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Citrus tristeza virus: An increasing trend in the virus occurrence and distribution in citrus fruits of Northwest, Pakistan

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Citrus tristeza closterivirus (CTV) is one of the most damaging fruit viruses playing havoc in citrus orchards around the world. Here, we report, an ELISA-based indexing of citrus trees over a period of eight years (2002 to 2010) in Northwest Pakistan, revealing that the incidence of CTV is increasing mainly with the distribution of infected rootstocks, putting citrus industry at the verge of complete annihilation. The surveys revealed that the average incidence of CTV in 10 major citrus growing districts in the Northwest of Pakistan has steadily increased from 24% (in 2002) to 31, 35, 39 and 44 in 2004, 2006, 2008, 2010, respectively. Maximum per cent increase of CTV was in citrus orchards in district Haripur, that is, 27.50% and followed by 26% increase of the virus incidence in district Swat during 2002 to 2010. The incidence of the virus was correlated with use of sour orange as root stock with rough lemon. In orchards, where rough lemon was used as root stock; the incidence of CTV was comparatively low. A comparative study of the virus incidence conducted during 2006 to 2012 in sweet orange trees grafted on sour orange and rough lemon in Northwest indicated an average per cent incidence 43 and 37, respectively. This is the first comprehensive study on citrus fruits to determine an increasing trend of CTV in Northwest of Pakistan where citrus industry has been a major source of income for local farmers. An extensive rescue plan needs to be placed to avoid complete destruction of this export-based industry.

Key words: Citrus, Tristeza, citrus tristeza closterivirus (CTV), ELISA, incidence, Pakistan.

INTRODUCTION

Citrus fruits represent approximately 40% of all fruit crops growing in Pakistan and mainly concentrated in Punjab and Khyber Pakhtunkhwa (KP) Province (Catara, 1987; Catara et al., 1988). The range of cultivated citrus

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Abbreviations: CTV, Citrus tristeza virus; **DAS-ELISA,** double antibody sandwich-enzyme-linked immunosorbent assay; **KP,** Khyber Pakhtunkhwa; **PARC,** Pakistan agricultural research council.

cultivars in Pakistan is limited. Kinnow is a major citrus cultivar in Punjab, whereas sweet orange (blood-red) is mainly grown in KP. Many cases of citrus decline have been reported from Punjab and KP, Pakistan in 1970s and citrus tristeza was considered as the major cause of the decline (Bove, 1995). In a preliminary survey conducted by a group of Italian and Pakistani experts in 1988 with the co-operation of Ministry of Foreign Affairs and Pakistan Agricultural Research Council (PARC), citrus was reported to be infected by a number of virus and virus-like diseases in KP and Punjab, regions Pakistan and only CTV was confirmed by enzyme-linked immuno-sorbent assay (ELISA) and electron microscopy (Catara et al., 1988). After a period of about 15 to 20 years, the situation had been deteriorated to the extent that most of the citrus orchards are about to collapse in KP (Arif et al., 2005a) as well as in Punjab Provinces (Iftikhar et al., 2009) of Pakistan. Almost 100% of citrus trees in most parts KP are infected with one or more virus and virus-like diseases (Arif et al., 2005a) that resulted in high economic losses. The major virus and virus-like diseases of citrus trees reported in Pakistan are tristeza, infectious variegation, exocortis, cachexia-xyloporosis, greening and stubborn (Catara, 1987; Catara et al., 1988; Bove, 1995; Arif et al., 2005a; Iftikhar et al., 2009). Most of these diseases are wide spread in new plantations due to un-certified infected bud wood being used as scion. As a result, the citrus industry that could earn foreign exchange in billion is on decline and is almost collapsed. New plantation would also be un-successful until pathogen-free bud wood is provided to the citrus growers.

Tristeza caused by citrus tristeza virus (CTV) is the most economically destructive disease of citrus fruits world-wide. The virus is restricted to phloem tissues of the infected plants. It occurs in most citrus producing areas of the world, especially in the areas where CTV-sensitive sour orange (*Citrus aurantium* L.) is used as rootstock (Bar-Joseph et al., 1989; Rocha-Pena et al., 1995). CTV was originated in the Orient from where it was spread world-wide through infected bud-wood and plants in the quest for new citrus varieties (Bar-Joseph et al., 1979; Roistacher et al., 1991; Roistacher and Moreno, 1991; Rocha-Pena et al., 1995). Several epidemics of CTV have been reported from Argentina and Brazil during the 1930s, over 30 million citrus trees were killed (Bar-Joseph et al., 1989), in Spain during 1960s and in Venezuela during 1980s, about 10.0 and 6.6 million trees were killed, respectively (Cambra et al., 1988; Rocha-Pena et al., 1995).

