha. During the last 35 yr, the area under citrus cultivation has increased by 5 times. With fast growing domestic market and high potential for export of processed juice, the Indian citrus industry is expanding rapidly. This has created a demand for the introduction of superior varieties of citrus species in order to produce high quality fruits in different parts of the country. The absence of domestic quarantine free movement of bud wood is mainly responsible for the spread of virus and virus like diseases in the country.

With increasing emphasis on increased production along with improved fruit quality competitive at international standards and subsequently keeping an eye on global markets for exports in coming years warrants a thorough stock taking of constraints associated with citrus production in India and identify the future thrust areas to be tackled in a systematic manner. In order to establish the citrus industry of the country on a sound scientific footing it is necessary to develop agro–techniques which improve productivity as well as quality of the produce.

The productivity of the citrus fruit in India is comparatively low owing to many biotic stresses of which some fungi, bacteria, viruses, viroids and
phytoplasma play a very significant role. More than 30 virus diseases, two phytoplasma diseases, one spiroplasma disease, three viroid diseases, 11 fungal diseases, 3 bacterial diseases and two nematode diseases are known to occur in citrus throughout the world along with some diseases of uncertain etiology like citrus blight etc. Some of these diseases are of high economic significance as they can debilitate or wipe out the whole citrus industry if not managed in time. The roles of these pathogens are well established in declining tree health and production.

Among virus diseases, tristeza, ringspot, mosaic and citrus yellow vein clearing virus are important in India whereas vein enation and impatiens diseases are important only in certain regions. Among phytoplasma diseases, witch’s broom of lime and rubbery wood are major diseases but are endemic in nature. Citrus exocortis and yellow corky vein are two most important viroid diseases of citrus. In addition to these pathogens, citrus greening disease is also very important and wiped out the citrus cultivation in parts of Karnataka. This disease is caused by a bacterium, Liberobacter asiaticum. The major virus and virus like diseases of citrus worked out in India are summarized in the following paragraphs.

VIRUS AND VIRUS LIKE DISEASES

Tristeza

Tristeza (which means sadness in Spanish and Portuguese) was the name originally used to describe the rapid and widespread decline and death of millions of trees on sour orange rootstock in Argentina and Brazil following introduction and spread of the disease in the 1930s (69, 93). Later, a virus like agent was established in 1946 which was aphid transmitted and was named as Citrus tristeza virus (CTV) (17). It causes decline of trees grafted on sour orange (Citrus aurantium L.) as originally described. It produces stem pitting on limes [C.aurantifolia (Christen) Swingle and C.latifolia Tan.], grapefruit (C.paradisi Macf), and oranges [C.sinensis (L.) Osbeck] which debilitates trees and reduces yield.

Tristeza virus has wiped out the citrus industry in many countries before it was actually established and managed. For example, in Argentina and Brazil where the citrus industry has expanded after first world war, tristeza destroyed about 30 million trees. Similar situations were also reported from Spain, Japan and United States. The estimates indicate that tristeza destroyed about a million trees in India (1).
In India, Brown (22) recorded the failure of malta sweet orange on sour orange rootstock, providing evidence of flourishing tristeza disease in India. It was first reported from Maharashtra state (82). Later, its transmission was demonstrated by Toxoptera citricidus (88), Aphis gossypii, Myzus persicae (90), Aphis craccivora and Dactylopus Jacae (89). The virus was reported to be non-persistently transmitted from citrus to citrus. CTV is reported from almost all the citrus growing regions in India. It is less common in Northern India where T. citricidus does not occur although other species are found (29). Tristeza virus occurs almost in all citrus growing regions in the world and has been reported in the form of various strains. However, destructive strains are known to occur only in places wherever the vector T. citricida is present and active.

The decline symptoms of CTV are due to phloem necrosis in the bark of sour orange rootstock just below bud union. Movement of carbohydrates to the root system from the canopy is prevented. Once the carbohydrate reserves in the root system are exhausted, new fibrous roots can not be generated and root system degenerates resulting in tree decline (93). This decline is very fast and lethal and symptoms appear 1-2 yr after infection and the disease is called 'quick decline'.

Kitazima et al. (47) showed that thread-like virus particles were constantly associated with tristeza infection. Bar-Joseph et al. (14) for the first time partially purified tristeza virus (CTV) which lead to the development of serological methods for CTV detection. Kagzi lime (C. aurantifolia) is commonly used as a indicator host for biological detection of the virus and trifoliate orange can be used to filter tristeza from mixed infection of other viruses already present in field trees (83). A major breakthrough in CTV research was the production of virus specific polyclonal and monoclonal antibodies against CTV for successful detection in enzyme-linked immunosorbent assay (ELISA), (15) and the use of dsRNA technology (31) which later helped in identification of CTV-free planting material.

Tristeza virus infects nearly all citrus species, citrus relatives and hybrids. The only non-rutaceous host of CTV is Passiflora sp. In general, mandarins are normally tolerant to CTV infection but some strains of CTV can even infect certain mandarins such as Coorg mandarin in India. Tristeza virus was apparently originated in Asia where it might have existed for centuries but was unrecognized because many of the commonly propagated citrus cultivars in Asian countries are tolerant to CTV and were propagated by seed. Citrus was introduced to new world as seed and since CTV was not seed transmitted, the seedling trees were free from this virus. Eventually, vegetative propagation was
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started to maintain best horticultural traits citrus. When moved as vegetative propagation material nationally or internationally, CTV definitely moved through such materials resulting in wider distribution of the disease.

CTV is more damaging to Indian sweet oranges than to other cultivars (6). Kagzi lime is used as an indicator host for CTV detection, but the diagnostic vein–flecking symptoms on this host depend on temperature. No foliar symptoms are developed at temperature higher than 27°C and hence its biological indexing on large–scale is difficult, if controlled conditions are not adequate.

