RNA-Seq Analysis Reveals Host Plant Transcriptomes in Response to *Agrobacterium*-mediated Transformation

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Background and Rationales

- *Agrobacterium*-mediated T-DNA transformation has become a predominant tool
- However, many crop species are still difficult to be transformed using this approach
- Genomics and genome editing demand for efficient T-DNA transfer in more crops
- Studies on mechanisms of T-DNA transfer shall benefit crop transformation
- Functions of most of the *Agrobacterium* genes in T-DNA transfer have been well studied
- By contrast our knowledge of plant genes involved in this process is very limited
- This project has been undertaken to discover plant genes involved in T-DNA transfer
- There have been transcriptomics studies previously using subjective cDNAs and microarrays
- No study has used RNA-Seq to reveal whole plant transcriptomes in response to T-DNA transfer

Advantages of RNA-Seq

- Allows discovery of complete transcripts and even splicing variants
- Doesn’t need prior knowledge of transcript sequences
- Is highly sensitive and enables to discover low abundant transcripts
- Is accurate, fast, and now becomes economically feasible

Experimental Approach

- *Agrobacterium* avirulent A136 and virulent At804 strains were used
- Mock inoculation was used as negative (blank) control
- AGROBEST (2014) was used for whole Arabidopsis seedling infection
- RNA samples were collected at 0, 3, 6, 12, 24, 48h post infection
- RNA-seq was performed at MU DNA Core Facility
- NextSeq 500 sequencer was used
- Sequences were trimmed and mtDNA and rDNA were removed

Time Course RNA-Seq: Comparison at each time point

0 3 6 12 24 48h pi

Mock	Negative (blank) control
Avirulent	Pathogen-Associated Molecular Pattern-Triggered Immunity (PTI)
Virulent	Elicitor-Triggered Immunity (ETI) + PTI

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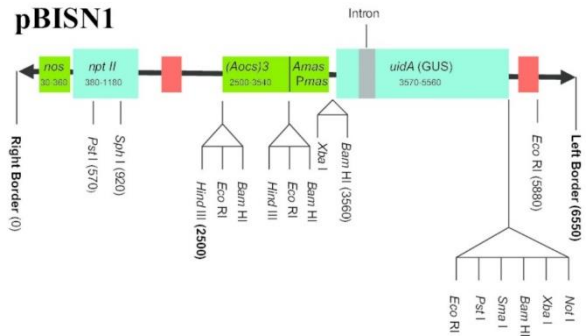
Results and Discussions