The virus particles are flexuous, approximately 2000 × 11 nm in size (Gonsalves et al., 1978; Bar-Joseph and Lee, 1989), having single stranded positive-sense RNA of about 20 Kb (Gonsalves et al., 1978; Bar-Joseph et al., 1985; Bar-Joseph and Lee, 1989) and encapsidated by a coat protein (Bar-Joseph and Lee, 1989; Sekiya et al., 1991). Molecular characterization of the CTV isolates in various countries has been established (Biswas, 2010; Al-Sadi et al., 2012; Davino et al., 2013). CTV is transmitted by several aphid species in a semi-persistent manner (Raccah et al., 1989; Yokomi et al., 1994). However, each aphid species vary in transmission efficiency. The most efficient aphid vector is *Toxopetra citricida* (Roistacher and Bar-Joseph, 1987; Rocha-Pena et al., 1995) where *Aphis gossypii* Glover is also an efficient vector of severe strains of CTV in many areas around the world (Roistacher and Bar-Joseph, 1987; Yokomi and Garnsey, 1987). *Aphis spiraecola* Patch (Yokomi and Garnsey, 1987), *Toxoptera aurantii* (Norman and Grant, 1958), *Aphis craccivora* Koch and *Dactynotus jacae* (Roistacher and Bar-Joseph, 1987; Bar-Joseph and Lee, 1989) have also been reported as vectors of CTV. *A. gossypii* and *A. spiraecola* are the major vectors of CTV in Pakistan (Catara, 1987).

In Pakistan, occurrence of CTV has long been suspected in citrus fruits but virus identity was confirmed by using ELISA and electron microscope from samples collected from various parts of KP and the Punjab provinces (Catara et al., 1988; Catara et al., 1991; Anwar and Mirza, 1992). In Northwest (KP province) of Pakistan, comprehensive surveys were conducted to report virus and virus diseases of citrus fruits and CTV was emerged as major pathogen (Arif et al., 2005a). The occurrence and distribution of CTV was also reported from selected orchards of KP and the Punjab provinces by Iftikhar et al. (2009). In this paper, we report an increasing trend of CTV in citrus fruits in Northwest of the country including KP province, based on more than eight years of comprehensive survey study, requiring a rescue plan to avoid complete annihilation of citrus industry.

MATERIALS AND METHODS

Field surveys: sampling and indexing of mother citrus trees for CTV

Field surveys were conducted from March to September 2002 to 2010 in 10 major citrus growing districts (Peshawar, Nowshera, Charsadda, Mardan, Swabi, Haripur, Malakand, Swat, Dir, D. I. Khan) of the KP province, Pakistan. Two citrus orchards were selected in each district and orchards were selected where 100 to 200 citrus trees were present at 10 to 20 years of age. During surveys, trees were examined diagonally as described by Hughes and Gottwald (1998) or by selecting 10 × 10 m, 20 × 20 m area at two-three sites in an orchard (Arif et al., 2005a). In some cases where total numbers of trees were in hundreds, then a scheme of random assessment was made (Arif et al., 2005a). The total number of trees in selected sites or in some cases total numbers of trees in an orchard were counted, assessed and trees showing symptoms of the virus were recorded. Trees showing characteristic symptoms of CTV were tagged for future reference. For serological indexing for CTV, leaf samples were collected from infected and healthy trees from all 20 orchards in 10 districts surveyed. Samples were collected in plastic bags, kept in ice bags and transported to the laboratory for serological detection. The specimen of insects associated with citrus plantation were collected in Petri dishes containing moistened blotting paper and kept for identification. Detailed information on insecticides sprays, source of nursery plants and rootstocks, cultivars and symptoms of other biotic effects

were also recorded. Percent incidence of CTV was determined in each orchard/site or district using ELISA based surveys as: (% CTV incidence = total trees infected/total trees tested × 100). Mean values were calculated for each district surveyed.

