Analysis of small RNA production patterns among the two potato spindle tuber viroid variants in tomato plants

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Abstract

In order to analyze the production of small RNA (sRNA) by viroids upon infecting the plants, the tomato plants (Solanum lycopersicum cvt. Rutgers) were inoculated with the variants of Potato spindle tuber viroid (PSTVd). After 21-days of postinoculation, total RNA was extracted and subjected for deep-sequencing using Illumina HiSeq platform. The primers were trimmed and only 21- to 24-nt long sRNAs were filtered after quality check of the raw data. The filtered sRNA population was then mapped against both the genomic (+) and antigenomic (−) strands of the respective PSTVd variants using standard pattern-matching algorithm. The profiling of viroid derived sRNA (vd-sRNA) revealed that the viroids are susceptible to host RNA silencing mechanism. High-throughput sequence data linked to this project have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE69225.

Keywords:
Viroids
Potato spindle tuber viroid
Viroid derived small RNA
RNA silencing

1. Direct link to deposited data

2. Experimental design, materials and methods

Viroids are the non-coding, single stranded, circular RNAs molecules with sizes range of 246 to 401 nucleotides (nt). They can infect certain plants. Recent findings, however, show that viroid infection is associated with the appearance of viroid-derived small RNA (vd-sRNA). These vds-RNAs have sizes similar to endogenous small interfering RNA and microRNA (miRNA) indicating the possibility of connection of vds-RNA to the induced symptoms. Interestingly the symptoms vary dramatically, depending on both the plant cultivar and the viroid strain [1].

In order to verify the production of small RNA (sRNA) by the PSTVd variants: PSTVd-mild (PSTVd-M, synonym PSTVd-Dah; GenBank Acc. No. AB623143) and PSTVd-intermediate (PSTVd-I; GenBank/Ac. No. AY937179) upon infection, the tomato plants were inoculated with the respective viroid variants as described previously [2]. Mock inoculated plants were used as control. At 21-days post inoculation (dpi) leaf samples were collected and subjected to total RNA extraction using the mirVana™ miRNA isolation kit (Ambion, Austin, TX, USA), with slight modifications. Briefly, 3 to 4 leaf discs were homogenized with 400 μl of lysis/binding buffer. Then, 60 μl of miRNA homogenate additive was added. The resulting solution was purified using acid phenol/chloroform (5:1) followed by DNase I (Promega, Madison, USA) treatment. The RNA was precipitated by adding 2.5 vol of absolute ethanol. RNA integrity was examined in a 2100 Bioanalyzer (Agilent Technologies, California, USA). The total RNA obtained was subjected for northern blot analysis in order to confirm the accumulation of PSTVd-specific sRNA as described previously [2]. By referring to the RNA size marker, a gel slice containing the sRNAs of 15 to 50 nucleotides were excised and eluted from gel using 1 ml gel elution buffer (0.5 M ammonium acetate, 1 mM EDTA [pH 8.0], 0.1% [w/v] SDS) as described previously [3]. All the recovered sRNAs were sent to Hokkaido System Science Co. (Sapporo, Japan) for deep-sequencing using a Genetic Analyzer IIx platform (Illumina, San Diego, CA, USA). The samples were quantified in an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and were processed simultaneously in the Illumina system using an index-sequence. The adapter sequences were trimmed...
from the ends of the resulting raw data. Filtered sequences where re-
grouped after eliminating the sequence reads less that 21 nt and longer
that 24 nt [3]. Approximately 4.3 million reads of sRNA were obtained
from the leaf samples of mock inoculated (GSM1695655), PSTVd-M
(GSM1695656) and PSTVd-I (GSM1695657) inoculated plants.

Sequence analysis of over 4.28 million sRNAs obtained from mock
inoculated plants identified about 108 and 106 viroid speci-
fics small RNAs (vd-sRNA) of PSTVd-M and PSTVd-I type, respectively, matching
the genomes of PSTVd-M and PSTVd-I. Analysis of 4.54 million sRNA
reads obtained from the PSTVd-M inoculated plants against both the
(+) and (−) strands of PSTVd-M revealed 103,933 vd-sRNAs. Similarly,
analysis of 4.88 million reads obtained from the PSTVd-I inoculated
plants against both the (+) and (−) strands of PSTVd-I underscored
488,146 vd-sRNAs. That said, PSTVd-I inoculated plants showed more
vd-sRNA (11.3%) than PSTVd-M (2.3%) inoculated plants. This differ-
ence in the vd-sRNA recovery can be attributed to the lower accumu-
lation of PSTVd-M compared to PSTVd-I, as described previously [2]. All
21- to 24-nt long vd-sRNAs were profiled on both polarity strands of
the respective PSTVd variants using the standard pattern-matching al-
gorithm in order to understand the production of the vd-sRNAs
(Fig. 1). Although PSTVd-M and PSTVd-I produced different amount
of sRNAs, both showed similar vd-sRNA profile. Further, profiling of
vd-sRNA on the PSTVd genome revealed that certain region of PSTVd
produced more sRNA than others, indicating that these regions are
more susceptible to RNA silencing.

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