Despite some diversity at the molecular level most CTV isolates share a number of common properties. All are phloem limited and host range is mainly confined to citrus only except a species of Passiflora (13). All CTV isolates are readily transmitted by grafting. No mechanical transmission is known but can be transmitted by stem slash inoculation. No seed transmission is reported. All isolates of CTV are aphid transmitted.

CTV is a member of the clostero virus group (11, 49) and the complete sequence and genetic organization of one isolate has recently been determined (45, 63). The virus particles are 10–12 x 2000 nm and highly flexuous. It is easily sheared by mechanical forces but is relatively stable otherwise. The genome is single, positive sense RNA of about 19,256 nucleotides which encodes 12 open reading frames that potentially code at least 17 proteins. Virus coat protein is about 25 kDa. Serological relationship has been established among nearly all isolates and highly conserved epitopes have been identified (39, 70).

The CTV coat protein gene (CPG) was selectively identified in PCR and was cloned (51). The coding region of CPG was expressed in Escherichia coli (Migula) Castellani and Chalmers. In addition to the biological indexing on kagzi lime and virus specific antibodies, diagnostic reagents based on CTV–coat protein and genomic RNA have been developed for quick and reliable indexing of CTV. Strain specific monoclonal antibodies (MAbs) such as CTV–MCA 13 are being used to identify protective mild strains for the management of CTV by cross protection against severe and disastrous strains.

Indian Citrus Ringspot Virus

A ringspot disease of citrus was first described by Wallace and Drake (91). Later it was found that ringspot shares properties with psorosis (73). In India, a disease with ringspot symptoms was first reported as a strain of psorosis–A disease (2) but it was observed that field trees in India were devoid of bark...
lesions, a characteristic symptoms of all psorosis strains. Another major
difference was the characteristic flecking or vein clearing of young leaves of
inoculated sweet orange plants which unlike psorosis persisted until the leaves
became mature rather than for a short while, a characteristic of most psorosis
strains. These observations revealed that the disease in India was apparently
different from other known psorosis diseases. The incidence of ring spot
disease was observed up to 100% in most of the kinnon mandarin orchards in
North India especially in Punjab. The incidence in sweet orange ranged from
20–50% in Maharashtra, Andhra Pradesh and Karnataka. The incidence of the
disease was also evaluated on different rootstocks with kinnon as a scion variety and it was found that the disease incidence was more on Sohsarkar (C.
*rana* Raf (56%) and Karna khat (54.7%) as compared to Troyer citrange
(7.3%) in a root stock trial at the Indian Agricultural Research Institute, New
Delhi. The mature leaves of affected trees or inoculated plants showed distinct
ringspot symptoms without bark scaling.

The maximum incidence of the ringspot disease was observed in
kinnon–mandarin trees in India. The yield loss (number of fruits) in 7 yr old
kinnon trees was from 20.54 to 98.38%. The health of the affected trees
jeteriorated year after year and finally the affected trees collapsed.

Except bud transmission, no other mode of natural spread of the disease
could be established so far (23). However, citrus ring spot viorn were trapped
in immuno–electron microscopy (IEM) from the pollen of ringspot affected
kinnon trees, indicating the possibility of its transmission through pollen (1).
The virus was mechanically transmitted from citrus to herbaceous hosts like
*Cenopodium quinoa* and *Phaseolus vulgaris* var. *saxa*, singtamey, gheusami
and alapatri (61).

The disease can be easily recognized in field trees by its characteristic
ringspot symptoms. Affected trees show conspicuous rings on mature leaves
which may be one to several per leaf. Most of the ICRSV affected trees show
decline or die–back symptoms and the quantity and quality of fruits is greatly
affected. The glasshouse inoculated plants of sweet and mandarin show
shock reaction after 2–3 Abbrev. of inoculation. Young leaves of mosambi
sweet orange plants also develop vein clearing symptoms on young leaves
which persist till the maturation of the leaves. The virions of ICRSV both from
field and glasshouse infected plants can be detected in electron microscope in
leaf dip preparations with negative staining. The DAS–ELISA and DAE–
ELISA has been standardized for this virus and the virus is detected from the
planting materials. Recent studies have shown that the citrus ringspot virus in

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India is the second largest producer of citrus fruits in the world.

In the cultivation of citrus, the most popular species are *Citrus reticulata* and
*Citrus *tropical* and *Citrus aurantium.* The disease is also known as
“black spot” or “ring spot.”

In Indian citrus orchards, the disease is caused by a virus known as the
*Citrus ringspot virus* (CRSV). The disease is transmitted by the citrus
aphid, *Mesaphis persicae.* The virus can also be transmitted through
mechanical means, such as the use of infected plant material or by contact
with infected foliage.

The disease affects all citrus species and can cause severe damage to
orchards. Symptoms include the appearance of yellow or brown spots on
leaves and twigs, which develop into black lesions. In severe cases, the
leaves may fall off, and the tree may eventually die.

The disease is most prevalent in warm, humid climates, and it is
important for growers to implement good cultural practices to manage
the disease. These practices include regular pruning, proper irrigation,
and the use of disease-free planting material.

In conclusion, the citrus ringspot virus is a serious threat to citrus
orchards in India. growers must be aware of the symptoms and take
appropriate action to prevent the spread of the disease.
India is distinct from other ringspot viruses and hence named as Indian citrus ringspot virus (ICRSV).

Indian citrus ringspot virus (ICRSV) infects most of the commercial citrus cultivars and rootstocks used in India causing variable symptoms (23). The virus associated with citrus ringspot disease in India is purified from citrus and singtamey bean. The purified and leaf dip preparation of ICRSV affected leaf samples showed flexuous filamentous particles measuring 650 x 15 nm. The genome of ICRSV consists of ssRNA of about 7.5 Kb and the coat protein is 34 kd in size. Short amino acid motifs in the CP sequence revealed limited similarities with the genera Potex, Carla, Fovea and Allexiviruses but no strong similarity to any one of these. This shows that the virus does not belong to any of the known groups of viruses and needs to be placed in a new group (76, 77).