Serological studies

DAS-ELISA was used for the detection of CTV (Clark and Adams, 1977). The tests were performed in polystyrene micro-plates containing 96 wells (NUNC, Immunoplate II, Thermal Scientific, Waltham, MA, USA). ELISA-plates were coated with 100 µl aliquots of CTV-specific antibody (Agdia, USA) with coating buffer, pH 9.6. Plates were kept at room temperature in a humid box for 3 to 4 h. Leaf samples were extracted by crushing through pestle and mortar in extraction buffer, pH 7.4. Leaf tissues were extracted in extraction buffer at 1:10 ratio (w/v), and 100 µl of prepared sample was dispensed in each well after washing. After first incubation was completed the plates were washed with 1 × PBST buffer, pH 7.4. Positive control wells were filled with using same amount of sap from known CTV-infected and healthy citrus plants as negative control. ELISA-plates were incubated inside a humid box for 2 h at room temperature or overnight in refrigerator (4°C). After washing, 100 µl of enzyme conjugate (Agdia) was dispensed in each well of the plate and incubated in a humid box for 2 h at room temperature. The plates were washed four times with 1 × PBST. 100 µl of OPD solution [(100 ml of OPD solution, pH 5.0 was prepared by dissolving hydrogen peroxide (30%) 0.4 ml, citric acid (anhydrous) 5.1 g, sodium phosphate, dibasic (anhydrous) 7.33 g in 900 ml of distilled water and volume was adjusted to 1000 ml by adding more distilled water)] was added per well and the plates were incubated 1 h in humid box at room temperature or overnight (that is, 16 h) at 4°C. The reaction was stopped by adding 50 µl 3 M sulphuric acid to each well. The reaction was assessed visually or measured at A_{405} nm using Titertek Multiskan, (Model MC-Photometer) (Flow Laboratories, Covina, CA, USA). Inc.). The samples were considered to be positive when the $A_{405\ nm}$ values exceeded the mean of the virus-free samples by at least a factor of three.

RESULTS

CTV was present in all major citrus growing areas of the Northwest of Pakistan (KP province) surveyed (Table 1). The characteristic symptoms of CTV were commonly observed on sweet oranges grafted on sour orange. The infected trees exhibited vein clearing in leaves and developed phloem necrosis and inner face tiny projections (Figure 1). The affected wood produced tiny projections going in to small holes in the inner face of the bark. Vein clearing and stem pitting were also observed on sweet orange trees sour orange rootstocks. ELISA-based indexing of citrus trees revealed that the incidence of CTV is increasing, with the distribution of infected rootstocks from 2002 to 2010 (Table 1). The average percent incidence of CTV in the major citrus growing districts of the Northwest (KP province) was recorded as: 24% (2002), 31% (2004), 39% (2006), 41% (2008) and 44 % (2010), respectively (Table 1). In KP province, the minimum and maximum range of CTV was 16 to 31% during 2002, 25 to 38% during 2004, 27 to 42% during 2006, 35 to 47 during 2008 and 40 to 48% during 2010, respectively. Therefore, the range of increase of CTV during 2002 to 2010 in KP province was from 16 to 48% (Table 1). Sweet orange was the major commercially grown citrus species in the province, therefore, ELISA-based indexing was done mainly in the orchards. The incidence of CTV was at maximum range (44%) and widely spread in 10 districts surveyed. In KP province, in most cases, where the incidence of the virus was high, sweet orange was grafted on sour orange root stock. In a few orchards at District Haripur, rough lemon was used as root stock; and thus, the incidence of CTV was comparatively low (Table 2). A comparative study of the virus incidence conducted during 2006 to 2012 in sweet orange trees grafted on sour orange and rough lemon in KP province indicated an average per cent incidence of CTV was 43 and 37, respectively (Table 2).

Maximum per cent increase of CTV was reported in citrus orchards in Haripur and followed by virus incidence in district Swat. In 2002, the virus incidence at Haripur was only 16.50%, while at Swat district was 20%, respectively. The results reported in Table 3 shows an increasing trend of the virus in 10 major citrus growing districts of KP province during 2002 to 2010. Figures 2 and 3 show the increasing trend of CTV in each districts/division and KP province as a whole, Pakistan, during 2002 to 2010. Further details of CTV increase in citrus fruits in major district/divisions of KP province, Pakistan such as Peshawar, Mardan, Malakand, Hazara D. I. Khan, and as a whole in KP, are given as Table 3. The identity of the virus was further confirmed through graft inoculation (by taking buds from selected infected trees) on different citrus species (*C. aurantium*, *Citrus lemon* cv. Eureka and *Citrus sinensis*). The graft inoculated citrus species mainly exhibited vein clearing and chlorosis symptoms. A comparison of CTV symptoms on naturally infected citrus species in field and reactions of the plants artificially inoculated in screen house are given in Table 4. Citrus species (*C. limon*) cv. Eureka was reported better indicator plant for the detection of CTV due to the production of vein clearing and chlorosis symptoms comparatively less time period after graft inoculation.

DISCUSSION

The results confirmed the wide spread infection of CTV in all citrus growing areas of the Northwest of Pakistan. Citrus tristeza along with other graft-transmitted diseases has been reported in Northwest, Pakistan (Arif et al., 2005a). Citrus tristeza virus, exocortis viroid and greening posed a real threat to citrus cultivation, not only in Pakistan but also in most citrus producing areas of the world (Catara et al., 1988; Bar-Joseph et al., 1989; Lee et al., 1994; Rocha-Pena et al., 1995). In Northwest of Pakistan, CTV was reported in a few locations with low intensity during 1987-1988 (Catara, 1987; Catara et al., 1988), however, greening was reported to be potential threat to citrus in this area (Coehran, 1976; Catara et al.,

Table 1. Percent incidence of Citrus tristeza virus in major citrus growing areas of Northwest of Pakistan during 2002 to 2010.

