Prospects of classical cross protection technique against *Citrus tristeza closterovirus* in Pakistan

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ABSTRACT: In Pakistan citrus groves in general are facing a serious problem of decline that is attributed to different causes. The major cause, however, is the prevalence of citrus virus and virus-like diseases; *Citrus tristeza virus* (CTV) is of utmost concern. Although CTV has been identified and characterized on the basis of serological and physical properties, no information is available on the strains of CTV in Pakistan. The identification of CTV strains will be helpful in developing strategies to control the decline of citrus trees to a great extent. Many citrus growing countries have successfully used the technique of cross protection to minimize the drastic effect of severe CTV strains. By pre-immunization of the citrus tree with mild strains, the decline can be controlled to increase the life span of the citrus tree. In this study we focus on the possibility of establishing a cross protection technique in Pakistan against the CTV strains.

Keywords: *Citrus tristeza virus*; cross protection

Among all fruits cultivated in Pakistan, citrus holds the top position both in area and production. Kinnow mandarin (*Citrus nobilis* × *Citrus deliciosa*), Feutrell's early (*C. reticulata* Blanco), sweet orange; blood red and Musambi (*C. sinensis* (L.) Osbeck), grapefruit (*C. paradisi* Macf.) and sweet lime (*C. limettioides* Tan.) are the main citrus species grown in Pakistan, whereas lemon and lime take up a relatively small area. Chapot was the first who surveyed citrus orchards in 1970 and observed dieback and decline symptoms in a large number of citrus trees without attributing any cause. He reported the stubborn disease of citrus as well as the presence of Psylla, *Diphorina citri* vector of greening disease but not of stubborn disease. On the contrary, Cochran (1976) reported the greening disease in Pakistan for the first time. Extensive surveys by Bove (1995) and Catara et al. (1988) revealed the presence of several virus and virus-like diseases of citrus and their identity was based on symptom expression, enzyme linked immunosorbent assay (ELISA) and electron microscopy (EM). Later on, surveys and the ELISA indicated that citrus trees were infected by CTV to an extent of 23% (Anwar, Mirza 1992).

The prevalence of these diseases in citrus groves must be responsible for health deterioration because they are systemic ones, plants remain infected throughout their life, and many viruses take a long time to manifest symptoms. The different strains of CTV also take a long time except the yellow strains of CTV that can be diagnosed as early as possible. CTV is reported to comprise many strains or cluster of strains. Although the sour orange and sweet orange and mandarin combination is stimulatory for CTV, it can also occur in other ways.

Recent surveys and our work on disease-free citrus plants (Mazhar 2002; Mazhar et al. 2005) based on ELISA test clearly revealed the presence of CTV in citrus groves, mainly in mandarin as well as in sweet orange. It is very likely that sudden death and general decline may becaused by CTV.

Citrus decline in Pakistan

Citrus decline is a gradual deterioration process exhibiting loss of vigour, death of twigs and branches, reduction in yield and ultimate death of the whole plant. It seems to be a major problem in Pakistan. Viral diseases in citrus received no or little attention for a long time. The presence of citrus viral disease in Pakistan was first recognized in 1985. Bove (1995) reported that greening was responsible for citrus decline along with dieback caused by poor cultural practices, gummosis and root rot. Other typical viral symptoms such as bark scaling, blind pockets, bud union crease and cracking, etc. were noticed. Viruses (CTV, IVV and YVC) and viroids (Cachexia-Xyloprosis, exocortis) are totally dependent on the host metabolism and the host parasite interaction is not breakable.

CTV and its strains

Citrus tristeza closterovirus (CTV) is one of the most common, important and serious pathogens worldwide. By 1991, over a 100 million trees had been destroyed by CTV quick decline in Argentina, Brazil, Spain, California, Venezuela and other areas. As the virus is aphid transmitted, eradication procedures are not very effective. The two CTV types which affect citrus production most are: (a) quick decline and the associated necrosis of phloem cells just below the bud union of trees on sour orange, lemon and grapefruit rootstocks, and (b) severe stem pitting in the scion or rootstock and the associated tree decline, reduction in yield and poor fruit quality. Some CTV strains are mild and have no effect on citrus while others are severe and can cause tree decline and/or death. A tree often becomes infected with more than one strain. Certain strains prevent the infection or expression of symptoms of other strains. Infected trees can harbour several strains and the results may be quite different than the effect of any single strain infection. The severity of the disease also varies with variety and rootstock. Mandarins are very tolerant to CTV regardless of the rootstock. Grapefruits and oranges, which will decline quickly on sour orange rootstocks, are tolerant to most California CTV strains if grown on commonly used citrange, trifoliate or lemon-type rootstocks (Kallsen 2002). Mild strains of CTV can be used occasionally to protect trees from more severe strains similar to immunizations to protect animals from diseases.