**Citrus Yellow Mosaic**

A mosaic disease of citrus was reported from Andhra Pradesh by Murthy and Reddy (55) on satdugdi sweet orange and on khasi mandarins from North–eastern states (8). The incidence of the disease was recorded to an extent of 5.84%. Recent surveys, however, showed the incidence of the mosaic disease from 10 to 70% in citrus orchards and nurseries in Andhra Pradesh (7). The disease can cause yield reduction of fruits up to 77% and the juice was 10% less with 1.3% less ascorbic acid having more acidity. Rao and Narasimhan (65) reported decrease in chlorophyll a, b and IAA in mosaic affected leaves. The mosaic disease from Andhra Pradesh was reported to be transmitted by aphid, *Toxoptera citricida* and that from north–eastern region by aphids, *Myzus persicae* and *Aphis craccivora*. However, no further information is available on these two mosaic diseases. In view of the occurrence of the mosaic disease only in India and its wide spread distribution and the losses caused, it was studied in detail at the Advanced Centre for Plant Virology, IARI, New Delhi.

Unlike earlier reports, citrus mosaic disease could not be transmitted by aphid vectors but was transmitted by a mealybug, *Planococcus citri*. The characteristic symptoms of the disease in field infected sweet orange and pummelo trees consisted of bright yellow motting of leaves and yellow flecking along the veins. It was transmitted to various citrus species by graft and to sweet orange, galgal and sugarcane by mechanical inoculations using phosphate buffer. In samples, collected from mosaic affected citrus trees from Andhra Pradesh, a mixed infection of three viruses, ICRSV, CTV and mosaic was often observed. Therefore, sap inoculation facilitated to obtain the pure culture of the virus.
In purified and leaf dip preparations of mosaic affected leaf samples, the bacilliform virions measuring 130 x 30 nm were constantly observed (9, 61). This bacilliform virus reacted specifically in ISEM tests to antisera of seven badnaviruses, sugarcane mosaic badnavirus, commelina yellow mottle virus, banana streak virus, cacao swollen shoot virus, Dioscorea bacilliform virus, canna yellow mottle virus and Kalanchoe top spotting virus but not to rice tungro bacilliform virus. Based on its bacilliform morphology and serological reactivity, the citrus mosaic bacilliform virus was identified as a member of badnavirus group. Its badnavirus nature was further confirmed by amplifying its genomic nucleic acid in PCR using degenerated primers located on consenses sequences of badnaviruses (9). These experiments confirmed that citrus mosaic disease is caused by a badnavirus which is the first record of a dsDNA virus in citrus.

SDS–PAGE and western blot analysis of purified virions showed one major band of 32 kDa and two minor bands of 22 and 19 kDa. The 32 kDa is the major capsid protein and two minor bands are degraded product of 32 kDa. The nucleic acid extracted from purified virions showed two bands of 21 kb and 6.6 kb in non–denaturing agarose gels. The faster migrating band of 6.6 kb is linear form of viral DNA while slow migrating band of 21 kb may be circular form of DNA. Electrophoresis of viral DNA digested with S1 nuclease gave two faster migrating bands of approximately 5.0 and 2.2 kb, besides 6.6 kb linear DNA band. These two bands arose due to the presence of two discontinuities on the viral DNA. The presence of two discontinuities on the genome is hall mark of reverse transcription process in pararetroviruses to which badnaviruses belong. The viral DNA was cloned at Hind III site in pUC18 vector and transformed into competent E. coli strain NM522 cells. The recombinant colonies were screened and finally two virus specific clones of 2.0 and 4.0 kb were identified. The cloned fragments were radiolabelled and used as probes to detect the virus in field trees by dot–blot hybridization. The virus was also detected in mealy bug vector using this probe. The 2.0 kb probe can detect the mosaic virus in DNA–DNA hybridization in even 25ng of the total DNA or 95 pg of viral DNA or 25 ng of tissue per spot (66).

A mosaic disease of citrus has been reported from Japan (42) and two from India (8). To avoid confusion, it was decided in the 13th IOCV conference held in China in 1995 that the name of the disease be changed as citrus yellow mosaic based on its characteristic symptoms, which was accepted.

*Citrus Yellow Vein Clearing Virus*

Yellow vein clearing disease of citrus is a new disease discovered recently from Experimental Research Orchard at Abohar in Punjab on a citrus cultivar etrog
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Later the disease was observed from Ludhiana, Ahmedabad, Pune and Anand on several commercial and germplasm plants of citrus showing its wider distribution in India. It is distinct from Indian citrus ringspot virus (ICRSV) and found in a mixed infection with ICRSV in naturally infected citrus trees. The typical symptom of the disease was yellowing of veins and veinlets and water soaking of veins on ventral side. The disease is graft transmissible to all commercial cultivars and mechanically transmitted to mosambi and herbaceous hosts like Phaseolus vulgaris var. sasaka, singtamey, ghwassami and Chenopodium quinoa but not transmitted by three species of aphids, mealy bugs and whitefly. Phaseolus vulgaris var. singtamey was identified as systemic host and C. quinoa as local lesion host. The disease is also not transmitted by seed or dodder.

The virus was purified from singtamey bean plants and antibodies were produced. The virus had flexuous particles measuring 685 x 14 nm. It has a coat protein of 32 kDa. The virus was detected in DAS–ELISA, DAC ELISA and ISEM using virus–specific antibodies. It is serologically distinct from 9 filamentous viruses viz. Indian citrus ringspot virus (ICRSV), henbane mosaic virus (HMV), potato virus–Y (PYY), potato virus–X (PVX), papaya ringspot virus (PRSV), garlic latent virus (GLV), shallot latent virus (SLV), carnation latent virus (CLV), garlic mosaic virus (GMV) and hence considered a new virus which is normally associated with ICRSV in contaminated trees.

