Characterization of small RNAs derived from *Tomato spotted wilt virus* infection by deep sequencing

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Abstract

RNA silencing is a conserved eukaryotic surveillance mechanism thought to play a role in protection against invading nucleic acids such as viruses, transposons and transgenes. Virus infection leads to accumulation of viral small RNAs (vsRNAs) at high levels. The processing of vsRNAs can be from viral dsRNA replicative intermediates, self complementary regions of the viral genome or from the action of RNA-dependent RNA polymerases on viral templates. The overall composition of the populations of vsRNAs generated by most plant viruses remains unknown. We have used deep sequencing techniques to characterize vsRNAs of *Tomato spotted wilt virus* (TSWV), a member of the genus *Tospovirus*, which causes economically important diseases in numerous crops in many parts of the world. The vsRNA profiles from TSWV-infected pepper, tomato, and tobacco plants were generated. Analysis of the vsRNAs indicate multiple hot spots for small RNA production from the TSWV genome, the location of these hot spots are predominantly conserved across infections of different host species. Details of the origin, distribution and abundance of TSWV vsRNAs in infected plant tissue were compiled.

Introduction

- Losses due to tospoviruses exceed US $1 billion worldwide (Pappu et al., 2009).
- TSWV is one of the most widely occurring and economically important plant viruses.
- Wide host range, with more than 1000 plant species reported to be susceptible (Peters, 1998).
- Transmitted by several species of thrips. Crops affected by TSWV include tobacco, tomato, pepper, bean, lettuce, peanut and potato.
- In infected plants, viral double-stranded RNA (dsRNA) induces RNA interference (RNAi), mediated by small, double-stranded RNA (dsRNA) molecules.
- There are two families of small RNAs that repress gene expression at transcriptional or posttranscriptional levels: short interfering RNAs (siRNAs) and microRNAs (miRNAs).
- These small RNAs play critical roles in a large variety of biological processes, such as development or plant responses to biotic and abiotic stresses.
- An essential component of this gene regulation is RNAse III Dicer, that cleaves dsRNA into 21- to 24-nucleotide (nt) duplexes siRNA or miRNA.
- It is believed that there are four Dicer-like (DCL1-4) present in plants which generates virus-derived siRNAs of 21, 22 and 24 nt respectively (Donaire et al. 2008).
- Development of high-throughput pyrosequencing technology has allowed the discovery of several sRNAs through deep sequencing.
- Several studies using RNA and DNA viruses have reported the characterization of viral siRNA from multiple genomic regions (Donaire et al. 2008, Qi et al. 2009).
- However, the siRNA profiles were characterized mainly in the case of positive-sense RNA viruses and no reports of sRNAs from tospovirus are available.

Objective

In this study, we employed the next generation sequencing technology to characterize the sRNAs associated with Tomato spotted wilt virus following its infection of tobacco (*Nicotiana benthamiana*) and tomato (*Lycopersicon esculentum*).

Materials and Methods

- Tobacco and tomato plants were grown from seeds under green house conditions at 26ºC with 16h day and 8h night.
- Seedlings were mechanically inoculated with TSWV using phosphate buffer.
- Leaves showing symptoms were collected and virus infection was confirmed by enzyme linked-immunosorbent assay (ELISA) and RT-PCR using N gene and coat protein specific primers.
- Non-inoculated symptomatic leaves were collected and snap cooled in liquid nitrogen and stored in -80ºC.
- To check the sRNA concentration during TSWV infection leaf samples were collected at the interval of 10, 17 and 24 dpi and RNA was ran in 17% denaturing polyacrylamide gel.
- To check the sRNA accumulation profiles the total RNA was send for deep-sequencing at BGI, China.

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References