Small RNA profiling in two PSTVd infected Bulgarian pepper cultivars

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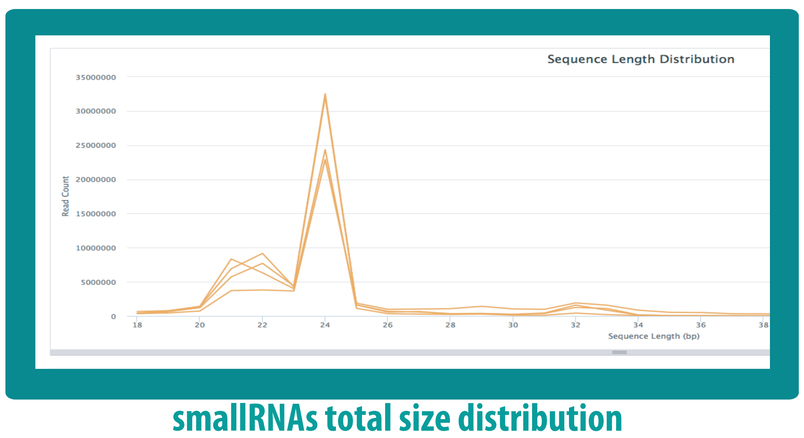
Abstract

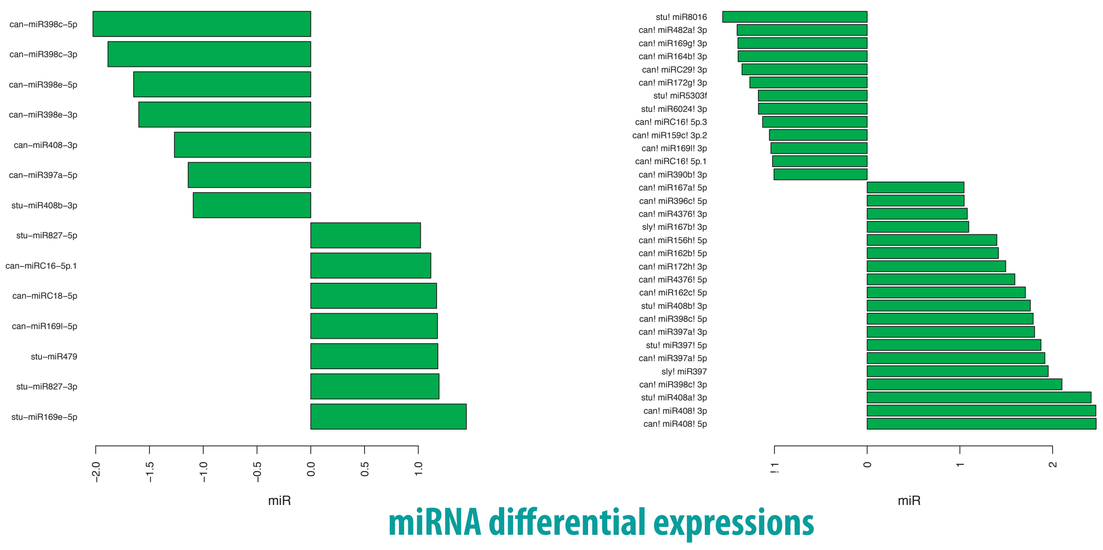
Potato Spindle Tuber Viroid invades many species of the Solanaceae family in varying degrees of infectivity, with pepper remaining tolerant and developing mild symptoms.

Though viroid pathogenesis is not understood in details, we can assume that certain cultivar-specific or species-specific host- pathogen interactions might be implicated.

Fifteen Bulgarian pepper cultivars were infected with PSTVd KF440-2. Two of them (Kurtovska kapia and Djulunska shipka) showed a specific phenotype with a characteristic leaf spreading upwards upon PSTVd infection and were selected to study viroid-host interactions at RNA level.
Total RNA was isolated from healthy and infected plants of both cultivars and was subjected to a small RNAseq assay. Assessment of differentially expressed PSTVd derived sRNAs and pepper miRNAs was performed. Molecular-biological approaches are underway to verify
the bioinformatics predictions.



smallRNAs total size distribution



miRNA differential expressions

Methods

Bioassay: Pepper plants of two Bulgarian cultivars (Kurtovska kapia (KK) and Djulunska shipka (DS)) were mechanically inoculated with appx. 100ug in vitro transcribed PSTVd KF440-2 (+) RNA at the two true leaves stage. Mock plants were inoculated with 5% K2HPO4 buffer. All plants were grown at 22C, 16h/8h day/light period for 30 dpi and upper leaves were collected for further analyses.