Greening Disease

The presence of greening disease in India was suggested by Lilian Fraser, a scientist from Australia during her visit to India in 1965 (33). Following this suggestion Dr. S. P. Capoor and his team at the Regional Station of Indian Agriculture Research Institute, Pune, experimentally demonstrated that the so-called greening “virus” was transmitted by oriental psyllid, Diaphorina citri (25). Their experiment showed that possibly a “virus” was involved with the greening disease of citrus as BLOs and MLO’s were not known at that time. Occurrence of greening disease was later reported from various parts of the country mostly based on field symptoms and limited transmission tests. Most of these reports were based on zinc deficiency type symptoms on the leaves of affected trees but leaf mottle, a typical symptom of greening disease has been overlooked by subsequent workers.

Until the reports of Garnier et al. (35, 36) that the greening pathogen was phloem restricted Gram negative bacterium, the organism was considered to be a virus. Although the bacterial nature of the greening organism (GO) was established several years ago but it has not yet been cultured in cell free
medium and hence called as bacterium–like organism (BLO). Efforts were made all over the world to characterize the BLO associated with greening disease, but no one succeeded till Jagoueix et al. (43) experimentally confirmed that the greening organism was a true bacterium. They classified it as *Liberobacter* genus with two species, *L. asiaticum*, the organism responsible for Asian greening and *L. africanum*, the organism associated with South African greening. These studies were based on 16s rDNA sequences as well as sequences of the rplKArL–rpsBC operons with two probes In 2.6 (Asian greening) and 1.7Kb (South African greening).

Another breakthrough in the study of citrus greening disease was its experimental transmission to periwinkle (*Catharanthus roseus*) by dodder (*Cuscuta campestris* L.). In periwinkle GO multiplies quickly with much higher concentration than in citrus. Therefore it was easy to isolate it from phloem tissue of inoculated periwinkle plants for various studies.

In the absence of reliable diagnostic reagents and tools like electron microscope, the actual incidence and distribution of citrus greening bacterium (CGB) in India could not be achieved till a collaborative Indo–French project was developed at IARI, New Delhi (1991–94) with a view to prepare diagnostic reagents and its effective detection by serological and molecular techniques.

During the period of Indo–French project, 51,331 trees from 98 orchards were examined in 8 different states of India (Andhra Pradesh, Delhi, Haryana, Karnataka, Orissa, Punjab, Rajasthan and Uttar Pradesh) and the presence of citrus greening bacterium (CGB) was confirmed by electron microscopy or immunofluorescence or DNA–DNA hybridization in leaf samples collected from these places. Using these tests, it was established that leaf mottle was the symptom of greening disease and not the Zn deficiency type as reported by earlier workers (86).

Psyllids can acquire CGB during feeding on diseased source (25) but the percentage of the transmission is extremely low. After acquisition the BLO passes through intestinal valve and travels to acini in the salivary glands from where it is transferred to the plants. This is normally a long process (1–3 wk) for most of the prokaryotes. Except in few endemic regions for greening like Coorg in Karnataka and Hindupur in Andhra Pradesh, the field spread of CGB was extremely low in spite of the presence of the psyllid vectors (*D. citri*) in abundance which suggested the possibility of the presence of different biotypes in psyllid vector.

Recent experiments showed that adult psyllids were unable to acquire CGB from known CGB infested pineapple sweet orange plants after variable
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acquisition feeding as determined in DNA-DNA hybridization tests (1). It appears that psyllids either acquire and transmit CGB at their nymphal stages or there are biotypes in D. citri that alone act as efficient vector. Further comparative DNA-DNA hybridization results revealed that biotypes of Indian psyllids were less efficient vector than that of Malaysian psyllids. Different serotypes of the CGB were also determined with the help of monoclonal antibodies. The MAbs prepared from Pune strain of the CGB also recognized the Malaysian CGB showing similarities in the pathogen found in two countries but the presence of more number of viruliferous psyllids in citrus plantations in Malaysia further confirms the distinct biotypes in the vector, D. citri.

Two important reagents, the monoclonal antibodies and CGB specific nucleic acid probes, were developed from a Pune strain of bacterium (20, 86). However, the trees with typical die back symptoms in Nagpur region were without leaf mottle and gave negative results for the presence of CGB in electron microscopy and DNA-DNA hybridization. These results were in contrast to the observations made by Fraser et al. in 1966 (33) and hence more intensive testing is needed. The nucleic acid probes also detected the bacterium in individual psyllid vector.

Among the commercial cultivars, group of sweet oranges [C. sinensis (L.) Osbeck] is more susceptible to the greening bacterium than the group of lemons [C. limon (L.) Burm.f] and limes [C. aurantifolia (Christm) Swingle]. Trifoliolate orange [Poncirus trifoliata (L.) Raf.] is known to be tolerant to greening bacterium but it was also found infected with greening in Coorg region. Hence, the prospects of successful breeding of new scion varieties inheriting workable resistance is still remote. However, the disease symptoms can be suppressed by injecting the affected trees with 500 or 1000 ppm tetracycline or penicillin under pressure (10 Kg/cm²) (44). Regular treatment with these drugs can reduce the incidence and intensity of the greening disease.

Field diagnosis of greening disease is difficult because the symptoms on affected trees are often confused with that of zinc and other nutritional deficiencies. Varma et al. (86) tested citrus trees showing variable symptom with electron microscopy, ELISA and DNA-DNA hybridization and concluded that motting of the leaves on affected trees was the main foliar symptom of the greening disease. Unusual colouring of the fruits and aborted seeds were the additional symptom. Electron microscopy of ultrathin sections is an important technique to detect the bacterium in sieve tubes of affected leaves. No polyclonal antibodies could be developed for this bacterium so far because of its pleomorphic nature and has not been possible to culture in synthetic media. Monoclonals have, however, been prepared and used for strain-specific
detection (20, 86, 5). The nucleic acid probes detected most bacterial strains of the greening present in India. It is, therefore, now possible to detect more strains of greening bacterium in ELISA, immuno-fluorescence and DNA–DNA hybridization (86, 5).

