RNA-seq analysis of resistant and susceptible cucumber plants infected with *Pseudomonas syringae* pv. *lachrymans*

RENATA SŁOMNICKA, HELENA OLCZAK-WOLTMAN, GRZEGORZ BARTOSZEWSKI

Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture, Biotechnology and Landscape Architecture, Warsaw University of Life Sciences, WULS-SGGW, Warsaw, Poland

Introduction

One of the factors limiting open-field production of cucumber (*Cucumis sativus* L.) is angular leaf spot disease caused by *Pseudomonas syringae* pv. *lachrymans* (*Psl*). It is an economically important pathogen, as cucumber is grown throughout the world over an area of 2,1 million hectares with a total production of 71,3 million tons mainly in China but also in EU and the USA. Recently, destructive outbreaks of this disease have been reported in Poland, China and USA (Meng et al. 2016, Newberry et al. 2016, Olczak-Woltman et al. 2008). In Chinese provinces, disease occurrence varied from 15 to 50% in different fields, causing 30% to 50% of yield reduction (Meng et al., 2016). Therefore, better understanding of *Pseudomnas syringae* pv. *lachrymans* and *Cucumis sativus* pathosystem is needed.
In this study RNA-seq was applied to compare transcriptomic response to the highly virulent *Psl* strain 814/98 of two cucumber lines, i.e.: partially-resistant Gy14 and susceptible B10.

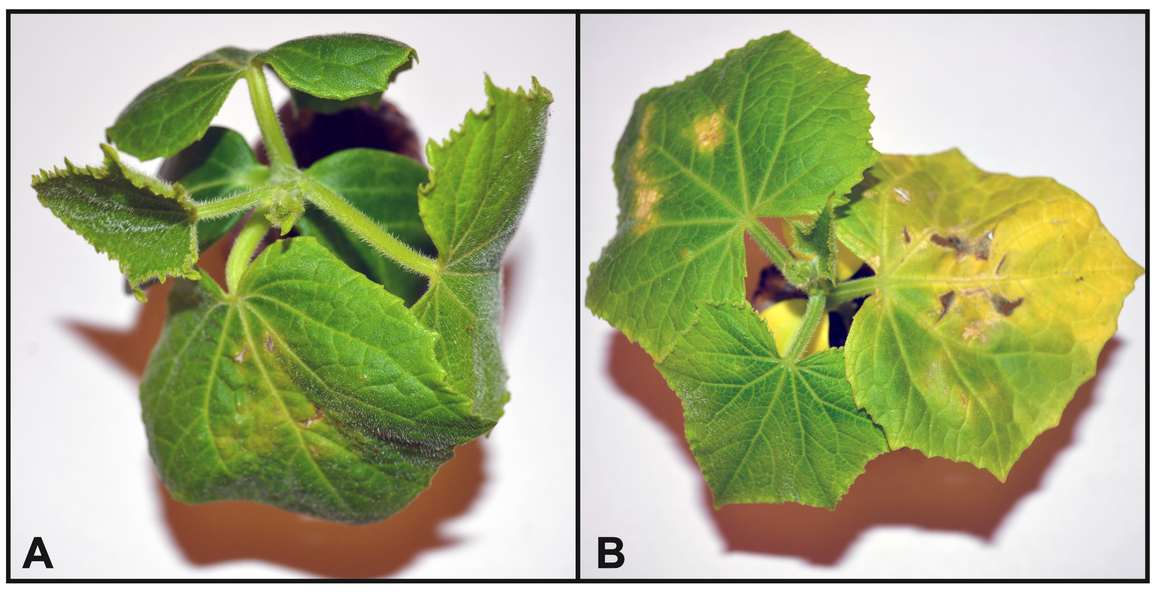


Figure 1. Angular leaf spot symtopms on cucumber accessions caused by *Pseudomonas syringae* pv. *lachrymans* highly virulent strain 814/98 three days post inoculation (3 dpi). (A) Gy14 line partially resistant to *Pseudomonas syringae* pv. *lachrymans*, (B) B10 line susceptible to *Pseudomonas syringae* pv. *lachrymans*.