Total RNA extraction

Total RNA was extracted from leaves of mock and PSTVd-infected plants using Spectrum plant total RNA kit, Sigma with the following modification. Briefly, the extraction was performed following a protocol A by adding 2% polyvinylpyrrolidinone K 30 (PVP) to the lysis buffer. RNA integrity was proved by agarose gel electrophoresis.

Detection of PSTVd (+/-) RNA in infected plants: For detection of (+) and (-) PSTVd strands in infected plants, an RT-PCR with PSTVd weid Fw/Rev primers pair was performed. RT reaction was accomplished with Script cDNA Synthesis kit (Jena Bioscience) according to instructions of manufacture.

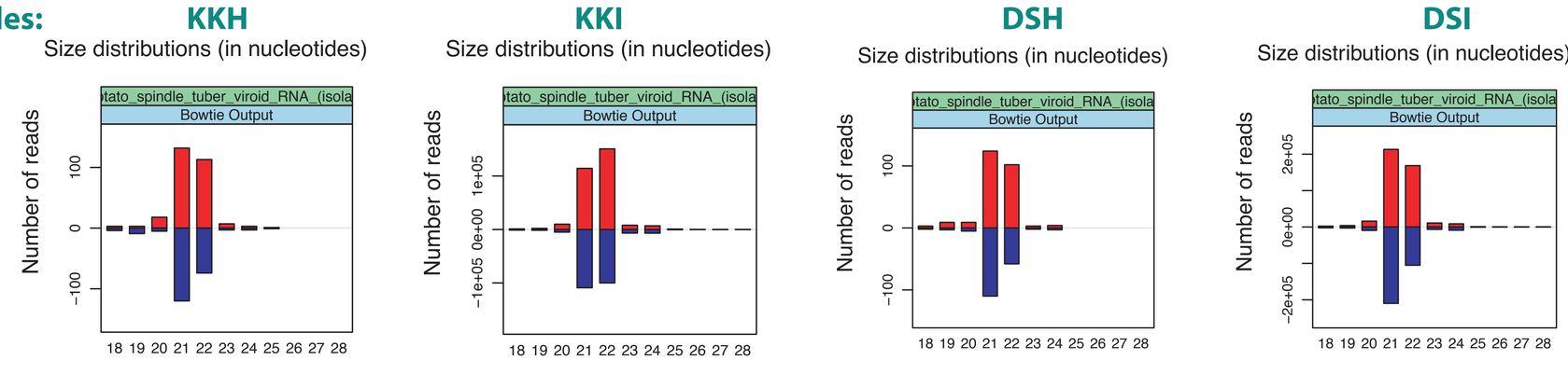
NGS sequencing

Small RNA sequencing was performed on Illumina HiSeq 2500 (rapid run mode), 50bp SE lane-based sequencing done by Macrogen Inc, Rep. of Korea.
Four small RNA libraries were prepared from healthy and infected samples per cultivar (KKH, KKI, DSH, DSI). After trimming of adaptors, rRNA seq filtering and discard of ambiguous bases (Ns), we obtained 62M, 59.5M, 51M and 49M reads for KKH, KKI, DSH and DSI,

Main outcomes:

1. PSTVd specific reads were identified through mapping against PSTVd KF440-2 reference sequence and the distribution of (+) and (-) sRNAs was evaluated.
2. The hot spots of PSTVd specific reads were defined.
3. Identification of conservative miRNAs and their expression levels
4. Establishment of differentially expressed miRNAs in response to PSTVd infection.

Samples: **KKH** **KKI** **DSH** **DSI**



Size distribution and PSTVd mapping of sRNAs

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Results