**Witches Broom**

Witches, broom disease of lime (WBBL) was first observed in north coastal plain of the Sultanate of Oman near the border of United Arab Emirates (UAE) in 1970. Since then the disease is spreading within Oman and extended all along the coastal plain from UAE to Muscat (34). In India, it was first observed in Nagpur district in eastern Mahashtra in 1995 (40). Later, it was seen in other major lime growing states of Andhra Pradesh, Tamilnadu and Karnataka. The characteristic symptoms of WBBD include chlorotic leaves, highly proliferated shoots and shortened internodes. Leaves drop prematurely and infected twigs are distorted. In some cases infected branches develop dieback.

In 1986, phytoplasma was reported to be associated with witches broom disease of lime. The organism is transmitted to periwinkle (*Catharanthus roseus*) by dodder (*Cuscuta spp.*) (19). It is graft transmitted to lime, troyer citrange lemon, rough lemon and trifoliate orange (21) but not to sweet or sour orange (34). The natural hosts of this phytoplasma in Oman are citron, sweet lime and Indian and Palestine sweet lime.

Monoclonal antibodies and DNA specific probes of the phytoplasma have been developed and used for specific detection of the phytoplasma in trees and insect vectors (21). Among several leaf hoppers, *Hishimonus phyctis* reacted positively with phytoplasma–specific probes which was finally proved to be the vector of the disease. *H. phyctis* was also found to feed on lime trees in Sultanate of Oman thus facilitating the spread of witches, broom phytoplasma under natural condition. In India, *H. phyctis* is a vector of the little leaf of brinjal, a phytoplasma disease (18). The WBBL–phytoplasma specific monoclonal antibodies and DNA probes did not react with the egg plant little leaf phytoplasma thus showing the two phytoplasma distinct from each other. Recently, witches, broom disease of lime has also been established in India (40).

**Rubbery Wood**

Rubbery wood (RB) disease is known to occur in India for a long time (4). The limbs of affected trees bend downward and are abnormally flexible. The trees become unproductive and may die. The disease is transmitted by graft and
dodder, *Cuscuta reflexa*. Phytoplastas were seen in EM in ultrathin sections of diseased tissue but not in tissues of healthy plants. Phytoplastas were seen only in sieve tubes of phloem cells (3). Rubbery wood has been tested with a panel of MAbS produced against the Indian, Chinese and South African greening bacterium and also with the MAbS produced against witches, broom of lime to establish its identity but no positive relationship could be established with these pathogens. The RW phytoplasma is detected either by electron microscopy or through biological indexing on lemon seedlings. However, the relationship of RW phytoplasma and recently established WBDL phytoplasma needs to be established.

**Exocortis**

Bark scaling and stunting of infected trees are the main symptoms of citrus exocortis disease as first described in 1948 by Fawcett and Klotz (32). However, mild to moderate symptoms were interpreted as a reaction of citrus exocortis viroid (CEVd) isolates on indicator plant, etrog citron. These include epinasty of leaves, browning of petiole, leaf tip and mid–rib (71). The affected trees remain stunted to various degree but CEVd is rarely lethal and fruit quality is not much affected. Trifoliate orange, citranges and Rangpur lime are desirable tristeza tolerant root stocks but are susceptible to CEVd. Mild strains of CEVd have been used to induce tree dwarfing for high density plantations in Australia and Israel but were later replaced by dwarfing root stocks.

Citrus exocortis disease is transmitted by graft and also through mechanical inoculations. The most common method of its natural spread is through contaminated tools employed during orchard operations specially while pruning. Seed and vector transmission have not yet been confirmed experimentally. It is highly transmissible by tools from tree to tree. CEVd is transmissible to *Gynura aurantiaca*, petunia and tomato plants and caused distinct and characteristic epinasty symptoms on these hosts. Recently it has been reported that in addition to CEVd there are four other discrete groups of citrus viroids based on molecular weight, sequence homology, host range and symptom reaction on etrog citron. In India, two viroid species causing diseases in citrus have been identified (64).

CEVd can infect most citrus species and several non–citrus hosts such as tomato (Rutgar) and cucumber (Suyo). The most sensitive citrus species are trifoliate orange, Rangpur lime, some citron specially etrog citron and lemons which develop stem blotching or bark splitting symptoms. CEVd infected sweet orange, grapefruit and mandarins are symptomless but when grafted on sensitive rootstocks characteristic disease symptoms develop.
CEVd is an infectious, circular, ssRNA with 302 nucleotides, (53) which are highly base paired forming a stable rod like structure. Some regions of the CEVd molecule have homologies with potato spindle tuber and chrysanthemum stunt viroids. CEVd is highly resistant to heat inactivation and also to chemicals used to inactivate viruses. It may survive for long periods in dry tissue but can be inactivated by hydrolysis or by ribonucleases under appropriate conditions.

*Citrus Yellow Corky Vein*

A disease of citrus characterized with yellowing of veins and formation of corky tissues on the lower surface of affected leaf veins has been reported as two different names in India, yellow corky vein and yellow mid vein by Reddy et al. (68) and Sharma and Pandey (80), respectively. The disease was also reported earlier as citrus yellow vein (75). Based on the typical symptoms all the three diseases were found similar and hence to avoid confusion the name of the disease was retained as yellow corky vein disease.

Yellow corky vein disease of citrus in India is known to cause yield loss up to 89.7% in kagzi lime in Assam. The disease is widely distributed in India and is being disseminated inadvertently through vegetative propagation. Experimentally, the disease was transmitted by wedge and bud grafting and infected 19 hosts in Rutaceae family. Kagzi lime (*Citrus aurantifolia*) has been identified as an indicator host of yellow corky vein disease of citrus. Transmission of the disease was highest when maximum and minimum temperature remains 41.7 and 22.7 °C, respectively. Polyacrylamide gel electrophoresis and return polyacrylamide gel electrophoresis (PAGE and R-PAGE) and nuclease treatment of samples revealed the association of viroid like RNA with yellow corky vein disease which is a new record (74).