Verification of AGROBEST transformation system

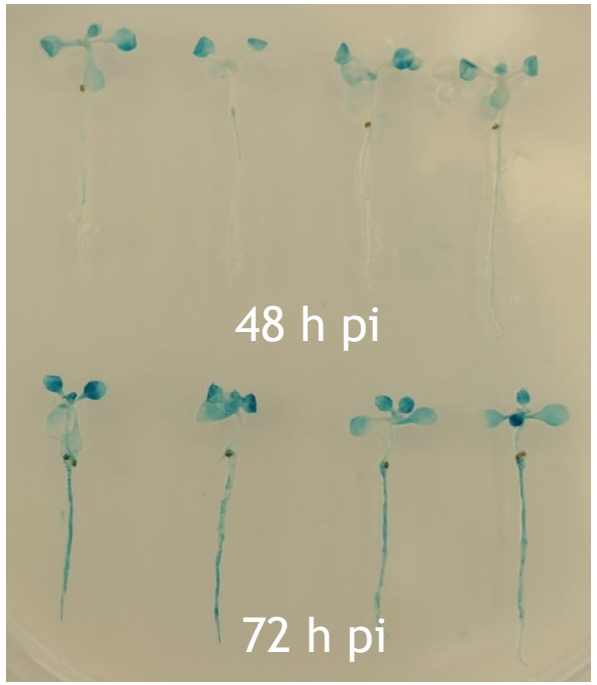
A

At804

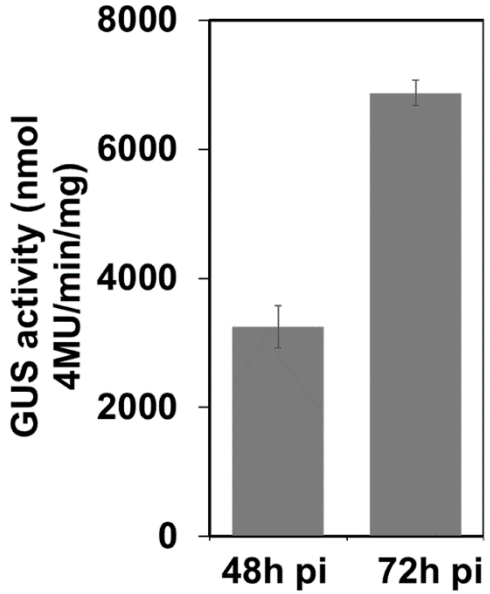


(Narasimhulu et al. 1996)

B



C



3 independent experiments = 1 RNA-seq replicate > 2 RNA-seq replicates

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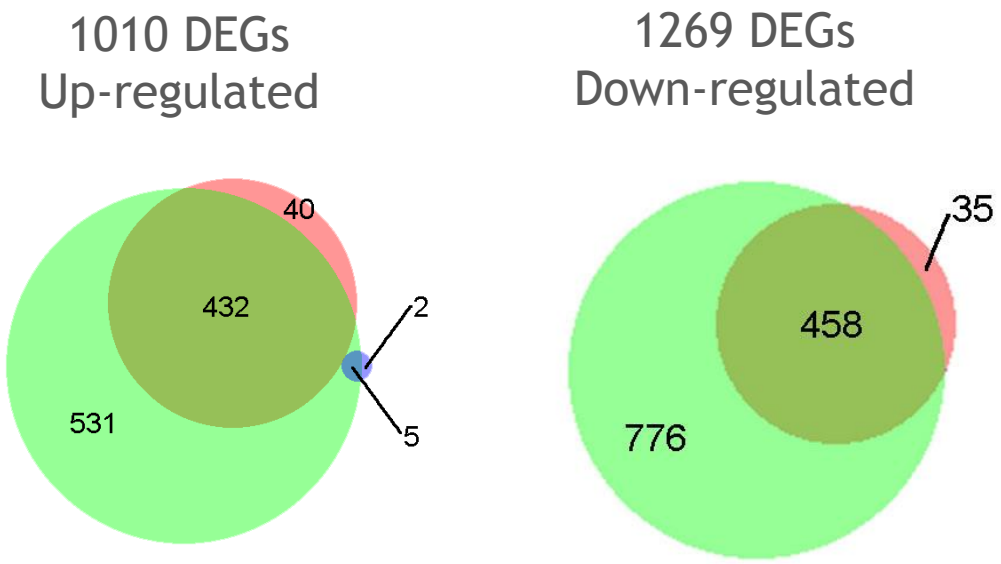
Number of differentially expressed genes (DEGs)

A



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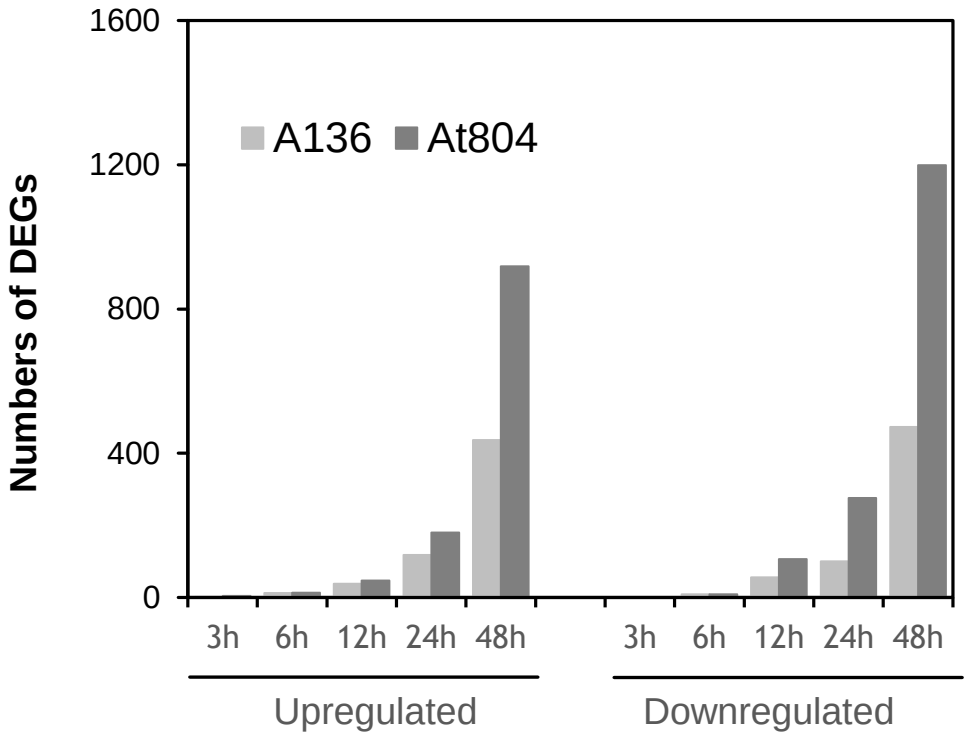