Division	District	Location	Year-2002		Year-2004		Year-2006		Year-2008		Year-2010	
			Tested/ Infected	% Incidence	Tested/ Infected	% Incidence	Tested/ Infected	% Incidence	Tested/ Infected	% Incidence	Tested/ Infected	% Incidence
Peshawar	Peshawar	Malakandher	45/10	22	40/12	30	30/10	33	20/8	40	35/15	45
		ARI Tarnab	55/14	25	52/20	38	40/16	40	45/19	42	40/16	40
	Charsadda	Tangi, Abazo	80/20	25	60/18	30	38/13	34	16/7	44	38/17	44
		Charsadda	70/16	23	50/14	28	42/14	33	25/9	36	42/18	43
	Nowshera	Manki Sharif	110/27	25	90/24	27	54/18	33	24/10	42	54/24	44
		Akora Khattak	90/24	27	85/23	27	23/7	30	15/7	47	23/11	48
Mardan	Mardan	Bakshali	194/60	31	100/34	34	54/20	37	24/11	46	56/26	46
		Palo Deri	190/54	28	102/33	32	44/14	32	34/15	44	44/18	40
	Swabi	Marghuz	180/61	34	110/40	36	30/11	37	24/10	42	30/13	43
		Dook	160/33	21	102/29	28	30/12	40	25/11	44	30/14	46
Hazara	Haripur	Haripur-1	162/27	17	56/16	29	50/18	36	30/12	40	50/22	44
		Haripur-2	145/23	16	40/12	30	25/9	36	40/14	35	25/11	44
Malakand	Malakand	Dargai, Dobandai	80/25	31	60/18	30	42/16	38	40/16	40	42/18	43
		Dargai, Kal Dera	80/17	21	44/14	32	45/12	27	42/17	40	45/20	44
	Swat	Barikot	80/16	20	28/8	29	30/8	27	50/21	42	30/14	46
		Fazalabad	100/20	20	50/16	32	24/10	42	30/11	37	24/11	46
	Dir	Timergara	62/14	23	40/10	25	20/8	40	26/10	38	20/9	45
		Khungi Payan	58/12	21	56/18	32	18/5	28	15/7	46	18/8	44
D. I. Khan	D. I. Khan	D. I. Khan-1	36/11	31	30/8	27	20/7	35	24/10	42	24/11	46
		D. I. Khan-2	44/9	20	18/6	33	25/9	36	30/12	40	25/12	48
Average			2021/493	24	1213/373	31	576/223	39	579/237	41	695/308	44

[I]Results based on DAS-ELISA detection of citrus tristeza virus from leaf samples collected from fields.

1988). During the course of about 15 years of these investigations, comprehensive and systematic surveys and indexing of mother plants revealed that the average incidence of CTV has increased from 24 to 44% and is almost prevalent in all areas surveyed. This rapid and consistent increase in incidence and distribution of CTV shows the potential threat of CTV to citrus fruits in Northwest of Pakistan. The common practice of

