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Survey and Biological Indexing of *Citrus tristeza virus* Infecting Citrus Species in Vidharba Region

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ABSTRACT

Citrus tristeza virus (CTV), a member of genus Closterovirus, is an important pathogen which has destroyed more than one million citrus trees in India till date. Field surveys were carried out in major citrus growing regions of Vidarbha region (Nagpur, Amravati, Akola and Wardha). Under field conditions, the CTV infected trees of citrus exhibited stunted growth, vein clearing, vein flecking, chlorosis with decline and reduced leaf size. The CTV incidence of 13.5 % in sweet orange followed by 10.0 % in mandarin and 6.0 % in acid lime was reported during survey. Among three cultivars surveyed sweet orange found higher susceptible to CTV. In the present investigation biological indexing was done by graft inoculating acid lime as a indicator host with tissues from suspected citrus trees. Highly predictable symptoms were developed on indicator host acid lime viz., seedling yellow after 8 week inoculation.

Key words: *CTV, Survey, Biological indexing.*

INTRODUCTION

Citrus is one of the important fruit crop growing among the world. It occupies an important place in the wealth and economy of India as third largest fruit industry after mango and banana. In Maharashtra state Citrus is grown on about 253.4 thousand hectares of area under Citrus with production of about 3800.6 MT fruit annually reported Anonymous[3]. *Citrus tristeza virus* (CTV), important viral pathogen under genus *Closterovirus*, causes huge losses to over 100 million citrus trees worldwide reported by Bar-Joseph and Dawson[5]. CTV spreads into new regions (long spread) via movement of infected propagating materials such as seedlings and bud woods. Aphid dispersion of virus is important within a citrus growing area (short spread). The principal vectors of CTV are *Toxoptera citricida* (Kirkaldy), the brown citrus aphid (BrCA), *Aphis gossypii* (Glover), the melon cotton aphid, and *A. spiraecola* (Patch), the spirea or green citrus aphid reported Herron *et al* [12]. Roistacher and Bar-Joseph[15].

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Efficiency of CTV transmissibility is affected by the species of aphid, by the source plant at acquisition feeding and the CTV isolate. The BrCA is the most efficient aphid vector of CTV. In areas where Br CA is not present, *A. gossypii* is the most efficient vector implicated Bar-Joseph and Loebenstein[4]; Bar-Joseph *et al* [6]. Infected planting material is known to be the most potent primary source of spread of CTV into new areas and plays an important role in the secondary spread of the virus. Therefore, use of virus-free planting materials and control of BrCA subsequently, would prevent severe crop losses and keep citrus industry viable and profitable. CTV is a phloem limited, longest known plant virus with particle sizes of 10–12×2000 nm and transmitted by aphid vector *(Toxoptera citricida)* in semi-persistent manner. The viral genome contains positive sense ssRNA of about 20 kb nucleotide length with 12 ORFs encoding at least 19 putative proteins Bar-Joseph and Dawson[5]. In Maharashtra state citrus is grown on about 253.4 thousand hectares of area under citrus with production of about 3800.6 MT fruit annually Anonymous[3], The most important commercial citrus cultivars in India are the mandarin (*Citrus reticulata* Blanco) followed by sweet orange (*Citrus sinensis* Osbeck) and acid lime (*Citrus aurantifolia* Swingle). CTV causing various disease symptoms like mild symptoms to seedling yellows stem pitting, severe stunting and ultimately decline of the plant depending on citrus species, virus strains, scion/rootstock combinations and environmental factors Lee and Bar-Joseph[14]. *Citrus tristeza virus* (CTV) is one of the most devastating diseases in citrus producing regions. The symptoms appear by CTV in the form of vein clearing / vein flecks on the leaves of acid lime is seen intermittently when viewed against light (characteristic symptom). *Tristeza* affected look chlorotic and sickly in the early stage gradually the leaves drop and defoliated twigs show die –back infected plants show stunted growth.