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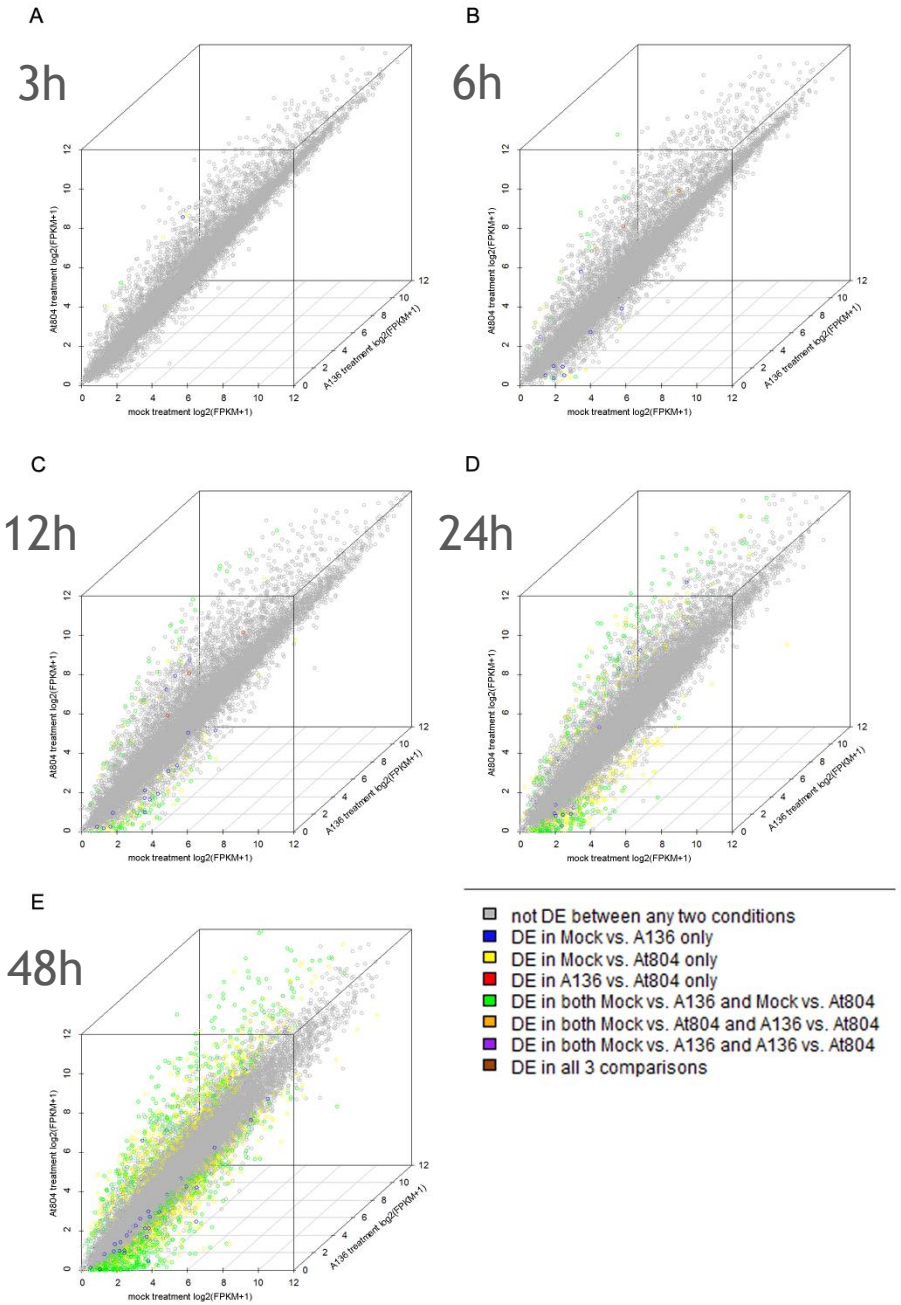
Three-dimensional scatterplots showing DEGs