**DIAGNOSIS**

*Biological Indexing*

Sensitive diagnostic methods are very important for developing strategies to avoid pathogens in propagative materials. Virus and virus like diseases of citrus have been identified by graft inoculation to various plant species which develop characteristic symptoms when they are inoculated by one or more pathogens. Biological indexing is done by inoculation to specific indicator plant by graft or mechanical inoculations. Biological indexing requires insect proof glasshouse with controlled conditions. Some of the citrus viruses like CTV, ICRCV, CMBV require low temperature 18-22/26-28°C for symptom development
while some pathogens like exocortis viroid and phytoplasma require higher temperature (32°C for symptom expression). The indicator hosts and symptoms expression of various virus and virus like diseases are given in Table 1.

Table 1. Indicator hosts of important virus and virus–like diseases of citrus.

<table>
<thead>
<tr>
<th>Name of virus</th>
<th>Indicator host</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tristeza, closterovirus</td>
<td>Mexican lime</td>
<td>Flecking of the leaf veins and stem pitting</td>
</tr>
<tr>
<td>Yellow mosaic, badaivirus</td>
<td>Sweet orange, Citrus decumana</td>
<td>Bright yellow mosaic on leaves of inoculated plants</td>
</tr>
<tr>
<td>Indian citrus ring spot virus (new group)</td>
<td>Sweet orange</td>
<td>Shock reaction, vein clearing of young leaves and chlorotic rings on older leaves</td>
</tr>
<tr>
<td>Chenopodium quinoa</td>
<td>Necrotic local lesion on inoculated leaves</td>
<td></td>
</tr>
<tr>
<td>Greening bacterium</td>
<td>Pine apple sweet orange</td>
<td>Morining of leaves</td>
</tr>
<tr>
<td>Witches’ broom, Phytoplasma</td>
<td>Mexican lime</td>
<td>Proliferated shoots and shortened internodes</td>
</tr>
<tr>
<td>Yellow corky vein, Viroid</td>
<td>Kagzi lime</td>
<td>Yellowing of mid vein and lateral veins and formation of corky tissue on lower surface of leaves.</td>
</tr>
<tr>
<td>Exocortis, viroid</td>
<td>Etrug citron</td>
<td>Epinasty, browning of petiole, leaf tip and midrib</td>
</tr>
</tbody>
</table>

Mexican lime (Citrus aurantifolia) is only suitable indicator host for detection of CTV. It shows vein clearing or vein flecking symptoms on leaves of inoculated plants. At low temperature sometimes leaf cupping symptoms are also noticed. Grapefruit seedlings are often used to differentiate stem pitting and seedling yellow strains of tristeza. Seedling yellow strains produce small yellow leaves and stunted growth of seedlings of grapefruit and sour orange. Vein corking is seen in Mexican lime when inoculated with severe seedling yellow tristeza isolates. Grapefruit and Madom vinous sweet orange are used as indicator plant to identify stem pitting strain. Mild isolates of CTV rarely show any conspicuous symptoms but the virus can be detected in ELISA systems.

Viroid diseases are detected by grafting infected budwood on citron selection 861–S1. The temperature ranging from 32–40°C during the day and 27–30°C during the night is ideal for symptom development on this indicator plant. Severe symptoms associated with CEVd appear 8–12 wk after inoculation in the form of classical leaf epinasty. Petiole may be wrinkled or
cracked and discoloured. Cracking on bark may vary depending on the strain and environment. Indian citrus ringspot virus (ICRSV) can also be detected by mechanical inoculations to Phaseolus vulgaris cv. Saxa and Singtamey. Whereas, citrus yellow mosaic can also infect sugarcane by mechanical inoculations.

**Serological Indexing**

Indexing of citrus planting material by serological techniques is more easy and quicker than indicator hosts as it requires large number of plants, controlled conditions and infrastructures like glasshouses etc. For serodiagnosis of viruses a high titred polyclonal antisera would be required where as for citrus greening bacterium and witches' broom phytoplasma specific monoclonal antibodies would be required for their successful detection.

**Table 2.** Indexing of cross protected kagzi lime trees in the experiments at IIHR Bangalore and at All India Citrus Improvement Project, Tirupati.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>* OD values at 405 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 DF 1</td>
</tr>
<tr>
<td></td>
<td>Bangalore</td>
</tr>
<tr>
<td>Mild strain</td>
<td>0.78</td>
</tr>
<tr>
<td>Severe strain</td>
<td>0.92</td>
</tr>
<tr>
<td>Mild strain+Severe strain</td>
<td>0.80</td>
</tr>
<tr>
<td>Uninoculated (Healthy)</td>
<td>0.52</td>
</tr>
<tr>
<td>Controls Glasshouse (Healthy)</td>
<td>0.00</td>
</tr>
<tr>
<td>Severe strain (T- 36)</td>
<td>1.13</td>
</tr>
<tr>
<td>Mild strain (T – 30)</td>
<td>1.26</td>
</tr>
</tbody>
</table>

* The OD values are the average of 6 trees of each treatment.

Although several serological techniques have been used for virus indexing but ELISA, immunofluorescence (IF) and immunosorbent electron microscopy (ISEM) are being used widely to detect pathogens in citrus. However, pathogens which are restricted to phloem tissues such as greening bacterium and phytoplasma are best detected in IF ELISA has been more commonly used
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to detect CTV and its strains. In India, cross protection experiments done at Bangalore and Tirupati were evaluated in ELISA using monoclonal antibodies (MAbs) (28). The results are given in Table 2.

During this experiment, two MAbs, 3DF1 and MCA–13 were used. 3DF1 recognizes all CTV strains but MCA–13 recognizes only severe strain. Therefore, a strain recognized by 3DF1 but not by MCA–13 was considered as mild strain. However, all the trees of these experiments were contaminated with severe strain of CTV and no mild strain was detected. The use of ELISA for detection and diagnosis of tristeza is well tested and proven technique. Number of monoclonal antibodies (MAb) and polyclonal antibodies (PAb) have been prepared against different isolates of CTV in different laboratories. Some of the MAbs react only with limited number of isolates while other react to a broad range of isolates. These differences in reaction pattern and epitope mapping indicate that multiple antigenic sites are present in CTV coat protein. Monoclonal antibodies MCA–13 is commonly used to differentiate severe strains from mild strains. ICRSV and citrus yellow vein clearing virus are also detected by DAS–ELISA. The antibodies for yellow mosaic badnavirus and citrus ringspot virus have also been developed and are being used for the detection of these viruses.