Citrus tristeza virus is prevalent in many countries where citrus is grown. 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 rootstocks in Argentina and Brazil since the 1930's (Rocha-Pena et al. 1995; Wallace 1978). CTV is transmitted semi-persistently by several species of aphids, of which the brown citrus aphid, *Toxoptera citricida*, is the most efficient (Lee, Rocha-Pena 1992). CTV strains are broadly grouped according to how they affect certain plants or scion/rootstock combinations (Lee, Rocha-Pena 1992), i.e. those causing mild symptoms, seedling yellows symptoms, decline on sour orange, stem pitting of grapefruit, and stem pitting of sweet orange. The mild forms do not normally cause noticeable effects on most commonly grown citrus cultivars whereas the seedling yellow strains cause severe chlorosis and dwarfing of inoculated sour orange under greenhouse conditions. Declining strains cause death of trees grafted on sour orange rootstocks. However, these strains do not cause damage to trees grown on tolerant rootstocks such as sweet orange or Rangpur lime. In contrast, stem pitting strains of grapefruit and sweet orange cause significant damage to grapefruit or sweet orange regardless of their rootstocks. Control of CTV by cross protection is largely aimed at the stem pitting strains of grapefruit and sweet orange, although work is progressing in Florida to identify mild strains that might give protection against the decline strains that affect trees grafted on sour orange rootstocks (Fuchs et al. 1997).

Cross protection: definition and concept

Cross protection is a phenomenon whereby plants infected with one strain of the virus do not develop additional symptoms when inoculated with another strain of the same virus. In other words, prior infection with one (protecting) plant virus will prevent or interfere with super-infection by another virus (usually related virus). This phenomenon was first shown by McKinney (1929) with *Tobacco mosaic virus* (TMV). The two viruses can replicate and spread independently in isolation, but in the presence of the protecting virus, the host plant confess resistant to the challenging virus, or the symptoms of the challenging virus are suppressed. Success in virus control by cross protection depends on whether the avirulent virus can invade and replace the virulent virus, and whether the virulent virus is prevented from re-establishing. 'Invasion' of mild viruses usually involves artificial inoculation as many have very low transmission rates by vectors under natural conditions (Costa, Muller 1980; Lecoq et al. 1991; Yeh et al. 1988). Some examples of cross protection in annual and perennial crops are shown in Table 1.

Principle and procedure of cross protection

The terminology commonly used refers to the virus strain that induces cross protection as the 'protecting

Table 1. Examples of cross protection in some perennial and annual crops

Host plant	Protecting virus	Challenging virus	Transmission	Sources
Perennials				
Cacao	Mild strain of *Cacao swollen-shoot virus* (*budnavirus: caulimoviridae)*	severe strains of CSSV	semi-persistent by mealy bugs: *Planococcoides njalensis*, etc.	Posnette and Todd (1955)
Citrus	Mild strains of *Citrus tristeza virus* (*closterovirus: closteroviridae*)	severe strains of CTV	semi-persistent by *Toxoptera citricida*	Costa and Muller (1980)
Papaya	Mild mutant PRV strain (PRV HA 5-1) of *Papaya ring spot virus* (*potyvirus: potyviridae*)	severe strains of PRSV	non-persistent by *Myzus persicae*	Yeh et al. (1988)
Annuals				
Tobacco	Green mosaic strain of *Tobacco mosaic virus* (*tobamovirus*)*	yellow mosaic strain of TMV	mechanical inoculation	Broadbent (1976); McKinney (1929)
Tobacco	Mild strain of *Potato* virus *X* (*Potyvirus*)*	severe strain of PVX	mechanical inoculation	Salaman (1933)
Tomato	Very mild strain of *Potato spindle tuber viroid* (viroids)	severe strains of PSTVd	mechanical inoculation	Branch et al. (1988)
Potato	Mild strain of *Potato leaf roll virus* (*polerovirus*: *luteoviridae*)	severe strain of PLRV	persistent by *Myzus persicae, Macrosiphum euphorbiae*	
Cereal	MAV, *Barley yellow dwarf virus* (*luteovirus*: *luteoviridae*)	PAV, a BYDV	persistent by *Sitobion avenae, Rhopalosiphum padi*	Power (1996); Rochow et al. (1983)