Material and Methods

Total RNA was isolated from pooled leaves tissue (6 plants for each pool), collected from 2-3 weeks old plants in growth chamber conditions (Figure 1). The leaves were collected three times: before inoculation, one and three days after inoculation with highly wirulent strain Psl 814/98 (0, 1, and 3 dpi).

Illumina HiSeq2000 platform was used for RNA-seq sequencing. HISTAT v0.1.6-beta was used to perform genome mapping. Reconstruction transcripts, comparison reconstructed transcripts to reference annotation and prediction potential novel transcripts performed with using StringTie v.1.0.4, CuffCompare v.2.2.1 and CPC v.0.9-r2 program, respectively. The gene expression analysis were performed using Bowtie 2 v2.2.5 and RSEM v1.2.12. Based on gene expression results Differentially Expressed Genes (DEGs) were detected, i.e: between time points within each investigated line and

Results

About 4.47 Gb of clean reads were obtained for each sample and 93.18-93.75% of clean reads were mapped on reference genome (9930 line) confirming that samples are comparable (Table 1). Transcripts representing about 19,000 genes for each sample were identified with total mapping ratio 73.12-76.21% (Table 2).

Table 1. Summary of sequencing reads after filtering.

Sample	Total Raw Reads(Mb)	Total Clean Reads(Mb)	Total Clean Bases(Gb)	Clean Reads Q20(%)	Clean Reads Q30(%)
B10_0 dpi	48.11	44.81	4.48	99.09	96.88
B10_1 dpi	48.11	44.77	4.48	99.13	97.00
B10_3 dpi	48.11	44.56	4.46	99.09	96.91
Gy14_0 dpi	48.11	44.91	4.49	99.13	97.00
Gy14_1 dpi	48.11	44.57	4.46	99.00	96.69
Gy14_3 dpi	48.11	44.63	4.46	98.98	96.65

Table 2. Summary of gene expression.

Sample	Total Mapping Ratio (%)	Uniquely Mapping Ratio (%)	Total Gene Number	Known Gene Number	Novel Gene Number	Total Transcript Number	Known Transcript Number	Novel Transcript Number
B10_0 dpi	74.53	40.26	18859	18320	539	28822	17790	11032
B10_1 dpi	75.15	39.45	19039	18488	551	29081	17994	11087
B10_3 dpi	73.12	39.42	19149	18592	557	28663	17507	11156
Gy14_0 dpi	75.10	41.09	18807	18281	526	28817	17789	11028
Gy14_1 dpi	76.21	40.23	18871	18332	539	29075	18024	11051
Gy14_3 dpi	73.53	39.74	18981	18439	542	28752	17634	11118

Results

Generally, the examined samples at 1 dpi and 3 dpi indicate higher similarity of expression profiles in contrast to time point 0 dpi for both lines. This relateness illustrate a heatmap of pearson correlation and hieralchical clustering between samples, which are presented on Figure 2 and Figure 3.

Based on gene expression results, DEGs were revealed with higher number up-regulated DEGs. The increasing number of up- and down-regulated DEGs for both lines were detected comparing samples at 0 dpi vs at 1 dpi and 0 dpi and 3 dpi. However, the Gy14 line (which shows partially-resistance) indicated a higher number of DEGs at 0 dpi vs at 1 dpi than B10 line (Figure 4A and 4B). Moreover, the DEGs analysis revealed higher number up- and down-regulated DEGs between lines at 1 dpi (357 up- and 295 down-regulated DEGs, respectively) than at 0 dpi (230 and 311 DEGs, respectively) and at 3 dpi (238 and 213 DEGs, respectively) (Figure 4C).

The Gene Ontology classification and pathway functional enrichment indicated that a lot of DEGs take part in metabolic pathways and biosynthesis of secondary metabolites, exibit binding and cathalytic activity and are associated with cell and membrane component .

