Prevalence of *Citrus tristeza virus* in Mandarin of Sikkim Himalayan Region

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Abstract The assessment of *Citrus tristeza virus* incidence in mandarin of Sikkim, involving sampling techniques, was estimated by DAS-ELISA. Mandarin orchards had high CTV incidence (46.32%), however, differential prevalence with regard to age of plant and location was observed. The CTV prevalence was relatively high in older orchards (51.01%) than that of younger ones (40.80%). Under all the plant age groups, south district had the highest CTV incidence (52.50%) and east district had the lowest (37.71%). The spatial distribution of CTV in plants indicates high concentration in twig followed by leaf tissue, however, stem had relatively less concentration. High aphid infestation was observed in all mandarin growing groves with the maximum in south district and minimum in east district. *Toxoptera citricida* was the predominating aphid species followed by *T. aurantii*, however, *Aphis spp* population was significantly less. Aphid infestation and CTV prevalence were positively and significantly correlated.

Keywords *Citrus tristeza virus* · DAS-ELISA ·
Toxoptera citricida

Citrus tristeza virus (CTV) is the most widely spread and economically important viral disease of the world [1]. It is probably originated in Asia and has been disseminated to almost all citrus-growing countries mainly by the movement

of infected plant material and subsequent spread by aphid vectors with *Toxoptera citricida*, commonly called the brown citrus aphid, being the most efficient [13]. CTV is a phloem-limited 2000 nm long filamentous virus having single stranded RNA genome [11]. The virus is genetically and biologically diverse and can cause field symptoms ranging from vein clearing, stem pitting, yellowing, slow decline and quick decline, or no symptoms depending on virus isolate, time of infection, rootstocks, citrus cultivars and environmental conditions [4].

The classical identification procedure for CTV is to graft-inoculate indicator plant of *Citrus aurantifolia* (Mexican lime) and observe them for vein clearing, stem pitting and leaf cupping [17] which is relatively less reliable and time consuming, but Double antibody sandwich ELISA (DAS-ELISA) [3, 7, 8], has revolutionized the detection test, making it feasible to test large number of samples. In recent years, PCR based diagnosis [6, 10] has been used for the rapid diagnosis of citrus virus.

The citrus industry of Sikkim Himalayan region is dominated by mandarin (*Citrus reticulata* Blanco), which is cultivated under the humid temperate climate. The region has good potential of mandarin due to climatic suitability, however, high prevalence of CTV affecting industry considerably [14]. Moreover, use of uncertified planting materials further contributes significantly to the retrogression of production potential of mandarin in Sikkim [15].

Keeping in view the importance of disease-free, quality planting materials in improving the production potential of mandarin, DAS-ELISA tool was used to work out the prevalence of CTV in the major mandarin growing belts of Sikkim and in addition the infestation of aphids in mandarin was also studied. This investigation was aimed to work out the incidence of CTV in the state and to

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screen mother plants for production of disease-free plating materials.

Major mandarin growing groves of four districts of Sikkim (north, south, east and west) were surveyed in 2009–10, which were located in the mid hills between the altitudes of 800–1200 m amsl. Studies on the prevalence of *Citrus tristeza virus* (CTV) and infestation of aphids were carried out by identifying 3–4 important citrus growing areas of each district and from each area, two representative orchards of three different age groups i.e. 5–10, 11–20 and >20 years were selected. From each representative orchard 12–15 trees were randomly selected for examination. The selection of orchards was based on following criteria; (i) typically mandarin growing area, (ii) farmers following traditional farming system and (iii) have at least 300 mandarin trees.

From each randomly selected tree, 10 fully expanded young leaves of new growth from all sides of tree were sampled during the month of March–April and July–August. The samples were taken with the help of sterilized secateurs and kept inside the sterilized polythene bags and stored in a refrigerator at 4°C until processed. To study the distribution of *Citrus tristeza virus* (CTV) in different plant parts, five CTV positive plants of different representative orchards were tagged and from them three different samples; whole leave, twig bark and stem bark, were taken. Double antibody sandwich ELISA was used to determine the presence of CTV [3, 9]. The ELISA kit with polyclonal antibody (IgG) was procured from Bioreba AG (Switzerland). The reaction was considered positive only if the mean absorbance value at 405 nm was more than three times of negative control. The OD₄₀₅ values for buffer, negative control and positive control were 0.041, 0.083 and 0.831, respectively. The incidence of CTV in a representative orchard was calculated on the basis of number of positive trees and the mean of CTV incidence of different representative orchards reflected the prevalence of CTV in that area and accordingly prevalence of CTV in the state was worked out.

The aphid infestation was recorded during the study period and the genus or species was identified in the field using magnifying lens. The intensity of aphid infestation in plants was worked out by scoring system. Each randomly selected tree was scored between 0 and 3, according to intensity of infestation: 0 = no aphid, 1 = 1–4 twigs aphid infested, 2 = 5–10 twigs aphid infested and 3 = more than 10 twigs are aphid infested. The mean score was worked out in different orchards and accordingly, in different districts.

Data were subjected to analysis of variance (ANOVA) and Spearman rank correlations (r) was worked out by using SPSS statistical package (11.1 version) with level of significance at $P = 0.05$.