*Family unassigned (Mayo, Pringle 1998)

strain' and to the strain that is used to evaluate cross protection efficiency as the 'challenging strain' (Lecoq 1998). Challenging strains, inducing easily recognizable symptoms, are often chosen for laboratory experiments. They can be inoculated mechanically by grafting or using their natural vectors at different times after the inoculation of protecting strains.

In field experiments, the protecting strain is generally an isolate that induces mild symptoms and does not affect the potential yield of crop. Protecting strains used for controlling severe strains are often referred to as 'mild', 'attenuated', 'hypovirulent' or 'avirulent' strains (Fletcher 1978; Fulton 1986; Huss et al. 1989; Oshima 1975).

According to Lecoq (1998) an ideal mild isolate to be used in the field for cross protection should have the following characteristics:

1. It should induce milder symptoms than isolates commonly encountered in the fields and should not alter the potential yield and the quality of the crop.
2. It should be mild in all its cultivated hosts including those which are not targets for the cross protection.
3. The isolate should be genetically stable and not evolve towards more severe forms at times.
4. The mild isolate used for the cross protection should not be easily transmitted or disseminated by vectors.
5. It should provide a protection towards the widest possible range of severe challenging isolates.
6. It should be easy to produce, check for purity, store and provide to farmers. A simple inoculation procedure should be designed so that it does not require expensive equipment or specific training in order to be applied in the fields.

Mild strain selection: Some mild strains are obtained as naturally occurring variants. Plants with mild symptoms may be observed in the fields while most of the other plants show severe symptoms. Frequently mild virus subcultures may be isolated from such plants. Other mild strains have been obtained in the laboratory, either from single local lesion isolations from samples with severe symptoms or from plants inoculated by severe isolates, but which developed spontaneously axillary branches with mild symptoms (Lecoq et al. 1991). Heat or cold treatment may also yield mild isolates (Kosaka, Fukunushi 1993; Oshima 1975). After mutagenesis treatment, generally with nitrous acid, followed by local lesion selection, mild strains can also be produced (Rast 1972; Yeh, Gonsalves 1984).

After the selection of mild strain different steps should be followed in order to evaluate its potential for practical applications. A preliminary evaluation should be performed in the laboratory and or in a protected environment. Subsequently, experiments are conducted under field conditions in the area where natural epidemics caused by severe isolates occur.

Production and application of mild strain: According to Lecoq (1998), mild strain should be multiplied in highly protected environment under very strict phytosanitary supervision in order to eliminate the risks of contamination by undesirable viruses, bacteria or fungi. Many techniques have been developed for easy and efficient inoculation of mild strains. Mechanical inoculation with hair brushes, cotton swabs, sponge pads or forceps (Fulton 1986) is laborious and time consuming. It may also favour the non-intentional spread of other mechanically transmissible severe viruses. Different grafting techniques have proved to be very efficient to inoculate mild isolates to woody plants (Costa, Muller 1980; Muller 1980).

Use of spray guns (with adapted air pressures and nozzle sizes) is another useful alternative to inoculate the mild strains (Fletcher 1978; Yeh et al. 1988). An important parameter for the implementation of cross protection is the 'safety period' after inoculation of mild strain. It may be defined as the time necessary for the mild strain to invade its host before providing a full protection. This may depend on the host, the virus and the environmental conditions.

Potential examples of successful cross protection

Cross protection was widely used to establish relationships among virus strains. It was also of potential interest for protecting plants against viruses in the field (Matthews 1991). Cross protection has been observed to occur not only between viruses but also it was demonstrated to occur between viroids (Nibblet et al. 1978) or plant virus satellites (Jacquemond, Tepfer 1998). Cross protection is successfully used for:

1. *Tomato mosaic virus* in Japan
2. *Cucumber mosaic virus* in Japan, USA and Europe
3. *Plum pox virus* in Europe
4. CTV in India, USA, South America and South-East Asia
5. Cocoa swollen shoot in Europe
6. Papaya ring spot virus in South-East Asia
7. Potato spindle tuber viroid in South-East Asia.

