SYMPTOMATOLOGY, DETECTION, DISTRIBUTION AND MANAGEMENT OF VIRUS AND VIRUS-LIKE DISEASE OF CITRUS IN PAKISTAN

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Abstract

Virus and virus-like diseases induce a variety of symptoms in different citrus species grown in Pakistan. Frequent surveys of citrus orchards over a protracted period revealed the occurrence of complex disease syndrome comprising of yellowing, stunting, dwarfing and declining with short life span of citrus trees. Specific and overlapping symptoms were observed in case of infections with viruses, viroids and prokaryotes. On the basis of symptomatology in general and biological, serological and physical properties in particular, two viroids (CE, XY), six viruses and two prokaryotes were identified. CTV and GD were prevalent at all locations surveyed but CGD was predominant and appeared to be associated with the decline problem of citrus trees. High populations of citrus psyllid (*Diaphorina citri*), the vector of greening disease, were recorded on Kinnow, sweet orange and *Murraya* plants but *Aphis gossypii* was less active in the transmission of CTV. Management of these diseases, based on nursery improvement, phytosanitation, and production and use of virus-free propagative material is emphasized.

Keywords: Citrus species, viruses, viroids, prokaryotes, indexing, ELISA, phytosanitation, certified bud wood.

INTRODUCTION

Citrus occupies a leading position among the fruits grown in Pakistan. It represents about 35% of all the fruits produced, with major concentration in the Punjab (94%), followed by Sindh (28%) and NWFP (2.5%) and very little citrus is produced in Balochistan (0.3%). Main varieties are mandarin (Kinnow and Feutrell's early) representing 80% of citrus, sweet orange (Malta and Mosambi), grape fruit and sweet lime. Lemon and lime are grown on limited areas (Table 1). In general, range of citrus varieties in Pakistan is not very broad and the harvesting period is also limited.

Citrus is highly vulnerable to the attack of a number of diseases which are caused by different type of pathogens and favoured by certain conditions and practices. The root stock on which citrus is grown in the Punjab is rough lemon which is tolerant to Citrus Tristeza Closterovirus (CTV) but susceptible to Phytophthora root rot. On the contrary, sour orange root stock used in NWFP is susceptible to CTV but resistant to Phytophthora. Nurseries are managed haphazardly, unscientifically and unhygienically. The trees are often budded too low and planted quite deep with bud union line almost buried in the soil, which favours root diseases. The practice of intercropping with wheat,

berseem and often vegetables is very common and clean cultivation is obtained by heavy discing which destroys surface feeder roots and also results in compactness of the soil.

Background Information on Citrus Diseases

Some fungal and bacterial diseases of citrus are known to be present in Pakistan since 1920 as documented in different sources (Sattar and Hafiz 1955, Kausar 1960, Kamal and Mughal 1967, Hafiz 1985, Mughal 1985). However, virus and virus-like diseases infecting different citrus species did not receive due attention for a long time because proper facilities for their identification and characterization were not available. Capoor (1963) in India had reported decline problem in citrus trees mainly caused by th CTV and greening disease. Chapot was the first to visit the citrus orchards in Peshawar in 1970 but he found no evidence of the presence of CTV. However, he reported stubborn disease and noticed citrus psyllid (*Diaphorina citri)*, showing his unawereness that *D.citri* was the vector of greening and not of stubborn. The first evidence of greening disease came from Cochran in 1976 who observed its clear symptoms along with its vector on sweet orange. He also mentioned that a large number of citrus trees were declining and showing dieback symptoms but the cause of decline remained unknown. Catara *et al* (1988) and Bove (1989), made extensive surveys of citrus grooves in the Punjab and NWFP, identified diseases caused by viruses, viroids and prokaryotes and attributed greening as the main cause of citrus decline. Their findings constituted a strong base for additional surveys and investigations in citrus virology. This paper reviews the previous work, highlights the present status of investigations and deals with overall situation of virus and virus-like diseases of citrus in Pakistan.

General Methodology

Investigations presented in this paper were carried out over a protracted period of several years. Citrus nurseries were examined at selected places in the Punjab and NWFP and their operations and management were critically investigated. Citrus orchards were also selected in these two provinces, and at least 50-100 citrus trees were examined at each place and symptoms induced by the virus and virus-like diseases or suspected diseases were recorded as suggested many workers (Bos 1970. Catara *et al*., 1988, Bove 1989). Symptoms were noted on roots, bark, trunk, twigs, foliage and fruits at appropriate times in different visits.