log2-transformed FPKM (fragments per kilobase of transcript per million mapped reads) values

Scatterplot3d R package

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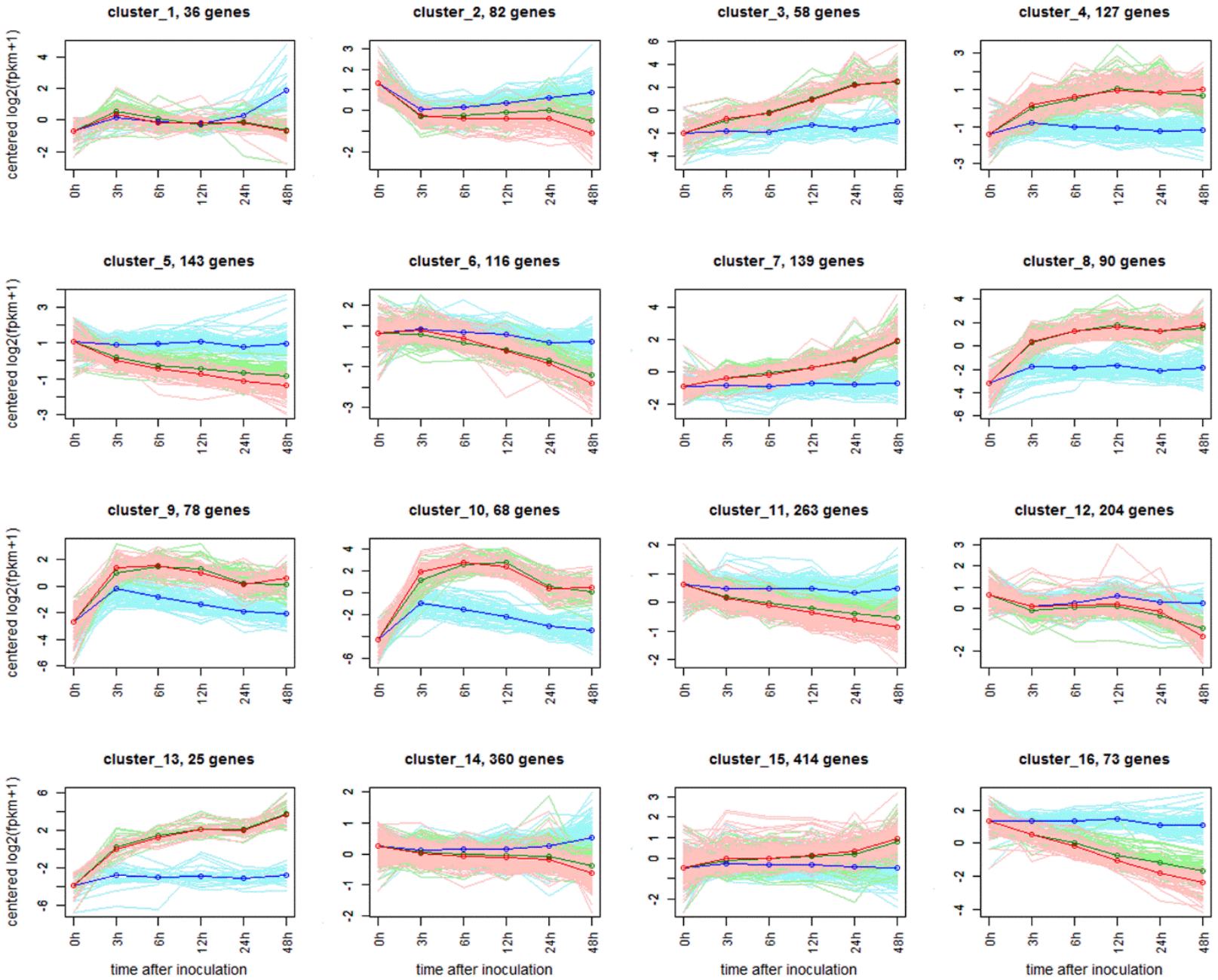
K-means clustering