MATERIALS AND METHODS

Roving Survey and collection symptomatic samples

Citrus is grown on large scale in Vidarbha region of Maharashtra. Survey was undertaken in citrus orchards (sweet orange, Nagpur mandarin and acid lime) at Vidarbha region of Maharashtra (Nagpur, Amaravati, Akola and Wardha districts) for CTV. Collections of leaf samples were made on the basis of symptoms appearance in field grown citrus trees during survey. The citrus trees showing symptoms of vein clearing, leaf necrosis, yellowing of leaves with stunted growth and vein flecking symptoms were collected and the samples were kept at -80°C in plastic bags in deep freeze with labels indicating name of the citrus cultivar and the location from where it was collected. Five –Ten infected samples were collected from each orchard. Twenty trees from each orchard were selected for observation and these collected samples were kept in plastic bags and brought to the laboratory, later kept in the deep freezer for diagnostic assay. CTV incidence recorded by using number of infected plants by total number of trees with multiplication by 100. Biswas *et al*[8] reported that the Disease incidence was estimated using standard method: number of samples infected divided by number of samples tested and multiplied by 100. Ahmad *et al* [2] reported the percentage of disease incidence by using method total number of plants infected by total number of plants observed and multiplication by 100.

Biological indexing

Biological indexing of test samples were done by bud wood grafting of suspected CTV field samples into one year old Acid lime seedlings, preferred indicator hosts for detection of CTV. Citrus plants (Nagpur mandarin, sweet orange and acid lime) showing abnormal deteriorating conditions were selected and indexed to know presence of CTV. The inoculated plants kept in green house condition for symptom appearance and inspected twice a week for observations. One bud was grafted onto each of the indicator host and made duplicate. The bud woods were budded at 20cm height to each of the acid lime seedlings. Ghosh *et al* [11] studied indexing of CTV and greening was done biologically by using one year old acid lime and sweet orange

indicater plants respectively. A minimum of 3-5 plants graft inoculated and the inoculated plants were kept in glass house under controlled temperature condition. Williams *et al*[17] studied strain of *Citrus tristeza virus* (CTV SY568) that caused severe stem pitting and stunting and occasional vein corking in sweet orange and grapefruit. Vein corking was common on the leaves of the two most severely stunted plants, but was rare or absent on other plants.

RESULTS AND DISCUSSION

Roving Survey based on symptoms for the year 2014-2015 and Symptomatology

Citrus is grown on large scale in Vidarbha region of Maharashtra. Incidence of virus disease is one of the important components for citrus decline and it's depending on climatic conditions of the region. Hence the survey was undertaken to know the virus disease in the Vidarbha region. During survey 07 sweet orange, 08 Nagpur mandarin and 05 acid lime orchards of different age group trees from Akola, Amravati, Nagpur and Wardha districts of Vidarbha region survived, randomly 20 trees from each orchards were selected for observations. During the period of survey, in field condition, *tristeza* virus infected trees showed characteristic symptoms of vein clearing and vein flecking in Kagzi lime. However, these symptoms were not observed in Nagpur mandarin and Sweet orange. Brlansky *et al*[11] reported the symptoms of decline and death of sweet orange on sour orange rootstock and the wood pitting of numerous *Citrus* spp. However, other CTV symptoms include seedling yellows (SY), vein clearing (VC), tree stunting and reduced fruit size and yield. The sweet orange trees showed yellowing of leaves with stunted growth and Mandarin infected trees showed chlorosis with decline symptoms. Such type of occurrence of CTV symptoms on citrus trees earlier reported by Bar-Joseph *et al* [6]; Singh *et al* [16].All the suspected samples were brought to plant pathology laboratory, at department of plant pathology, Dr PDKV, Akola for further investigation. Incidence of CTV was noticed in the range of 6.0% -13.5%. However highest incidence of CTV was noticed in sweet orange (13.5%) followed by Nagpur mandarin (10.0%) and acid lime (6.0%). Biswas[7], Ahlawat[1] reported the incidence of CTV ranging from 10-90% in citrus growing area in India. Biswas[8] again showed the overall 26.3% CTV incidence in Vidarbha region, 47.1-56.0% in Northeast (Assam, Meghalaya, Sikkim and the Darjeeling hills), 36-50 % in South (Andhra Pradesh and Karnataka) and 16-60 % in North-Northwest (Uttarakhand, Delhi and Punjab).