Immunofluorescence

It is generally used to detect phloem limited viruses and fastidious prokaryotes and to locate the pathogen in plant tissue. The nature of antigen epitopes recognized by the antigen–specific antibodies is determined by immunofluorescence. The principle involved is the specific binding of antigen and antibody which can be determined in section or plant sap taken from infected plants using fluorescein isothiocyanate (FITC) labeled with goat anti mouse/rabbit immunoglobulins. Immunofluorescence has been used for the detection of CTV and citrus greening bacterium. To conduct immunofluorescence test for greening disease strain specific antibodies have been developed and used for detection of different isolates as shown in Table–3.

The results of Table 3 showed variability in greening pathogen in Indian, Chinese and South African isolates. However, the MAbs were also highly specific to isolates.
Table 3. Detection of Greening Organism in immunofluorescence with MAbs

<table>
<thead>
<tr>
<th>Greening Isolates</th>
<th>INDIA</th>
<th>CHINA</th>
<th>S. AFRICA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2D12</td>
<td>10A6</td>
<td>10F4</td>
</tr>
<tr>
<td>India (Pune)</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bangalore</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>China</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Malaysia</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Thailand</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Taiwan</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indonesia</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>South Africa</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Philippines</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Immunosorbent Electron Microscopy**

Immunosorbent electron microscopy (ISEM) was originally described by Derrick (30). The method makes use of trapping of antisem–specific virus particle onto EM grids that have been coated by specific antiserum. The virus concentration is increased on the grids which, otherwise, is difficult to detect because of low concentration of the virus in host cells.

Decoration method of ISEM was introduced to plant virology by Milne and Luisoni (52). Decoration is a process whereby virus particles are first individually adsorbed to grids and placed over specific antibody so that the antibody halo is seen to have absorbed the stain and become electron dense.

**Nucleic Acid Based Diagnosis**

In citrus, most of the viruses are found at low concentration and have erratic distribution. Therefore, nucleic acid based diagnostic methods have been developed for indexing. Among them, nucleic acid spot hybridization and PCR are mainly used. CYMBV has been successfully detected by DNA–DNA hybridization using a virus specific 2kb probe (66) as shown in Table 4.

Nucleic acid hybridization using pathogen specific DNA probes labeled with P$^{32}$ (20) or digoxigenin (DIG) as non–radioactive probe has been used to detect greening bacterium L. asiaticum in plants which do not show any symptoms. Nucleic acid based probes have also been used to detect CYMBV from field samples and its vector Planococcus citri (66).
The technique was found to be very useful for virus detection in symptomless trees. Techniques of nucleic acid hybridization, and return polyacrylamide gel electrophoresis (R-PAGE) have also been used to detect viroids infecting citrus. Single strand conformation polymorphism (SSCP) has been adopted as a tool for viroid characterization during recent years. Differences among CEVd isolates were identified by SSCP analysis of full length viroid cDNA. In addition to CEVd, four other discrete groups of viroids are based on their molecular weight, molecular conformation and sequence homology as well as host range and symptom reaction on etrog citron and alternative host (78).

**Polymerase Chain Reaction (PCR)**

This technique was discovered by Mullis et al. (56) and its immense potential for the use in the diagnostic field was rapidly exploited. PCR has been found very useful because it can amplify targeted DNA up to $10^6$ - $10^9$ fold during 3-4 h reaction. PCR is used in detection assays with higher sensitivity far superior to all previously developed assays. It is highly specific. This technique has been modified in numerous ways to detect viruses. Among them RT–PCR, immuno-capture–PCR, Nested–PCR are used more frequently. The print capture PCR (PC–PCR) and squash capture PCR (SC–PCR) has also been used to detect CTV (24). PCR using virus or virus–group specific oligonucleotides...
as primers has been successfully used to detect citrus mosaic badnavirus (9). Citrus exocortis viroid from sweet and sour orange (leaves, bark, and fruit) has been detected by PCR (50, 94). CTV has been detected by RT–PCR from the twig and leaves (59, 73). Primers have been designed for Indian citrus ring spot virus (ICRSV) based on the sequence of CP gene. Using RT–PCR, a product of 500 bp was obtained in ICRSV infected plants of citrus and Phaseolus vulgaris. Using RT–PCR and PCR product as probe in dot–blot hybridization, ICRSV was detected even in symptomless trees (79).

Electron Microscopy

Electron microscopy of negatively stained leaf dip preparation is the quickest and reliable method of detection of viruses. Depending on size and morphology, a virus may be tentatively assigned to a particular taxonomic group. Indian citrus ring spot virus (ICRSV), citrus yellow mosaic badna virus (CYMBV) and citrus yellow vein clearing virus (CYVV) have been detected for the first time using EM in India at the Advanced Centre for Plant Virology, IARI, New Delhi. Citrus greening bacterium like Liberobacter asiaticum has been detected in thin sections of phloem tissues. Similarly phytoplasmas are detected in thin sections (86). When virus concentration is low in infected tissues and difficult to detect by usual leaf dip preparations, immunosorbent electron microscopy (ISEM) technique is applied to trap the virus using virus specific antibodies. Serological relationships between viruses are specifically established by this technique (ISEM–decoration). Citrus viruses like CTV, CMBV, CYVV and ICRSV have been detected in planting material (5, 61, 62). Decoration technique offers the most convincing demonstration by electron microscopy of specific combination between virus and antibody. The results of ISEM decoration are more sensitive than ELISA and hence can be used to identify virus free nucleus material.