Figure 2. Heatmap of Pearson correlation between samples. Both X and Y axis represent each sample. Coloring indicate pearson correlation(high: blue, low: white)

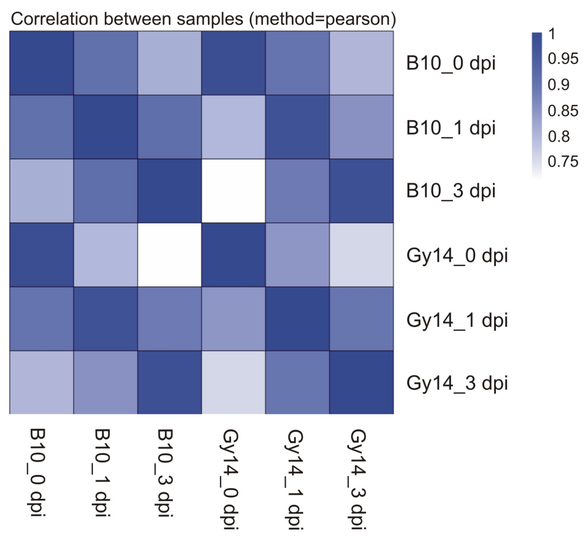


Figure 3. Hierarchical clustering between samples. More closer indicate more similar expression profile between samples.

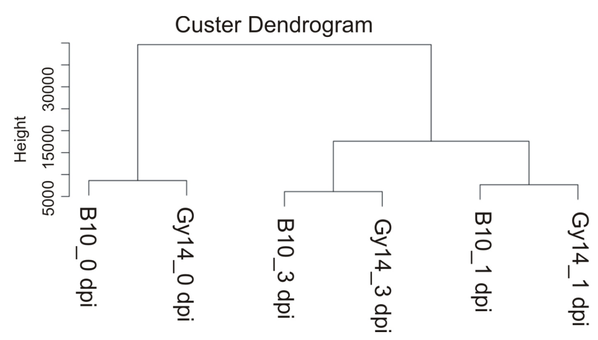
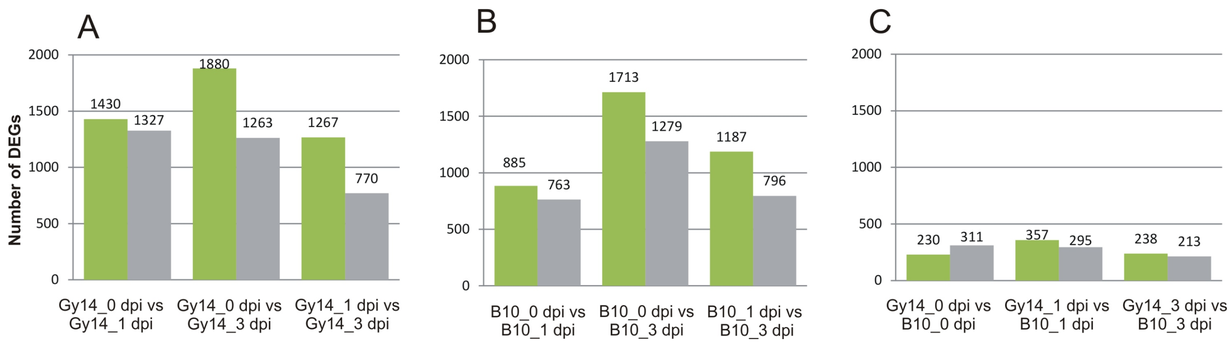


Figure 4. Summary of DEGs presents comparison between time points for Gy14 line (A), for B10 line (B) and between lines for each time point (C).
X axis represents comparing samples. Y axis represents DEG numbers. Green color represents up-regulated DEGs, down-regulated DEGs are marked in gray.



Summary

Transcriptomic profiles of Gy14 and B10 lines in response to Psl infection (0-3 dpi) has been revealed.
Massive transcriptomic response to Psl infection was observed 1 day after infection in resistant line Gy14 as compated to susceptible B10.
Improved functional annotation of cucumber genome will facilitate better understanding of transcriptomic response to Psl infection .

Acknowledgements

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Literature

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