Transcriptomic response of resistant and susceptible cucumber lines to *Pseudomonas syringae* pv. *lachrymans* infection

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A b s t r a c t

To understand better molecular mechanisms involved in cucumber (*Cucumis sativus* L.) response to *Pseudomonas syringae* pv. *lachrymans* infection, we analysed transcriptomic profiles of two cucumber lines: Gy14 and B10, that are characterized by different response to this pathogenic bacteria. We performed RNA-seq analysis and identification of differentially expressed genes (DEGs) for susceptible and resistant line at the early stage of infection. The higher number of genes up- and down-regulated (1430 up-regulated and 1327 down-regulated, respectively) at the early phase of infection was identified in resistant line Gy14 as compared to susceptible line B10. Moreover, for line Gy14 the higher number of uniquely expressed DEGs was found. This study provides insights into molecular basis of cucumber response to *P. syringae* pv. *lachrymans* and cucumber resistance to angular leaf spot disease.

K e y W o r d s: cucumber diseases, transcriptome, DEGs, plant pathogenic bacteria

Introduction

The current genomics technologies provide means not only for genome sequencing, but also for transcriptome sequencing and comprehensive analysis. Comparing transcriptomes of well characterized lines or varieties is one of the approaches to explore disease resistance mechanisms in plants and to reveal the role of specific biological pathways in building response to pathogen attack. In cucumber, the RNA-seq method has been employed so far for analysis of fruit development, flower sex expression, and plant responses to abiotic and biotic stresses (e.g. Ando and Grumet 2012, Kong et al 2015, Wang et al., 2018). However, to the best of our knowledge, no such research has been performed to study cucumber angular leaf spot disease that is related to leaf infection by *Pseudomonas syringae* pv. *lachrymans*. The aim of this study was to perform transcriptome profiling of two cucumber lines, i.e.: susceptible B10 and resistant Gy14 in response to infection of highly virulent strain 814/98 of *P. syringae* pv. *lachrymans*.

Materials and Methods

Plant material. The total RNA was isolated from pooled leaves tissue, collected from 2 to 3 weeks old plants grown in growth chamber conditions. The leaves were collected three times: before (0 dpi), one day after inoculation (1 dpi), and three days (3 dpi) after inoculation with a highly virulent strain 814/98 of *Pseudomonas syringae* pv. *lachrymans* (Słomnicka et al. 2018).

RNA sequencing and bioinformatics analyses. Illumina HiSeq2000 platform was used for RNA-seq. Sequencing was performed at Genomed S.A. (Warsaw, Poland). For each line and time point about 44,6 mln of reads were obtained. Clean reads were mapped using HISTAT on the cucumber reference genome 9930 v.2 (http://icugi.org/). Differentially expressed genes (DEGs) were detected and analysed using Cufflinks, StringTie, NOIseq and PossionDis software. Gene Ontology (GO) annotation and KEGG pathway analysis were used for DEGs classification and functional enrichment.

Results and Discussion

Genes differentially expressed in infected leaves of susceptible and resistant cucumber lines were identified. The biggest differences between lines and the highest number of DEGs were identified at the early phase of infection. One day after inoculation there were 2757 DEGs identified for resistant Gy14 line and 1648 for susceptible B10 line (Table 1). There were 1241 DEGs common for both lines. For resistant line Gy14 1516 unique DEGs were found, while only 407 DEGs were identified for susceptible line B10 (Table 2).

Table 1. DEGs identified for resistant and susceptible cucumber lines, time point 1 dpi.

| Line	Number of expressed genes	DEGs statistically significant*		Down-regulated		Up-regulated	
Gy14	19466	2757	14,2%	1327	48,1%	1430	51,9%
B10	19529	1648	8,4%	763	46,3%	885	53,7%

*log2FoldChange≥1.0

Table 2. DEGs unique for resistant or susceptible cucumber line, time point 1 dpi.

Line	Number of unique DEGs	Down-regulated		Up-regulated	
Gy14	1516	796	52,5%	720	47,5%
B10	407	240	59,0%	167	41,0%
Gy14 and B10	1241	531	42,8%	710	57,2%

For DEGs identified in resistant Gy14 line at the early phase of infection (0-1 dpi) we performed gene ontology analysis and functional enrichment. The GO annotation indicated that in the group of genes connected with biological processes there are 679 DEGs involved in metabolic processes and 512 DEGs involved in cellular processes. The group of cellular DEGs is divided mainly into genes connected with cell processes (647 genes), membrane processes (555 genes), and 263 genes involved in organelle processes. The major molecular functions of DEGs were binding (598 genes) and catalytic activities (764 genes). The detailed GO classification results are shown at Figure 1.

