New Approaches in Geographical Distribution, Recombination and Strain Demarcation of Citrus Tristeza Virus

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Dedicated to Baba and Fazal, for their immense Love and Support

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CTV	*Citrus Tristeza Virus*
ORF	Open Reading Frame
CTV-SY	Seedling Yellow
CTV-T	Rapid Decline
CTV-SP	Stem pitting
RdRp	RNA dependent RNA polymerase
NCBI	National Centre for Biotechnology Information
MEGA-X	Molecular Evolutionary Genetics Analysis
MP	Maximum Parsimony
RDP	Recombination Detection Program
SDT	Sequence Demarcation Tool

ABSTRACT

Citrus Tristeza Virus (CTV) is the causative agent of Disease Tristeza, a phloem limited Closterovirus predominantly within the Rutaceae family confined mostly plant genera (Citrus and Fortunella). Transmission of CTV is in a semi- persistent manner by aphid species specifically by oriental citrus aphid and also by graft inoculation. Different strains of CTV are capable of inducing quick decline, stem pitting and yellow seedling on a range of citrus species. CTV possess the largest genome of any plant virus and has a single stranded positive sense RNA of ~19.3 kb. It has longest filamentous virions (2,000×10-12nm). It encodes 12 ORFs and two untranslated regions (UTRs). ORF1a and ORF1b are 5' terminal half regions expressed from genomic RNA. ORF1a encodes replicase domains that play their role in virus replication and ORF1b encodes RNA dependent RNA polymerase (RdRp). Citrus tristeza virus (CTV) is the most damaging viral pathogen in citrus plants which causes significant economic casualties for the worldwide citrus industry. CTV is perhaps peculiar among the Closteroviridae as it possesses a variety of distinct strains, the isolates of which provide a wide range of phenotype combinations among its different hosts. There is no recognition of the link between genotypes and phenotypes, and to exacerbate further, such genotypes are identified worldwide as members of mixed populations within a single host plant. The CTV isolates display variable pathogenicity on their hosts that highlights a mixed CTV population. Several fragments within the CTV genome have been used to study the CTV genetic variation, but the auspicious region for speedy differentiation for the CTV strain was inaccessible. In this study, a systemic analysis was conducted to evaluate the region within the CTV genome for swift differentiation of Citrus Tristeza Virus strains. 74 Full Genome, ORF1a, ORF1b sequences were retrieved from NCBI and were analyzed to identify the geographical distribution of CTV. Full genome and ORF1a sequences were opted for the said purpose. Recombinant analysis were performed by RDP4.0 and recombinant sequences were identified. 9 strain were identified on the basis of the matrix scores that are RB, T36, T68, T3, T30, VT, New Strain 1, New Strain 2, New Strain 3. RDP results highlighted recombinants found in New Strain 1 and 2, VT, T68, T3 and RB and out of 74 complete genome sequences 23 isolates were recombinant. Thailand isolate JQ798289.1 was evaluated again for recombination as it does not fit upon the criteria of isolate of CTV formulated in this study Demarcation criteria by SDTv1.2 plots and matrix highlighted the demarcation criteria for isolate of CTV to be from 92-95% and for strain to be from 95-100%. This then established and explained the spatial trend of strain distribution. The findings have provided better perception into the evolution and

propagation of the virus and the knowledge required to establish a better strategy for disease management. Hence In-silico evolutionary analysis and recombination pattern of CTV in Pakistan which can lead to formulation of a strategy for the control of CTV in Pakistan and increase in the export of Citrus that can lead to better economic conditions.