Diseased specimens were collected in polythene bags and stored at 4°C until processed. Seeds of the indicator plants were grown in plastic pots in an insect-free environment. Mechanical inoculation and graft transmission were carried out as suggested by Roistacher (1991), and ELISA was performed for CTV using monoclonal antibodies (Clark and Adams, 1977). Viruses and prokaryotes were observed under an electron microscope at NARC Islamabad, as well as by Catara *et al*. (1988) and Bove (1989) in their respective laboratories. Viroids were analyzed through polyacrylamide gel electrophoresis (PAGE) by Catara *et al* (1988). PCR or DNA probes have not been applied so far in the detection of citrus viruses in Pakistan.

RESULT AND DISCUSSION

1. Citrus nurseries: Almost all the nurseries producing citrus plants and providing to the orchards were found to be suffering from faulty management. Major problems associated with the nurseries are that: i) the nurseries are not registered and function as a common profession with no scientific/ technical knowledge of different operation, ii) no standards and precautions are followed in the selection of seeds of root stocks and collection of bud wood tissue, iii) non availability of virus-free/certified bud wood, iv) phytosanitary measures or disinfection of tools are not carried out which results in the spread of viroid diseases as exocortis and xyloporosis and probably gummy bark and bud union crease, v) no treatment against soil-borne diseases and nematodes, vi) plants are budded or grafted too low on very young plants and vii) presence of citrus canker. It was safely concluded that most of the disease problems, especially those of viral origin, start from these nurseries.

2. Symptomatology: A great variety of symptoms were recorded in citrus trees infected by different virus and similar diseases (Table 2). It was experienced that symptomatology of citrus diseases is more complex than other diseases. Efforts made to distinguish virus-specific symptoms for field diagnosis were only partly successful because most of the symptoms either resembled or overlapped. Therefore, symptoms were classified in three categories: i). general symptoms such as stunted and suppressed growth, chlorotic or mottling of foliage, dwarfing of trees, declining, profuse flowering and fruit drop, ii). specific symptoms as ringspot, vein clearing and yellowing, concave gum blind pockets, cristacortis, pitting and pegging, bud union crease and bark scaling caused by viruses and viroids. Prokaryotic pathogens also produced specific symptoms as greening, lopsided and malformed fruits with insipid taste and dark purple aborted seeds, and iii) overlapping symptoms which are difficult to distinguish as a result of different diseases or mixed infection.

Knowledge of symptoms is essential for quick orientation and rapid diagnosis. Before the development of advanced sensitive techniques, viral diseases were diagnosed by symptoms expression or host reaction. As already stated symptomatology in citrus is complex, and therefore may not be regarded a good criterion for pathogen identification because: i) there is a great diversity of symptoms, ii) young citrus trees after viral infection do not manifest any symptoms for a long time, some viruses take 1-7 years for expression, iii). Symptoms are influenced by host, virus and its strain, time of infection, nutrition and environmental conditions. iv). symptoms in citrus are not expressed at one time but at different stages of tree growth. v) many trees remain asymptomatic and serve as source of infection. vi) deficiency diseases can be confused with viral symptoms and vii) virus diseases debilitate citrus trees and predispose them to fungal infection which may predominate under favourable conditions.

3. Detection and Distribution of Disease:

3.1. Citrus Tristeza Closterovirus: CTV is found in citrus species grafted on sour orange and thus a serious problem in NWFP but the disease is also present in the Punjab. It has been confirmed by indexing on lime, ELISA using monoclonal antibodies and electron microscopy which revealed presence of

bundle of virus in the cambium tissue. Disease incidence in the samples collected from Sahiwal, Sargodha, Faisalabad, Shaikhupura and Peshawar ranged between 7.4% to 18.2% with a mean incidence of 13%. (Catara *et al.,* 1991, Anwar and Mirza, 1992). Mosambi showed the highest infection (22.7%), Malta (17.85%) and Kinnow (4.65%). Characteristic symptoms of CTV are: vein clearing in lime, stem pitting and honey combing in oranges. Other symptoms include bronzing of leaves, dieback of twigs and roots, leaf drop, retardation of growth, foliage wilt, debilitation of trees, reduced fruit size and necrosis of cambial tissue. The infected trees may die suddenly. The brown aphid (*Toxoptera citricidus*) is not present in Pakistan but the disease may be transmitted and spread by *Aphis gossypii*. It is generally believed that only mild strain of CTV is present in Pakistan and may not be involved in citrus decline. Control measures include eradication of infected trees, use of tolerant root stock and certified bud wood, vector control and quarantine measures.