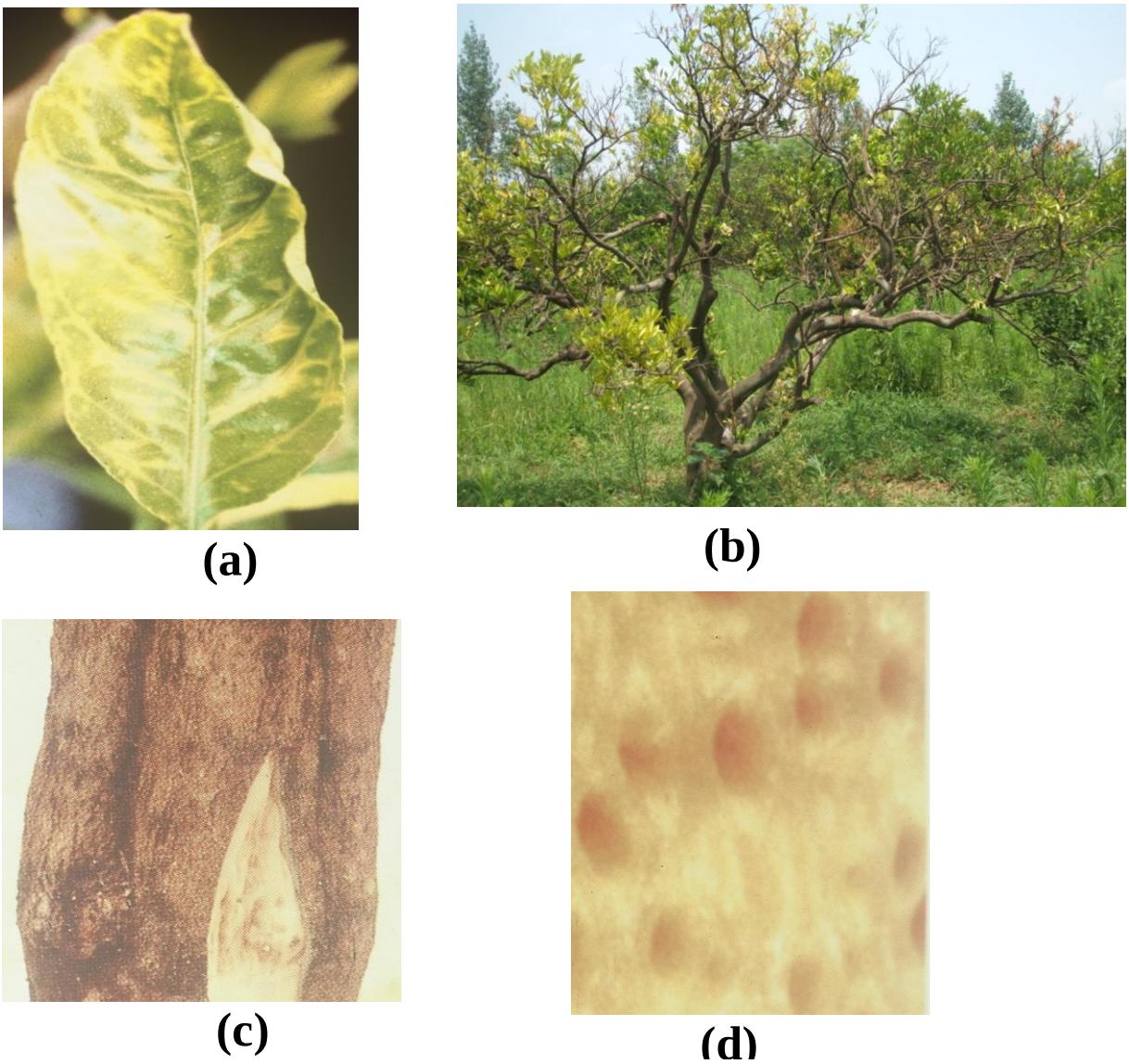


Figure 1. Field infection of citrus tristeza virus-sweet orange trees grafted on sour orange root stock. **(a)** Leaf showing vein clearing and chlorosis. **(b)** Decline and die-back. **(c)** Phloem necrosis. **(d)** Inner face tiny projections.

Table 2. Comparison of citrus tristeza virus incidence in sweet orange trees grafted on sour orange and rough lemon root stock in Northwest of Pakistan.

Year	Sweet orange[1] trees grafted on :			
	Sour orange [2]		Rough lemon[3]	
	Number of samples (tested/ infected)	% incidence	Number of samples (tested/ infected)	% incidence
2006	120/48	40	120/42	35
2008	130/53	41	130/44	34
2010	135/60	44	135/50	37
2012	140/66	47	140/56	40
Average % incidence		43		37

[1]Sweet orange (*Citrus sinensis* L. Osbeck), [2]Sour orange (*Citrus aurantium* L.). [3]Rough lemon (*Citrus jambhiri* Lush).

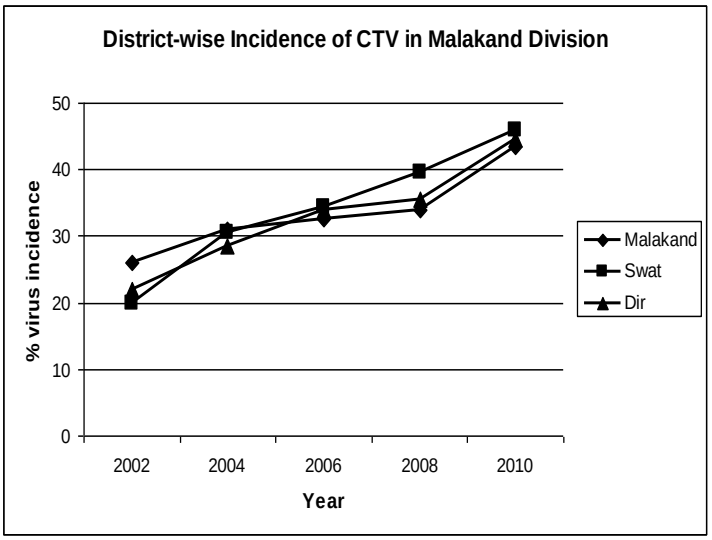
Table 3. Percent increase of Citrus tristeza virus in Northwest of Pakistan during 2002 to 2010.