mock

A136

At804

TrinityRNAseq



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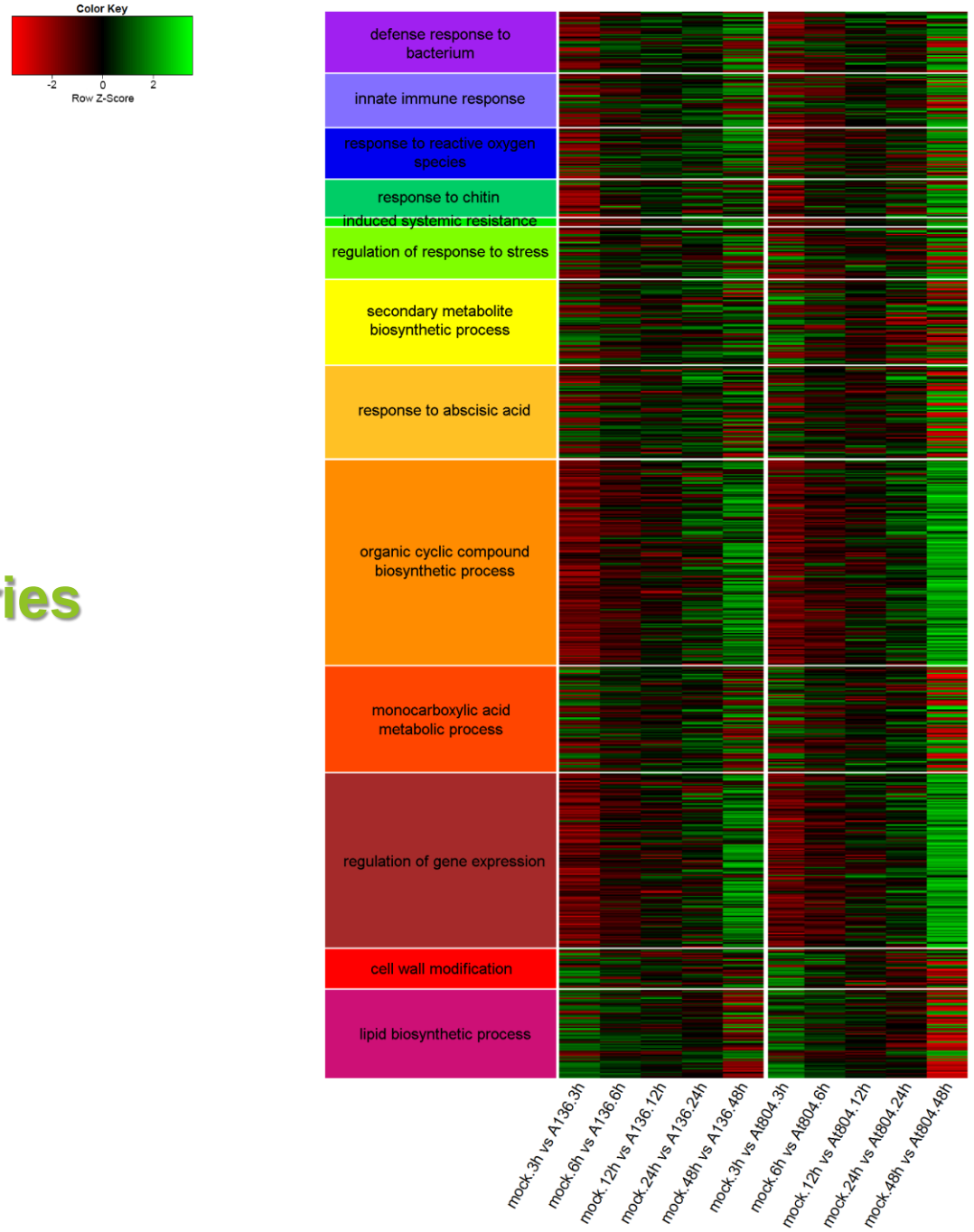
