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Abstract

A study was undertaken to find out the genomic variability in *Citrus yellow mosaic virus* (CMBV), a bacilliform virus under the genus *Badnavirus*, infecting different citrus species in India and their phyolgenetic relationship with other badnaviruses. Comparison of genome sequences of four isolates of CMBV infecting different species of citrus with previously sequenced three CMBV isolates indicated variability in coding region of ORFs 1, 2 and 3 of all the CMBV isolates infecting same or different citrus species with highest variability in coding region of ORF 3. Coding region of ORF 4, 5, were also highly variable in CMBV isolates but they were highly conserved in CMBV isolates infecting Acid lime. ORF 6 was comparatively conserved and was identical in CMBV isolate infecting Acid lime. All the CMBV isolates shared maximum identity with *Cacao swollen shoot virus* (CSSV) in ORF 1 and 3 indicating that CMBV isolates are more closely related to CSSV than other badnaviruses. This study has implication in determining the diversity and diagnosis of CMBV.

Citrus greening disease is a threatening disease of citrus in India and other citrus growing countries. Kinnow mandarin {*Citrus reticulate* Balanco ('King' X 'Willow mandarin')} is a major horticultural crop of Punjab and it is also grown in Rajasthan

and Haryana. During a survey in Punjab, symptoms of yellowing and mottling of leaves were observed up to 40% in kinnow mandarin orchard. These symptoms are often confused with nutrient deficiency and other stress related disorders. However, a fastidious greening bacterium has been attributed to cause the disease. The disease was graft transmissible and sequencing of 16S rRNA, 16S/23S intergenic spacer region and 23S rRNA of the greening bacterium associated with yellowing disease in kinnow mandarin confirmed it to be *Candidatus* Liberibacter asiaticus ('*Ca* L. asiaticus'). The 16S rRNA gene sequence of the greening bacterium under study showed an identity of 95.9 with '*Ca.* L. asiaticus'isolate of USA (Accession No. DQ471900), 95.4% with '*Ca.* L. asiaticus' isolate of China (Accession.No.DQ778016), 94.7 % with '*Ca.* L. asiaticus' Poona isolate (L22532), 93% with '*Ca.* L. africanus' isolate Nelspruit (L22533); '*Ca.* L. psyllaurous' (Accession No.EU812559); and 90.4% with '*Ca.* L. americanus' (AY742824) . Comparison of 5 sequences of 23S rRNA of *Ca.* L. species available in the GenBank and the present isolate of greening bacterium under study showed that 23S rRNA was highly conserved (99-100%) in all the 4 isolates of '*Ca.* L. asiaticus' and two isolates of *'Ca.* L. psyllurous'. But the sequence identity of 23S rRNA of '*Ca.* L. asiaticus' shared 91.5 % identity with '*Ca.* L. psyllurous' (Accession No.EU644449)) and '*Ca.* L. sp.NZ 082226' (Accession No. EU834130). The phylogenetic analysis of 16S rRNA of greening bacteria revealed that *Ca.* L. asiaticus from different countries grouped together while '*Ca.* L. americanus', '*Ca.* L. africanus' and '*Ca.* L. psyllouraus' formed a separate and distinct group.

A simplified cost effective technique was developed for template DNA preparation for PCR of *Citrus mosaic virus* and citrus greening bacterium. The protocol is simple, inexpensive, rapid and applicable to large scale of citrus trees. The spotted membrane methodology can also be used for short term sample storage for further testing. A cost effective multiplex PCR was developed for detection of mixed infection of Citrus *mosaic virus* and Citrus greening bacterium from citrus. Citrus being a vegetatively propagated crop, use of virus free planting material is very important. These diagnostic techniques can be used by the agencies given the responsibility of bud wood certification. It can also be useful for the phytosanitary assay in plant quarantine.