3.2. Citrus Greening Disease: Greening is the most serious and devastating disease everywhere. It is characterized by yellowing of veins and adjacent tissue, mottling of entire leaf, distinct yellow shoots, premature defoliation, die back of twigs and decay of feeder rootlets and lateral roots. Infected tree decline and ultimately die. Fruits on the infected trees do not fully colour and remain green at the stylar ends. According to Catara *et al* (1989, 1991), the disease is widely distributed in the Punjab and NWFP. Akhtar and Ahmed (1999) recorded its incidence, 0.5% - 4.5% in the Punjab and 5.3% in NWFP. The disease infects Kinnow mandarin (22-40%), Mosambi (25%), grape fruit (15%) and sweet lime (10%) as determined through non specific colour test. The disease is caused by a gram negative bacterium-like organism (BLO) which is located in the phloem sieve tubes (Bove, 1988). Citrus psyllid (*Diaphorina citri*) is the potential vector of greening disease which breeds on Kinnow, sweet orange and orange jesamine (*Murraya panniculata*). Vector develops high population in NWFP and Punjab. Citrus greening disease is believed to be definitely involved in citrus decline in India and Pakistan (Bove, 1989, 1991). Apart from other factors involved in decline, the impact of greening disease is quite obvious in Pakistan i.e mottling of leaves, vein clearing and vein banding, yellow shoots, die back of twigs, profuse flowering, heavy fruiting and sudden collapse of infected trees. The control of the disease may be achieved by use of healthy propagation material, eradication of infected trees from the orchards, vector control and injection of tetracycline-HCl (3g/tree)

3.3. Viroid diseases: The impact of viroids can be very serious because the infected trees do not show symptoms for many years. The nursery operations and warm climate are highly favourable for viroid diseases in Pakistan. Exocortis and Cachexia xyloprosis were identified through PAGE (Catara *et al*, 1988). In fact, 3-4 spp. of naked RNA with different molecular weight were distinguished which need to be further investigated. They induce symptoms of bark scaling; bark shelling, exudation of gum at the base of trunk near bud union, gum impregnation and stunting of the trees. The impact of viroid diseases has

not been assessed. To avoid spread of viroids it is recommended to use clean material for propagation and surface sterilized tools by dipping in a solution of 1.5 pints of household bleach (5.25% sodium hypochlorite/gallon of water). Do not use susceptible rootstock unless bud sticks come from healthy tested mother trees. Since the replication of viroid is favoured by high temperature, thermotherapy is ineffective, but micropropagation provides good results.

3.4. Citrus stubborn disease

It is caused by *Spiroplasma citri* and transmitted by leaf hopper *Circulifer tenellus*. It is favoured by warmer conditions. The disease is characterized by stunted and compressed growth, smaller leaves, and chlorotic mottle, partial infection of tree, distorted fruit with acorn shaped appearance, insipid taste and dark purple aborted seeds. It mainly infects sweet orange (Malta and Mosambi) and grape fruit. The disease is of minor importance. Control of the disease may be achieved by using bud sticks from healthy indexed mother trees (avoid any suspicious material, growing nursery trees where natural spread does not occur, pulling out affected trees from the orchards not heavily infested. Thermotherapy and microprapagation may be effective to recover infected material.

3.5. Other diseases: Some other viruses or probable viruses seem to be associated with diseases known as concave gum blind pockets, bud union crease, cristacortis, gummy bark etc. These diseases and their probable viruses/viroids still remain unidentified and uncharacterized. Citrus infectious variegation, Ringspot and yellow vein clearing have been investigated to some extent but they are of minor economic importance. (Catara *et al* 1993, Ashfaq *et al* 2004, Iftikhar *et al* 2004)

CONCLUSION:

The major virus and virus like diseases prevalent in Pakistan have been discussed in this paper, and the nursery and field problems have been highlighted. If the nurseries are scientifically and professionally operated and field problems are solved, it is possible to overcome 70-80% of the disease problems involved in citrus production. Some long and short term suggestions are presented as under:

- The citrus orchards in the main citrus production areas should be periodically surveyed to assess disease situation and to identify the priority problems.
- Virus-Vector Relationships should be investigated in detail with particular emphasis on identification of vectors and their population dynamics, transmission of viruses, biological control of citrus psyllid and development of sound and viable IPM strategy.
- Nurseries should be improved through registration, and scientifically operated with phytosanitary measures and trained manpower for the adoption of recommended horticultural practices. Nurseries should be provided with clean stock (mother trees, virus testing, thermotherapy, indexing)
- Bud wood certification program should be strengthened.
- Extension worker and nurserymen should be well educated and trained.
- In the existing orchards, infected plants should be eradicated. Intercropping, over watering and application of heavy doses of nitrogenous fertilizers should be

avoided. Proper pruning and protective sprays, use of balance fertilizers are essential.

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Table 1: Citrus species and cultivars grown in Pakistan*.

Citrus species	Botanical name	Distribution	Purpose
Kinnow mandarin (King and Willow leaf mandarin)	(*C.nobilis x C.reticulata*)	Punjab , NWFP	Commercial
Feutrell's Early (Mandarin x sweet orange)	*C.reticulata x C.sinensis*	Punjab	Commercial
Sweet orange (Malta and Mosambi)	*C.sinensis*	Punjab, NWFP	Commercial
Grape fruit	*C.paradisi*	Sindh, Punjab	Commercial
Sweet lime	*C.limettioides*	Punjab	Commercial
Acid lime	*C.aurantifolia*	Throughout Pakistan	Commercial
Lemon	*C.limon*	-do-	Limited
Pomello	*C.grandis*	Limited	-do-
Rough lemon	*C.jambhiri*	Punjab	Root stock
Sour orange	*C.aurantium*	NWFP	Root stock
Orange jasamine	*Murraya paniculata*	Throughout	Ornamental
Kumquats	*Fortunella sp.*	-do-	-do-

* Citrus species as mentioned by Chaudhary (1969) and Singh *et al* (1983)

Table 2: Expression of viral symptoms on foliage, trunk and fruit of citrus.

Foliage symptoms		Trunk symptoms		Fruit symptoms	
Blotches	+	Gummy bark	++	Greeninig	+++
Mosaic and mottling	+++	Gummosis	++	Lopsided	+++
Ringspot	+	Concave gum	++	Malformation	++
Vein clearing	++	Pitting	++	Stubborn	++
Vein yellowing	+	Pegging	++	Aborted seed	+++
Variegation	++	Cracking	+	Small fruits	+++
Smalling of leaves	+++	Blind pockets	++	Profuse flowering	+++
		Scaling	+	Fruit drop	+++
		Cristacortis	++	Heavy fruiting	+++
		Bud union crease	++		

Mild, = + Moderate = ++ Severe = +++

Table 3: Detection and distribution of virus and virus-like diseases of citrus in Pakistan

Disease	Pathogen & Acronym	Climatic conditions	Host plants	Detection methods
Cachexia-Xyloprosis	Viroid (CX)	Warm	Mandarin, Sweet orange (Mosambi, Malta)	PAGE
Concave gum blind pockets	Complex ?	Cool to warm	Mandarin , Sweet orange	—
Exoxortis	Viroid (EX)	Warm	Sweet orange , Sweet lime	PAGE
Ring spot	Virus (CRSV)	Cool	Mandarin, Sweet orange	Indexing
Cristacortis	Virus ?	Cool-warm	Mandarin , Sweet orange	—
Greening	Bacterium like organism (BLO)	Cool- warm	Sweet orange , Rough lemon , Lemon	EM, staining
Infectious variegation	Virus (CIV)	Cool	Mandarin, Sweet orange, Rough lemon, Lemon	EM
Psorosis	Virus ?	Cool	Mandarin, Sweet orange, Grape fruit	
Stubborn	*Spiroplasma citri*	Warm	Sweet orange, grape fruit.	EM
Tristeza	Virus (CTV)	Cool-warm	Sour orange, Sweet orange, Mandarin, lime	Indexing, ELISA, Lime test, staining (microscopic)
Yellow vein clearing	Virus (YVCV)	Warm	Lemon, Sour orange	Indexing, EM.