Division	District	Average % incidence	% increase			
		Year-2002	Year-2004	Year-2006	Year-2008	Year-2010
Peshawar	Peshawar	23.50	+10.50 (34.00)[1]	+13.00 (36.50)	+17.50 (41.00)	+19.00 (42.50)
	Charsadda	24.00	+5.00 (29.00)	+9.50 (33.50)	+16.00 (40.00)	+19.50 (43.50)
	Nowshera	26.00	+1.00 (27.00)	+5.50 (31.50)	+18.50 (44.50)	+20.00 (46.00)
Mardan	Mardan	29.50	+3.50 (33.00)	+5.00 (34.50)	+15.50 (45.00)	+13.50 (43.00)
	Swabi	27.50	+4.50 (32.00)	+11.00 (38.50)	+15.50 (43.00)	+17.00 (44.50)
Hazara	Haripur	16.50	+13.00 (29.50)	+19.50 (36.00)	+21.00 (37.50)	+27.50 (44.00)
Malakand	Malakand	26.00	+5.00 (31.00)	+6.50 (32.50)	+14.00 (40.00)	+17.50 (43.50)
	Swat	20.00	+10.50 (30.50)	+14.50 (34.50)	+19.50 (39.50)	+26.00 (46.00)
	Dir	22.00	+6.50 (28.50)	+12.00 (34.00)	+20.00 (42.00)	+22.50 (44.50)
D. I. Khan	D. I. Khan	25.50	+4.50 (30.00)	+10.00 (35.50)	+15.50 (41.00)	+21.50 (47.00)

[1]Average per cent incidence of CTV in each district/ year.

grafting of sweet orange on sour orange root stock could be one of the main reasons for its wide spread occurrence and distribution. Sour orange has been one of the most popular root stocks world-wide (Grosser et al., 2004) and in Northwest of the country, citrus growers mainly used it as root stock for the production of high quality sweet orange. Sour orange is known to be susceptible to various graft-transmitted pathogens including CTV (Grosser et al., 2004). The use of infected bud scion could also be the cause of its rapid spread in the target areas. This practice facilitates the growth both virus-host relationship and result in citrus decline in almost 10 to 12 years depending on host genotype, virus strains and suitable environmental conditions. These infected trees also served as reservoir hosts for viruses and its vectors. Another reason for its wide spread occurrence of the virus in Northwest of Pakistan is the abundance of aphid vectors. CTV is transmitted by many aphid species (Catara, 1987; Roistacher and Bar-Joseph, 1987). Although, the most important aphid vector, *T. citricida* (Kirdaldy) has not been reported in major citrus growing districts of Northwest of the country including KP province (Arif et al., 2005a) and the Punjab province, Pakistan (Catara, 1987; Iftikhar et al., 2009). However, the virus is also efficiently transmitted by *A. gossypii* and *A. citricola* in Northwest, Pakistan (Arif, unpublished; Catara, 1987). The efficiency of *A. gossiypii* as vector of CTV has been well documented (Roistacher, 1984; Roistacher and Bar-Joseph, 1987) Small scale transmission studies has also indicated that a single *A. gossypii* can efficiently transmits CTV from sour orange to sweet orange and back from sweet orange to sour orange (Arif et al., 2005a). In this study, *A. gossiypii* was found in abundance during March to April on succulent citrus shoots, together with a number of other insect species but their role in transmission of virus and virus-like pathogens have not been determined. Based on the wide spread and quick decline syndrome, citrus greening has been previously reported as potential threat (Catara et al., 1988). No doubt, greening is playing pivotal role in the deterioration of citrus industry in Pakistan and other parts of the world. Citrus decline seems to be a complicated disease syndrome and involvement of CTV, *Citrus exocortis viroid* (CEVd) and *Spiroplasma citri,* the other pathogens could also be a possibility (Arif et al., 2005a). Detailed studies are required for isolation and characterization of the pathogens associated with the quick decline syndrome of citrus in Pakistan.

