# Detection of Citrus tatter leaf virus by Real-Time RT-PCR

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ABSTRACT. Citrus tatter leaf disease caused by Citrus tatter leaf virus (CTLV) is a major disease leading to serious economic losses in the People's Republic of China due to the common usage of trifoliate rootstock. A real-time RT-PCR assay using SYBR Green I was developed to detect and quantify CTLV RNA-targets in our laboratory. A pair of primers, ASG-Pf and ASG-Pr, were designed within the conserved sequence of coat protein gene. The assay had specificity of detecting CTLV in different citrus hosts, and its sensitivity was 100 times higher than that of the conventional RT-PCR. It is rapid and reliable, and SYBR Green I is inexpensive, and thus this is a valuable tool to detect CTLV.

Citrus tatter leaf disease caused by Citrus tatter leaf virus (CTLV) is a major systemic disease of citrus in the world, which may lead to severe economic losses, and it was first discovered in latently infected Meyer lemon trees in the United States in 1962, (8). Most citrus cultivars are latent hosts for CTLV, while CTLV-infected cultivars grafted on trifoliate orange or its hybrids are usually stunted and chlorotic, and they also show a pronounced virus-induced bud union incompatibility (6, 7). It is known that CTLV is widespread in China, and it has caused tremendous economic loss in Zhejiang, Hunan, Fujian and Guangxi Provinces.

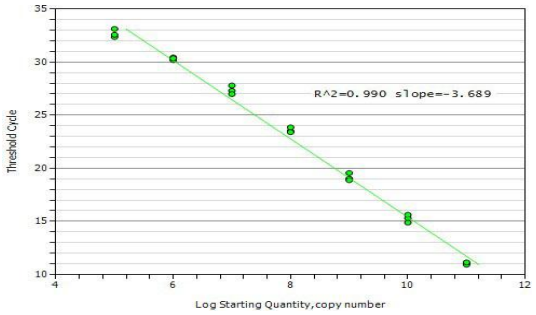
Although biological indexing, serology and reverse transcriptase polymerase chain reaction (RT-PCR) can all be used to detect CTLV (1, 2, 4, 5, 9), these methods either have limited requiring specialized facilities such as a greenhouse, or are less sensitive or costly. For rapid and reliable detection of CTLV, we established a system based on SYBR Green I dye.

Total nucleic acid extraction was carried out following the method described by Zhou et al. (10). Primers ASG-Pf (5' GAG TTT GGA AGA CGT GCT TCA 3') and ASG-Pr (5' TTG CAG AGA AGA AGG TAA AGC TC 3') were used to amplify a 165 bp region (3). For reverse transcription, 2 µL of nucleic acid extracts and 3 µL of water denatured at 95 ℃ for 3 min were used as templates. A 20 µL reaction mixture containing 5 µL of templates, 0.25 µM ASG-Pr, 4 µL of 5 × reaction buffer (TaKaRa), 0.5 mM dNTPs (TaKaRa), 0.5 µL RNAsin (Toyobo), and 0.5 µL of RTase (Toyobo). Reactions were incubated at 42 ℃ for 30 min. For real-time PCR, 25 µL reaction containing 1 µL of cDNA, 12.5 µL of SYBR Premix Ex Taq II (TaKaRa), and 0.08 µM of each primer were used. The real-time PCR program was performed as follows: at a temperature of 95˚C for 3 min, followed by 40 cycles at 95˚C for 20 s and then at 60˚C for 30 s.

Real-time RT-PCR can recognize all tested CTLV isolates, and no amplification was obtained from healthy plants or samples infected with other citrus viruses or viroids such as *Citrus tristeza virus* (CTV), *Satsuma dwarf virus* (SDV), *Citrus psorosis virus* (CPsV) and *Citrus exocortis viroid* (CEVd).

Two copies per µL CTLV recombinant plasmids were detected by real-time PCR,

and $2\times10^2$ copies per µL CTLV recombinant plasmids were detected by conventional PCR. The results showed that real-time RT-PCR was 100 times more sensitive than conventional PCR. Although it can detect as few as two copies per µL CTLV recombinant plasmids, the quantification range was established from $2 \times 10^2$ to $2 \times 10^8$ copies per µL recombinant plasmids due to the reliability of three repetitions (Fig. 1).




CTLV isolates from seven cultivars were successfully detected by real-time RT-PCR. The detectable $C_t$ values of CTLV RNA-targets ranged from 8.95 to 30.71 (Table 1). Great differences of the CTLV titer were found among several citrus species, with the lowest in grapefruit, and the highest in citrus hybrid.

TABLE 1
$C_t$ VALUE OF CITRUS TATTER LEAF VIRUS IN DIFFERENT CULTIVARS

| Cultivars | Threshold cycle ($C_t$) |
|---|---|
| Citrus hybrid | 8.95 |
| Lemon | 11.75 |
| Jincheng | 12.97 |
| Navel orange | 18.16 |
| Ponkan | 23.16 |
| Huyou | 28.90 |
| Grapefruit | 30.71 |

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