Limitations

Cross protection has also been associated with potential hazards (Fulton 1986; Hamilton 1985; Matthews 1991). Several limitations or possible risks associated with this control method (Lecoq 1998) are as follows:

1. An incomplete protection may occasionally be observed; indeed some apparent "breakdowns" of cross protection have been reported.
2. The protecting strain may possibly spread to other hosts in which it may have more severe effects.
3. Amplified disease symptoms caused by a synergism with other viruses might spread readily in the cross protected crop.
4. Heteroencapsidation or heteroassistance in mixed infection with another virus may modify virus transmission, specificity or efficiency.
5. Genetic recombination between the protecting strain and other virus(es) in mixed infection may occur.
6. The protecting virus may mutate into a more severe form that would cause a destructive disease.

Mild strain cross protection also occurs naturally in citrus areas with long histories of stem pitting, and no formal cross protection programmes (Garnsey et al. 1998). Each time growers select outstanding trees as budwood sources for propagation of new citrus orchards they are unknowingly selecting either trees infected with a protecting isolate or trees that have escaped infection by severe isolates.

The selection of protecting isolates is the easiest in areas where stem pitting is common and large populations of trees have already been screened by natural challenge. The risks associated with deliberate use of infected budwood are also minimal in these situations since no new virus component is added (Garnsey et al. 1998). The use of cross protection as preventive strategy for stem pitting in areas where stem pitting is uncommon is not recommended (Rocha-Pena et al. 1995). Successful mild strain cross protection presumably requires a certain minimum degree of the relationship between protecting and challenge isolates at least in certain areas of the viral genome, even though the mechanism is not fully understood. Examples of protection between different isolates and examples of apparent lack of protection between others were reported (Roistacher 1988; Roistacher, Dodds 1993).

Field observations indicate that protection is not generally permanent and that protective effects may break down over time, especially where the challenge pressure is high. From a commercial standpoint, cross protection is a means to extend the productive life of a planting faced with injury from stem pitting, but it does not provide a permanent cure to stem pitting problems.

Selection of mild protecting isolates has been largely empirical. With better knowledge of sequence differences between isolates and how the CTV genome is organized, it should be possible to select effective and mild protecting isolates more accurately (Niblett et al. 1993). It should also become feasible to genetically engineer attenuated isolates from severe sources with protective characteristics and to modify protective isolates so that they will not be aphid transmissible (Garnsey et al. 1998).

CTV cross protection

In the past, CTV cross protection was used in the context of 'mild strain' selection and the use of these 'mild strains' as protective isolates. These 'mild' CTV strains were defined by their mild reaction in indicator seedlings. This has proved to be a false assumption as a protective isolate in one cultivar may not be mild reacting in other cultivars (Mooney et al. 1994). For example, a very severe CTV stem pitting isolate in grapefruit was found to induce a very mild reaction in Mexican lime indicator seedlings (McClean 1977). Therefore, most scientists working on cross protection adopted the terminology advocated by Roistacher (1992) and for the purposes of cross protection they use the term 'protective' isolates or strains rather than referring to them as 'mild'. CTV cross protection or preimmunization consists in inoculating plants with a vigorous protective isolate of the virus to afford protection against the attack by a severe CTV strain. This has proven to be an economical and convenient means of reducing the effects of severe CTV in Australia, Brazil and South Africa.

Protective isolate selection procedures

Successful use of cross protection against CTV involves careful evaluation of specific host effects and protecting abilities. The usual procedures for the selection of a cross protecting strain are:

1. To select protective strains from healthy looking field trees in older orchards that have stunted or unhealthy trees showing symptoms of CTV infection.
2. By bud selection from milder reacting isolates on indicator plants.
3. By selection from field trees or glasshouse plants which had previously been infected with CTV-SY (seedling yellows) and exhibited seedling yellows symptoms but had recovered and lost this symptom.
4. By aphid transmission of CTV-SY or CTV-SP (stem pitting) in infected grapefruit, lemon or sweet orange seedlings to seedlings of grapefruit, lemon or Mexican lime, for production of attenuated isolates.
5. By aphid transmission of CTV-SY and CTV-SP from infected sweet orange to *Passiflora* species and then from *Passiflora* back to Mexican lime.