The results of this study indicate that CTV is quite prevalent in Northwest of Pakistan and its incidence is continuously increasing with time. Consistent and serious efforts are required to minimize the effect of the virus on citrus trees and for the successful production of citrus in Northwest and elsewhere in the country. It is essential that quarantine regulations should be strictly enforced to prevent introduction of CTV through exchange of and movement of infected nursery plants/ bud-wood from other parts of the country into the KP province and outside Pakistan. Awareness of citrus growers, farming community, researchers, extensionists on CTV problem and its prevention, is required. Eradication and immediate disposal of source of inoculum by removing old citrus trees and use of sour orange, *C. aurantium* for ornamental purposes should be discouraged. Rootstocks other than sour orange should be searched and tested for future plantation. Experience of rough lemon as root stock in many parts of Pakistan and elsewhere in world, is encouraging to prolong the life of CTV infected trees. Use of thermotherapy (Arif et al., 2005b) and cross protection (Abbas et al., 2005) techniques could be used to eliminate the virus in infected bud-wood and trees, respectively. Infestation of insects regardless of CTV vector, that is, *A. gossypii, Aphis citricolla,* on citrus nurseries and trees should carefully be monitored and controlled through insecticidal sprays. More precisely, an integrated approach could be adopted by using



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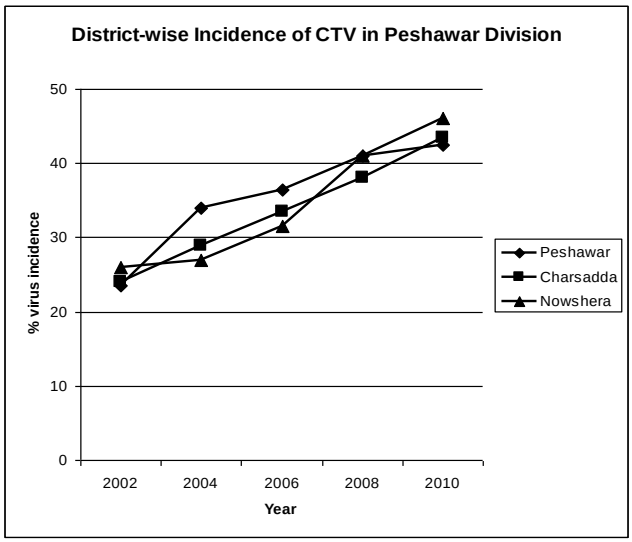

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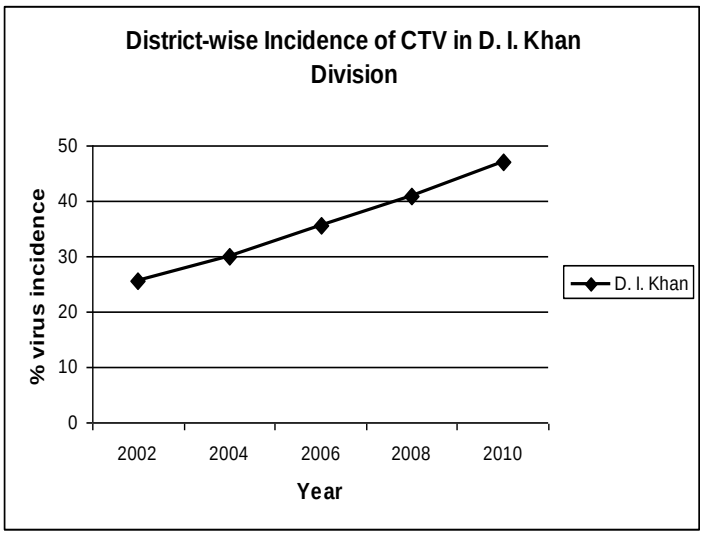

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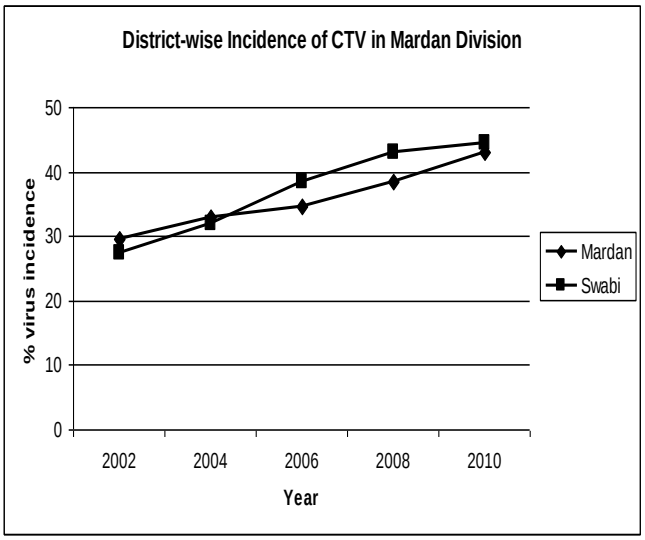