Figure 1. GO classification of DEGs. X axis represents number of DEG, and Y axis - GO term.
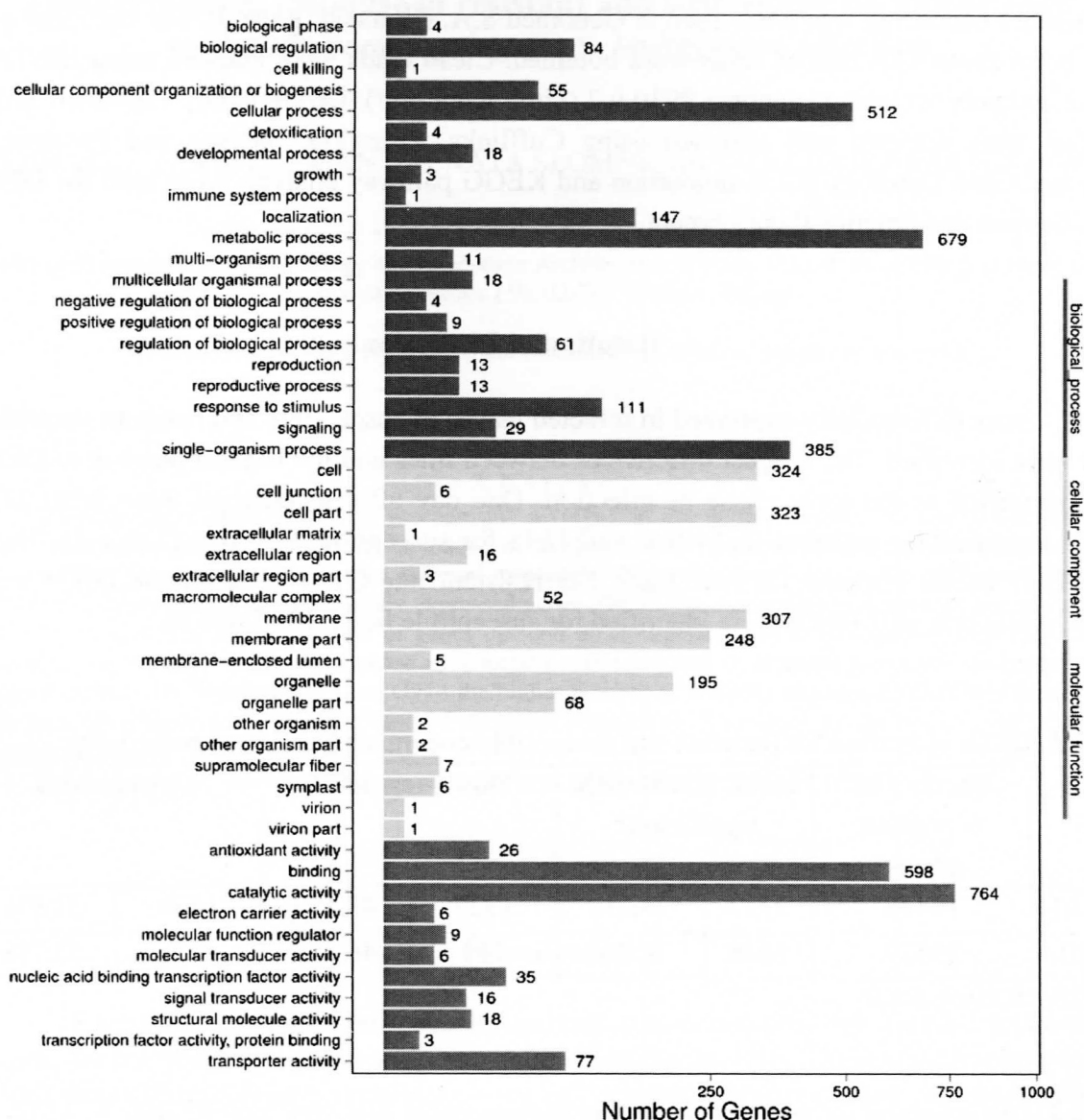
We performed also KEGG pathway classification and functional analysis. KEGG analysis resulted in classification of ca. 500 genes into the metabolic pathways and ca. 400 genes categorized to group of secondary metabolites biosynthesis. We identified DEGs connected with biosynthesis of: phenylpropanoids, flavonoids, carotenoids and anthocyanins, as well as plant hormones jasmonates (JA), salicylic acid (SA), and abscisic acid (ABA). There were also genes involved in ethylene pathway, ROS signalling and metabolism and chlorophyll degradation. We identified a small set of pathogenesis-related genes and genes connected with defence response to bacteria (Figure 2).