In the course of selecting and screening for potential protective CTV strains the following desirable traits are used as selection criteria (Lee et al. 1987).

Biological activity: The CTV strain should elicit mild symptoms not only in the target cultivar but also in other citrus cultivars, species and relatives.

Titre and movement within the plant: The distribution of the protective strain within the plant should be uniform and the virus should have the ability to quickly invade new growth flushes. Any part of the plant that is virus free, even temporarily, provides an opportunity for an aphid to vector in a severe challenge CTV strain, which could result in the breakdown of the cross protection.

Effect of environment on cross protection: Some CTV strains are better adapted to warm conditions, whereas some prefer cooler temperatures. Therefore, it is important to evaluate potential cross protection strains under environmental conditions similar to those in which they will have to perform.

Ability to be aphid transmitted: Strains of CTV differ in their ability to be rapidly aphid transmitted, an ideal protective strain of CTV should be easily and rapidly aphid transmitted.

Cross protection programs against CTV strains

Citrus growers have few other options since changing rootstocks does not prevent stem pitting in the scion, and lime, grapefruit and sweet orange cultivars tolerant or resistant to stem pitting are not available. MSCP is often only partially effective, and carries certain intrinsic risk; it can be useful in situations where heavy production losses are certain unless it is used.

CTV protective isolate/strain is currently used as control for grapefruit in South Africa and Australia and for Pera sweet orange in Brazil (Lee et al. 1994). In these countries protective isolates have been selected from vigorous trees that remained in areas where most trees had been severely affected by stem pitting (Carver 1989). Mildness of these isolates and their protective ability were confirmed in experimental tests. These protective isolates have been disseminated largely by using mild strain-infected budwood to propagate new trees. In South Africa, the certification programme was used to deploy mild isolates for protective purposes (von Broembsen, Lee 1988).

CTV cross protection in Brazil

Cross protection has been widely used with great success in Brazil, more than 8 million Pera sweet orange trees were cross protected in 1980 (Muller 1980) and more than 50 million trees in 1987 (Urban et al. 1990). After the introduction of CTV to Brazil in the 1920's, the Brazilian citrus industry converted to growing trees on CTV-tolerant rootstocks. However, strains of CTV were still causing significant damage to lime, grapefruit, and Pera sweet orange grafted onto CTV-tolerant rootstocks. Cross protection efforts were aimed at these strains. Mild strains were obtained by recovering CTV isolates from trees that grew well in orchards where severe infection was prevalent; the logic being that these trees were growing well because they were protected by mild strains. Indeed, six out of 45 isolates induced only mild symptoms and protected sweet orange, grapefruit, or lime trees against damage from stem pitting strains after challenge inoculation by aphids. Furthermore, protected plants produced good fruit yields. Once the efficacy of the mild strains was established, protected trees were obtained rapidly by grafting scion buds from mild strain-infected trees to healthy CTV-tolerant rootstocks. Cross protection to control CTV in Pera sweet orange in Brazil is still practiced (Lee, Rocha-Pena 1992).

CTV cross protection in Australia

Stem pitting induced by CTV is a severe problem affecting grapefruit in New South Wales. Stem pitting strains reduced yield and, more importantly, fruit size, which makes them less marketable. Cross protection experiments conducted at two locations over a 21–25 year period showed the beneficial effects of cross protection as well as the effects of the climate (Broadbent et al. 1991). As in Brazil, mild strains of CTV were selected from healthy appearing and non-stem-pitted grapefruit trees growing in severely affected orchards. The ability of two mild strains to protect inoculated grapefruit trees against a severe strain was compared at Somersby (humid area on the coast) and at Daredon (hot dry inland area), both in New South Wales. The overall data showed clearly that cross-protected plants yielded more and better quality fruits than severe strain-inoculated plants or plants that became aphid-infected by severe strains after planting in the field. The difference between cross-protected and severe strain inoculated or initially uninoculated trees was much more obvious at Somersby than at Daredon. Mean yield of mild strain No. 3135-inoculated trees was 204 kg as compared to 63 kg for the severe strain in the 19^{th} year after planting at Somersby. In comparison, the same mild strain-inoculated plants at Daredon had a mean fruit yield of 239 kg versus 145 kg for severe strain-inoculated plants at the same time period. Measurements of fruit size also showed significant differences. Cross-protected trees had a much lower proportion of small fruits than the severe strain-inoculated trees. Measurement in breakdown of cross protection from infection by severe strains indicated that 10 out of 117 (8.5%) plants showed deterioration of fruit quality at the cooler more humid Somersby site whereas no marked evidence of breakdown was noticed at the hotter and drier Daredon site.