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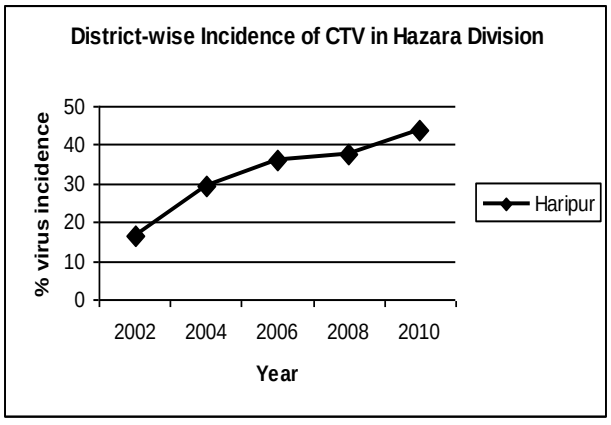

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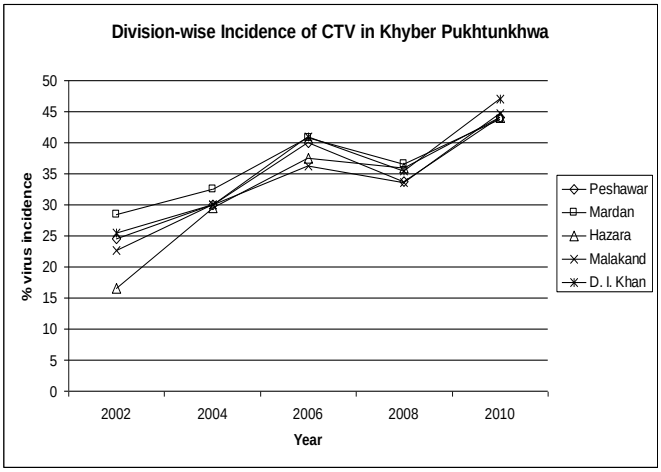

(f)

Figure 2. Curves showing increasing trend of Citrus tristeza virus in five major divisions and in Khyber Pakhtunkhwa Province. **(a)** Malakand. **(b)** Peshawar. **(c)** D. I. Khan. **(e)** Mardan. **(e)** Hazara divisions. **(f)** Overall in Khyber Pakhtunkhwa province.

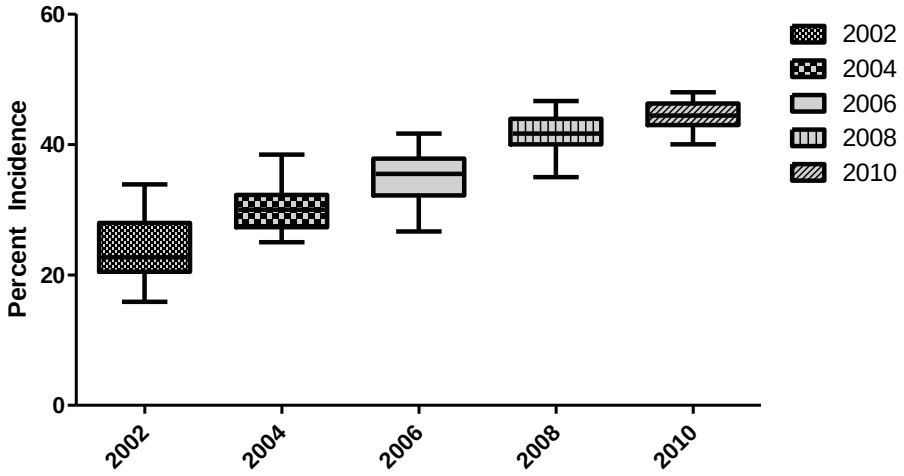

Figure 3. Box whisker plot showing percent incidence of citrus tristiza virus in Northwest of Pakistan during year 2002-2010.

Table 4. Reaction of citrus species against Citrus tristeza virus-prevalent isolates.

Pathogen	Naturally infected trees		Graft-inoculated plants	
	Host	**Symptoms**	**Host**	**Symptoms**
Citrus tristeza virus (CTV)	Sweet orange[1]	Vein clearing	*Citrus aurantium* (L.) [2]	Vein clearing and chlorosis
	Sweet orange grafted on sour orange	Phloem necrosis, inner face tiny projections	*C. limon*[3] (L.) Barm. cv. Eureka	Vein clearing and chlorosis
	Sweet orange / Mexican lime[4] / grapefruit[5]	Vein clearing and stem pitting	*C. sinensis* (L.) Osbeck	Vein clearing

[1]Sweet orange (*Citrus sinensis* (L.) Osbeck*);* [2]Sour orange (*Citrus aurantium* L.); [3]Eureka lemon *(Citrus limon* (L.) Barm.; [4]Mexican lime *Citrus aurantifolia* (Christm.) Swingle; [5]Grape fruit (*Citrus paradise* Macf.).

pathogen-free bud-wood, avoidance sources of infection, proper and timely use of insecticides, implementation of quarantine regulations and the use of resistant/ tolerant root stocks, for successful citrus production in Pakistan.

Conflict of interests

The author(s) did not declare any conflict of interest.

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