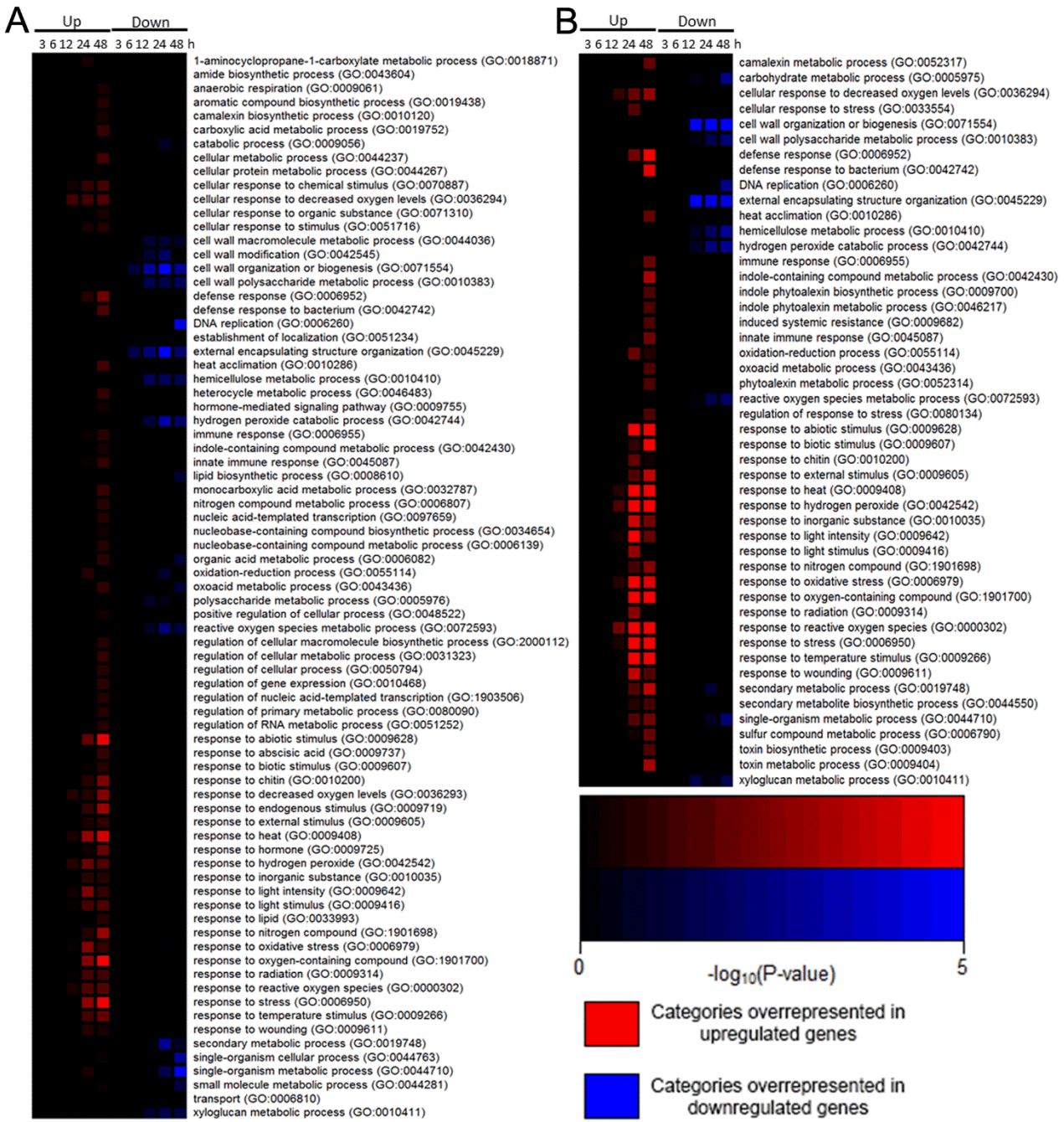
GO categories



log2-transformed FPKM (fragments per kilobase of transcript per million mapped reads) values