MANAGEMENT

Virus and virus like diseases of citrus are managed by an integrated approach of using virus–free planting material, use of host resistance, sanitation, cultural practices, control of insect vectors and regulatory measures.

Use of Virus–Free Certified Planting Material

Citrus being a vegetatively propagated crop, use of virus–free planting material is very important. This can be achieved by careful indexing of budwood collected from mother plants using sensitive diagnostic approaches described earlier in this chapter. Shoot tip grafting has been preferred to develop
virus-free planting material. It is an alternative to the use of nucellar seedlings (58). Citrus plants regenerated through in vitro grafting did not show the long juvenile phase of plants obtained from nucellar tissue (58). In view of the wide distribution of ICRSV, virus free planting materials have been developed by shoot tip grafting by the authors. Shoot tip grafting is known to produce virus free plants and to eradicate pathogens such as CEVd, CTV, CVV, psorosis, vein enation and cachexia (57, 58, 82)

Sanitation

The orchard using virus free plants should also be free from other sources of infection like diseased old plants and other weed hosts which are reservoirs of viruses and insect vectors. Therefore, it is very essential to eradicate the virus infected plants and other sources of infection. Citrus viroids have very wide host range outside family rutaceae. Murraya koenigii, Ruta graveolens, Evodia hupelensis are reported to be symptomless carriers of tristeza and periwinkle as an additional host for greening bacterium.

Cross Protection

Cross protection has been defined as the phenomenon by which a plant infected with a mild strain of pathogen is protected against subsequent challenge inoculation with a more virulent strain. This concept of cross protection has been successfully used in management of CTV with great success in Brazil where more than 8 million Para orange trees were cross protected in 1980 (54) and more than 50 million trees in 1987 (84). Cross protection was also very successful in South Africa. Cross protection is ideal for management of tristeza since it is often endemic vector borne virus and difficult to eradicate as there are no alternative control methods.

Viroid management by cross protection is also reported in tomato plants with a prior infection by a mild strain of PSTVd and challenge inoculation with a severe strain (46). However, indexing showed the presence of both the strains in cross protected plants. The severe strain was found to replicate faster than mild strain and eventually replaced by later (46). But increasing the gap between protecting and challenge inoculation decreased the number of doubly infected plants (81) Cross protection of PSRVd strain is a host dependent phenomenon and requires more information as how the cross-protection occurs in viroids in the absence of protein.
Vector Control

CTV is transmitted by brown citrus aphid *Toxoptera citricidus*, *Aphis gossypii*, *A. spiraecola* and *T. aurantii*. Greening bacterium is transmitted by psyllids *Diaphorina citri* in South East Asia, India and Saudi Arabia and by *Triozerythrea* in South Africa and Yemen. These insect vectors can be controlled by using biological and non-biological methods in order to stop spread of these diseases. Non biological methods include use of insecticidal sprays, insect traps, reflective mulches etc. Different biological agents include parasitoids, predators and microbes. Parasitoids are insect specific. The genera *Aphelinus*, *Mesidia* and *Mesidiopsis* of Aphelinidae (Super family Chalcidoidea) are of parasitoids of aphids and *Tamarixia radiata* is an effective parasitoid of citrus psylla. Similarly different coccinellides are useful predators of aphids. Entomopathogenic fungi like *Verticillium lecanii* and *Paecilomyces farinosus* are in practice to control *T.citricidus* and *Aphis gossypii* (85).

In Reunion Island, spread of greening disease could be checked within two years of release of eulophid ectoparasites *Tetrastichus dryi* and *T. radiatus* which reduced the population of psyllid vectors. *T. radiatus* could not reduce the population of *D. citri* in India because of secondary parasites (86).

Host Resistance

Citrus and its relatives show variable reaction to CTV. Most citrus species and cultivars are susceptible to infection but some are tolerant and do not show obvious symptoms of the disease. Mandarins (*C. reticulata* Blanco) are tolerant to stem pitting isolates of CTV where as acid limes (*C. aurantifolia*) are most susceptible and show vein clearing, stem pitting and stuntting when infected by most isolates of CTV.

Some citrus relatives are highly resistant or immune to infection (38). Trifoliate orange (*Poncirus trifoliata*), a citrus relative, is immune to CTV isolates and has been used as breeding parent for rootstocks and for development of CTV immune scions (16, 41). A large number of citrus species and hybrids with resistance or tolerance to CTV can be used to produce grafted trees tolerant to CTV–induced decline but most of the rootstocks are susceptible to other diseases (27).

Cultural Practices

Crop hygiene is a major cause of many mechanically transmitted diseases like ICRSV, CYVV, CMBV and exocortis. These diseases can be transmitted by contaminated field implements during orchard operations. Therefore, all the
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operational tools including pruning and grafting tool should be sterilized with 1–2% sodium hypochlorite before use.

Quarantine

It prevents the introduction of exotic, new and potentially destructive viral pathogens in the country or within the country. Only strict quarantine measures and restricted movements of citrus bud wood will limit the spread of viral pathogens. Quarantine often fails to recognize the importance of strain variation, pathotypes and alternative host in the introduction of new diseases. Therefore, care is required to be taken while granting permission for import of citrus spp./budwoods.

Budwood Certification Programme

Certification programmes are fundamental to the control of the transmissible diseases of citrus. When properly implemented, the certification programmes can provide protection against further spread of the graft transmissible diseases already present and safeguard against the introduction of exotic graft transmissible diseases (48). For management of virus diseases in citrus, it is essential to have mandatory bud wood certification programmes. To start with such a programme, it is essential to develop basic information on virus and virus–like pathogens, to prepare diagnostic reagents and to evolve techniques for quick detection of pathogen in the planting material. These diagnostic reagents can be used by the agencies given the responsibility of bud wood certification so that we can develop a strong bud wood certification system in the country. The bud wood certification programme will be successful by making policy decision and regulations for sanitization of the orchards, extension mechanism to popularize management practices for minimizing infection of plants and development of trained human resource for undertaking such programmes (85).

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