Table 1: Incidence of *citrus tristeza virus* in citrus species

Location	cultivar	Trees used for observation	No of orchards	% of incidence	Symptoms
Amravati Akola	Mandarin	160	8	10.0%	yellowing , stunted growth
Nagpur Amravati Akola	Sweet orange	140	7	13.5 %	Chlorosis, decline symptoms and stunted growth
Wardha Akola	Acid lime	100	5	6.0%	vein clearing and vein flecking

Biological indexing

Biological indexing of test samples were done by bud wood grafting of suspected CTV field samples into one year old Acid lime seedlings, preferred indicator hosts for detection of CTV. Each 24 samples of sweet orange and 16 samples of Nagpur mandarin and 16 samples of acid lime were indexed for the identification of CTV. Out of 24 samples of sweet orange, 16 samples of Nagpur mandarin and 16 samples of acid lime. Only one sample from Nagpur mandarin showed +ve reaction for CTV. The Nagpur mandarin sample expressed seedling yellow symptoms after 8 week inoculation on indicator host acid lime. Results of biological indexing assay on Acid lime are shown on Table 2. Similar results found by Borash *et al* [9] and reported that the symptom expression on indicator host as Kagzi lime was observed from 5th to 9th week after inoculation. Biswas[7] studied Kagzi lime indicator plants when graft inoculated with test samples develop mild to severe vein clearing and vein flecking symptoms characteristic for CTV infection.

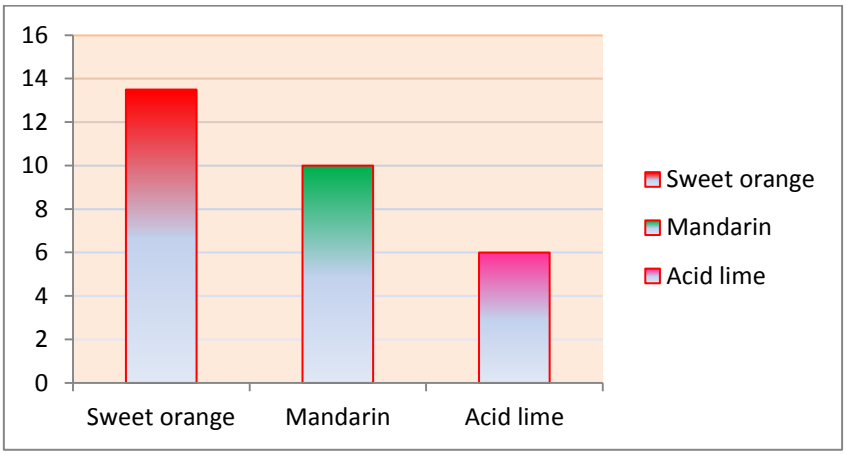

Fig.1. % of incidence of CTV

Table 2: Symptoms expression in biological indexing of *Citrus tristeza virus* on acid lime

Sr. No	location	Scion	No of samples inoculated	No of indicator host inoculated	Indicator host	No of indicator host infected	CTV recation	Types of symptoms	Incubation period	Remarks
1	Nagpur	Sweet orange	08	24	Acid lime	_	_	_	_	None of the sample showed +ve reaction for CTV.
		Nagpur mandarin	08	24	Acid lime	_	_	_	_	None of the sample showed +ve reaction for CTV.
2	Amarav-ati	Sweet orange	08	24	Acid lime	_	_	_	_	None of the sample showed +ve reaction for CTV.
		Nagpur mandarin	08	24	Acid lime	01	+ve 1	Seedling yellow	8 weeks	Only one sample showed +ve reaction for CTV.
3	Wardha	Acid lime	08	24	Acid lime	_	_	_	_	None of the sample showed +ve reaction for CTV.
4	Akola	Sweet orange	08	24	Acid lime	_	_	_	_	None of the sample showed +ve reaction for CTV
		Acid lime	08	24	Acid lime	_	_	_	_	None of the sample showed +ve reaction for CTV
	Total	Sweet orange	24				01			
		Mandarin	16				01			
		Acid lime	16							

Table 3: Asymptomatic plants and symptomatic plants comparison based on plant height and leaf area

Sr. No.	Samples	Scion	Leaf area (cm)2	Plant height (m)
1	Asymptomatic	Sweet orange	22 cm2	5 m
	Symptomatic		17 cm^2	3.6 m
2	Asymptomatic	Nagpur Mandarin	19 cm^2	4.5 m
	Symptomatic		15cm^2	3.2 m
3	Asymptomatic	Acid lime	18 cm2	4.8 m
	Symptomatic		14.5 cm2	3.8 m

Plant height of symptomatic plant is shorter than the asymptomatic plants. Asymptomatic plants produced normal leaf area but in symptomatic plants leaf area was greatly reduced. Jadhav *et al* [13] reported that the leaves of symptomatic plants were deformed (cup-like in appearance), dark green in colour and reduced in size ('little leaf').

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