CTV cross protection in South Africa

In South Africa, CTV is endemic and no program of enforced tree removal is in effect. South Africa still has a viable citrus industry. *Citrus tristeza virus* is controlled by means of cross protection. Trees are cross-protected by deliberately propagating them with infected budwood containing a mild strain of CTV, which prevents a more harmful, severe strain from infecting or expressing itself in the tree. The process is somewhat comparable to vaccination in humans. Cross protection does result in fruit yield and quality reductions in South African trees, and it allows the industry to continue. Tree longevity also appears to be reduced by cross protection. CTV strain monitoring continues to be a very important part in the South African CTV control strategy. New strains can appear which can overcome existing cross protection. Research efforts must be maintained to ensure that new cross-protective strains are available when needed. The CTV management program in South Africa remains an expensive program. Since CTV was endemic, tree removal was not an option for the South Africans.

Cross protection by mild populations of *Citrus tristeza virus* (CTV) in the South African citrus industry is essential because of the threat of introduction of severe strains into trees by the aphid vector. Populations (strain mixtures) or single strains should have specific characteristics to be suitable as good protectors. Two important traits associated with a good protector are rapid multiplication of the virus in order to invade all parts of the plant and its adaptability to different environmental conditions (van Vuuren, der Vyver 2001). It has been shown that the three cross-protecting populations used in the South African citrus industry are composed of different strains.

Table 2. Evaluation of mild CTV strains to maintain a 12 years old pineapple on sour orange rootstock in Flatwoods location where decline strains of CTV caused an annual loss of 20% (Lee et al. 1992)

Treatment	No. of trees	Average rating[1]	No. of dead trees
No mild CTV	7	3.25	2
Mild T30	7	2.32	1
Mild T26	7	1.78	0

[1]Trees were rated on a scale whereby 1 = healthy appearance, 4 = dead tree

CTV cross protection in Florida, USA

Some CTV strains cause decline of sweet orange trees grafted on the susceptible sour orange rootstock. In areas where these strains are prevalent, the industry generally adapts by abandoning the sour orange rootstock and switching to CTV-tolerant ones. However, there are still important citrus growing areas, such as Florida, where sour orange is still used because the dominant strains are the mild type, which does not cause quick decline.

Florida researchers have initiated experiments for selecting mild strains that might protect against CTV decline (Lee, Rocha-Pena 1992). These efforts are timely since decline-type isolates are present in Florida and have caused severe losses in some areas (Brlansky et al. 1986). Furthermore, should the expected invasion of the brown citrus aphid occur, it will facilitate the spread of decline type isolates and cause even more severe damage to citrus planted on sour orange (Lee, Rocha-Pena 1992). Although trials are in the early stages, there is hope that selected strains may help to control CTV decline of trees grafted on sour orange rootstock. The development of a monoclonal antibody, MCA13, which reacts preferentially to decline-type isolates, will help the researchers in their selection and evaluation of mild strains (Permar et al. 1990). Interestingly, efforts are being made to save mature trees on sour orange by deliberately infecting them with mild strains. If successful, this approach will be of significant economic benefit because about a third of the producing trees in Florida are still on sour orange rootstock.

According to Lee et al. (1992) several Florida mild strains were selected from Florida that demonstrated the ability to cross protect against both severe SP CTV strains in exotic locations and against QD on sour orange rootstock. Thus the immediate implementation of cross protection using these mild strains would prolong the economic life of trees already on sour orange rootstock and would also provide cross protection against severe SP strains. Lee et al. (1992) evaluated the efficiency of CTV mild strains to maintain a 12 years old Pineapple on sour orange rootstock against the QD strains (Table 2).