Data on the prevalence of CTV in different age group of mandarin orchard have been summarized in Table 1. Prevalence varied with the age and location of groves and ranged 34.92–59.01% with the average CTV incidence of 46.26 per cent in the state. The incidence showed increasing trend with the age group. The maximum prevalence was recorded in the age group of >20 years (50.80%) and minimum in 5–10 years (40.94%) and an intermediate range of prevalence (47.05%) was observed in 11–20 year old groves. The OD₄₀₅ values under different plant age groups and locations varied from 0.468–0.879. The spatial distribution of CTV in the mandarin groves of Sikkim Himalayan region showed the highest incidence in south district and lowest in east district under all the age groups of orchards. East district showed the CTV prevalence range of 34.92–39.68%, however, south district had 46.87–59.01%. Interestingly, the prevalence of CTV in north and west districts was at par under all age groups of groves (Table 1). Typical CTV symptoms: vein clearing, stem pitting, were not exhibited by mandarin trees which implies that mandarin does not show characteristic CTV symptoms, however, virus do multiply inside the plant tissues.

Similar type of studies were carried out by Biswas [5], who diagnosed CTV in Darjeeling hills through molecular tools and reported CTV infection up to 90% in mandarin trees at varying altitudes. A high incidence of CTV (>60%) was also reported by Thind et al. [16] in kinnow mandarin in Punjab. The high incidence of CTV in Sikkim Himalayan region may be due to the poor management of aphid vectors and use of virus infected planting material. The distribution and occurrence of CTV, clearly implies CTV an important disease in the region that might have been affecting production significantly.

Citrus tristeza virus shows significant spatial variation (Table 2) in different parts. The highest virus titre value (0.892) was recorded in twig followed by leaf (0.787); however, stem had the lowest titre value (0.543). The virus titre value in twig ranged from 0.839–0.928, in leaves 0.745–0.827 and in stem bark 0.512–0.622. [12] also reported high concentration of CTV in the twig portion of Kinnow in Punjab area of Pakistan. The variable amount of CTV in different plant parts may be due to uneven distribution of virus within the plant parts. The low concentration of virus in the bark of stem may be attributed to phloem-limited nature of the virus.

The intensity of aphid infestation in mandarin shows increasing trend with the age of tree (Fig. 1). The mean score of infestation varied from 1.32–1.68 in 5–10 year old orchard, 1.58–1.78 in 11–20 year old orchard and 1.86–2.45 in >20 year old orchards, however east district had the minimum score and south district had the maximum score

Table 1 Prevalence of CTV in different age group of mandarin of Sikkim

Districts	5–10 years			11–20 years			>20 years			Mean
	No. of sample	No. of +ve sample	% prevalence	No. of sample	No. of +ve sample	% prevalence	No. of sample	No. of +ve sample	% prevalence	
North	62	25	40.32 ^b	67	32	47.76 ^b	62	32	51.61 ^b	46.56 ^b
South	64	30	46.87 ^c	65	34	52.30 ^c	61	36	59.01 ^c	52.72 ^c
East	63	22	34.92 ^a	62	24	38.70 ^a	63	25	39.68 ^a	37.76 ^a
West	65	27	41.53 ^b	61	30	49.18 ^b	64	34	53.12 ^b	47.94 ^b
Total	254	104	40.94	255	120	47.05	250	127	50.80	46.26

In a column, means follow by same letter is not significantly different at 5% LSD

Table 2 ELISA reaction in different plant parts of mandarin

Plant parts	North	South	East	West	Mean
Leaf	0.784 ^b	0.793 ^b	0.745 ^b	0.827 ^b	0.787 ^b
Twig	0.878 ^c	0.926 ^c	0.839 ^c	0.928 ^c	0.892 ^c
Stem	0.514 ^a	0.527 ^a	0.512 ^a	0.622 ^a	0.543 ^a
LSD (0.05)	0.08	0.11	0.07	0.07	0.08

In a column, means follow by same letter is not significantly different at 5% LSD

Fig. 1 Intensity of aphid infestation in representative orchards of different districts of Sikkim

under all the age groups of orchard. The north and west districts had an intermediate intensity of aphid infestation.

The relative abundance of aphid species in mandarin is presented in Fig. 2. There were three vectors and among them, *Toxoptera citricida* predominated in all mandarin growing groves followed by *T. aurantii*, however, *Aphis spp.* were less than 5%. There was no significant temporal variation in the population dynamics of CTV vectors. The infestation of *Toxoptera citricida* was as high as 67.6% which is about two times high than that of *T. aurantii*. Correlation study shows strongly positive correlation ($r^2 = 0.893$) between the CTV prevalence and the infestation rate of aphid vectors. This observation corroborates the high prevalence of CTV in the Sikkim Himalayan region, which may be attributed to the high infestation rate of *T. citricida*. This finding is further substantiated by the report of Ahlawat and Raychaudhuri [2] and Biswas [5],

Fig. 2 Prevalence of aphid species in representative orchards of Sikkim

who observed *T. citricida*, the most efficient vector in transmitting CTV.

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