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Molecular Characterization of *Citrus tristeza virus* in Mandarin Orchards in Northeastern Himalayan State Sikkim

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Abstract—*Citrus tristeza virus (CTV), an aphid (Toxoptera citricidus) transmitted closterovirus, is one of the important causal agents causing decline disease in citrus worldwide. CTV is a phloem-limited, flexuous filamentous plant virus with particle size of 2000 X 11 nm. It contains positive sense, ssRNA genome, ~19.3kb in length comprising 12 ORFs (ORF1a-b and ORFs 2-11) potentially encoding at least 19 putative proteins and two UTRs. Sikkim which falls under Northeastern himalayan region of India is known to produce quality mandarin (C. reticulata) commercially. CTV is a major problem in this region causing decline of mandarin orchards which are being wiping out. Occurrence of CTV in mandarin orchards in Sikkim State has also been reported earlier but till to date CTV isolates of this State has not been characterized genetically. The different orchards of East and West Sikkim were surveyed. CTV incidence of 40-60% in East and 60-100% in West were estimated based on the testing with Direct antigen coated-ELISA (DAC-ELISA) and polymerase chain reaction (PCR). Seven CTV isolates; three from East and four from West were characterized based on cloning and sequencing of 5'ORF1a gene fragment (404nt) from L-ProI domain. The sequence analysis of 5'ORF1a gene fragment showed that the present isolates shared 98-100% nt identity among them and grouped together. Phylogenetic analysis showed that all of the present Sikkim CTV isolates are related with decline inducing Indian isolate Kpg3 and Israel severe isolate VT. CTV isolates of Sikkim were characterized for the first time and found to be genetically related with decline inducing Indian CTV isolate Kpg3; indicating decline inducing CTV isolates are common in Northeastern Himalayan region of India.*

Keywords*: Citrus tristeza virus, Sikkim mandarin, DAC-ELISA, RT-PCR.*