The infection by certain mild isolates of CTV can cross-protect grapefruit trees on sour orange rootstock from decline-inducing isolates of CTV that are prevalent in the Indian River region of Florida (Powell et al. 1999). The ability of three mild isolates of *Citrus tristeza virus* (CTV) to prevent natural infection of 84 Ruby Red grapefruits on sour orange rootstock by aphid-transmitted, decline-inducing isolates of CTV was assessed by symptoms and verified by enzyme-linked immunosorbent assay (ELISA) after 16 years. Out of 21 trees in each of four treatments protected by the DD 102 bb, Guettler HS, and DPI 1-12-5-X-E mild CTV isolates, 14, 10, and 14% were infected by severe isolates (MCA13 monoclonal antibody reactive) compared with 67% for unprotected control trees. The health of trees protected by the DD 102 bb CTV isolate was significantly better than that of unprotected control trees as measured by decline, tree ratings, and tree height.

Scope in Pakistan

The extent of decline in citrus groves was reported by many scientists (Bove 1995; Catara et al. 1988). Our preliminary work revealed that CTV was prevalent in Sahiwal and Faisalabad citrus groves where it was associated with decline. Many scientists reported the efficacy of MSCP to reduce the effect of severe strains. Introduction of MSCP into our citrus certification program will be useful to develop citrus plants tolerant to severe strains of CTV. The cross protection will be required only if severe strains of CTV are present in Pakistan. At present, no research work is conducted related to differentiation of CTV strains, and we do not know what types of strains are prevalent in Pakistan. Before the cross protection is initiated, it is important and of utmost concern to isolate and differentiate the CTV strains. If only mild strains are detected, then we are lucky enough that our citrus trees are free of severe strains. However, if the SP and QD strains of CTV are detected, then we have to adopt the control measures and strategies to minimize their effect. MSCP will be efficient to cope with the severe strains of CTV, as no alternative method is available to stop the effect of severe strains.

Program and planning

1. Selection of mild strains as indicated.
2. Multiplication of mild strains in a quarantined area.
3. Laboratory experiment on the efficiency of mild CTV strains.
4. Selection of citrus groves showing the highest incidence of CTV as well as in orchards free from CTV.
5. Pre-immunization of healthy citrus trees with mild strains and then inoculated with severe strains by releasing vectors.
6. To develop a population of viruliferous vectors.
7. Evaluation of cross protection by:
   a) Health of the tree;
   b) Symptom of CTV (pin holing, pegging, vein clearing, etc.);
   c) Fruit bearing and quality.

CONCLUSION

Cross protection has proved to be very effective in controlling SP and to some extent QD strains of CTV when no alternative control method is available. It was successfully applied to different species of citrus that were cultivated under a variety of conditions against severe strains of *Citrus tristeza closterovirus*. The versatility of this method makes it easy to use in a timely manner and it can also be applied easily to citrus species facing the threat of severe strains of CTV. Cross protection should be integrated into the citrus certification and management system as the use of specific cultural practices along with cross protection may enhance its field efficiency and counteract slight yield losses which are occasionally observed after protective strain inoculation.

Acknowledgement

We are highly acknowledged the support of the Ministry of Science and Technology, Government of Pakistan.

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Received for publication October 2, 2004
Accepted after corrections December 14, 2004

Možnosti použití techniky klasické křížové ochrany proti *Citrus tristeza closteroviru* v Pákistánu

ABSTRAKT: V Pákistánu je pěstování citrusovitých kultur vážně ohroženo z různých příčin. Významné místo zaujímají virové choroby a z virů svým významem a rozšířením převládá CTV. Ačkoliv byl virus identifikován již dříve a byly zjištěny jeho sérologické a fyzikální vlastnosti, chybějí stale informace o výskytu jeho kmenů na území Pákistánu. Identifikace kmenů pomůže stanovit strategii ochrany citrusovitých kultur vůči CTV. V řadě zemí, ve kterých se pěstují citrusovité kultury, byla s úspěchem použita křížová preimunizační metoda, při které inokulace mírným kmenem CTV může zabránit rozšíření silného kmene a tím do značné míry snížit ztráty jím působené. Ve studii je soustředěna pozornost na možnost zavedení techniky křížové ochrany u kmenů CTV.

Klíčová slova: *Citrus tristeza closterovirus*; křížová ochrana

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