Our results indicate that at the early stage of cucumber leaf infection by *P. syringae* pv. *lachrymans* the response of line Gy14 is much more complex and there are much more genes involved in this response as compared to suscetible line B10. Active transcriptomic response to infection and activation of many biological processes could possibly explain the resistance of Gy14 to *P. syringae* pv. *lachrymans*.

Figure 2. Functional enrichment of DEGs. X axis represents enrichment factor. Y axis represents pathway name. Colouring indicate q-value (high: white, low: blue), the lower q-value indicate the more significant enriched. Point size indicate DEG number (more: big ponit, less: small point).
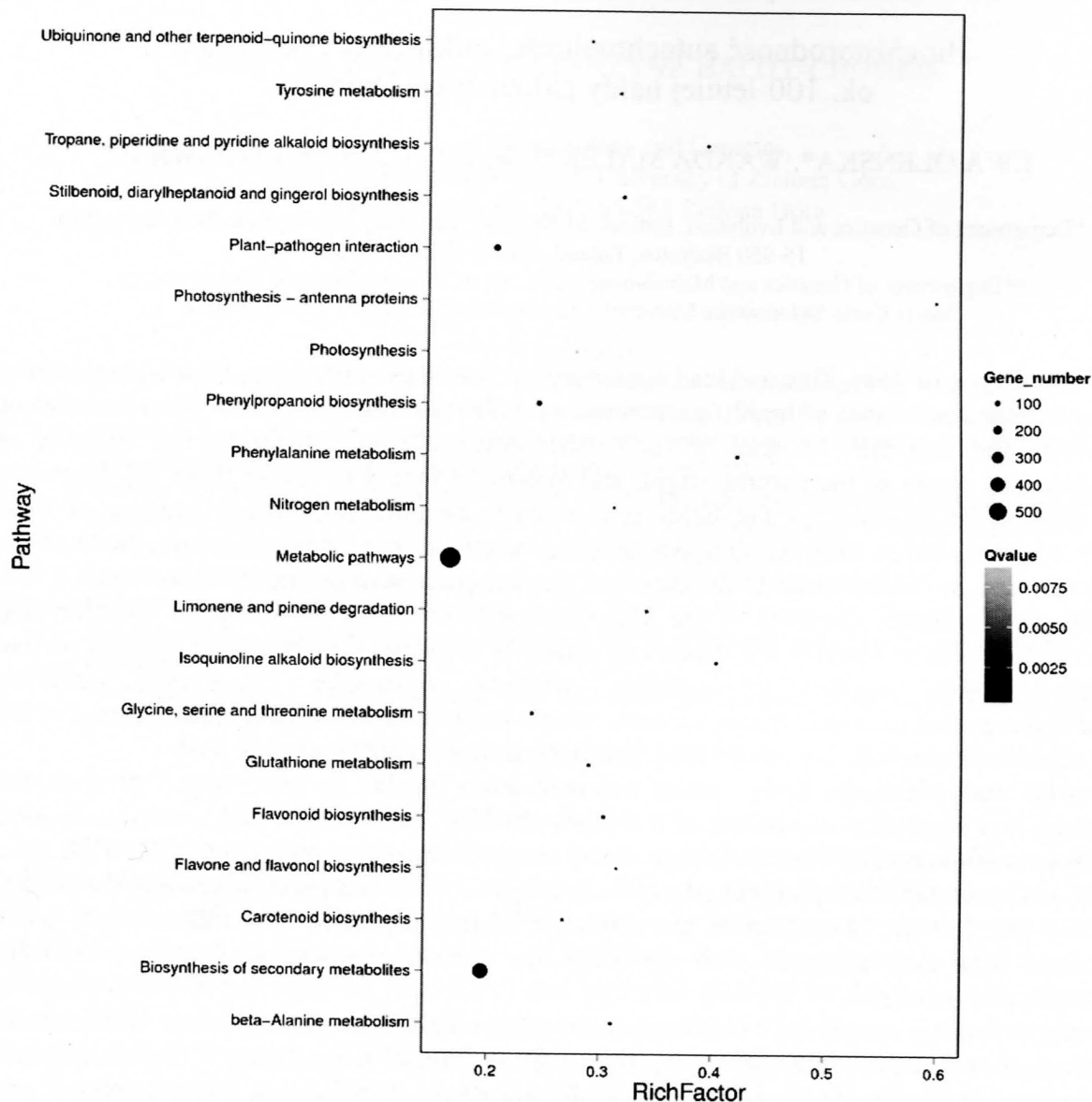
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