Gplots & RColorBrewer



Avirulent vs. mock

Virulent vs. mock

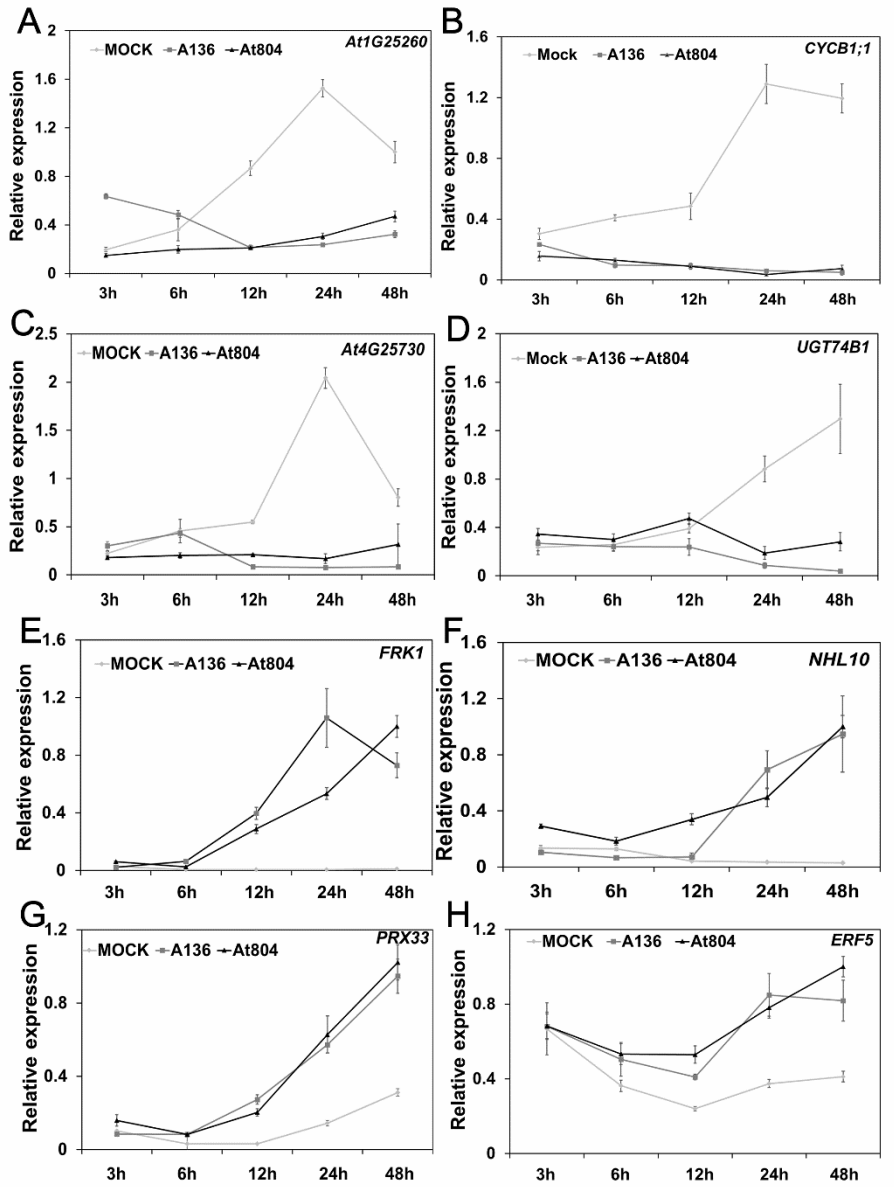
Validation of Transcripts by qRT-PCR

Ribosomal L10 family

DNA methyltransferase

FLG22-induced receptor-like kinase 1

PEROXIDASE 33



Cell cycle control

Cell wall organization and biogenesis

Arabidopsis NDR1/HIN1-LIKE 10

Ethylene-responsive transcription factor 5

A-D, growth and development

E-H, defense

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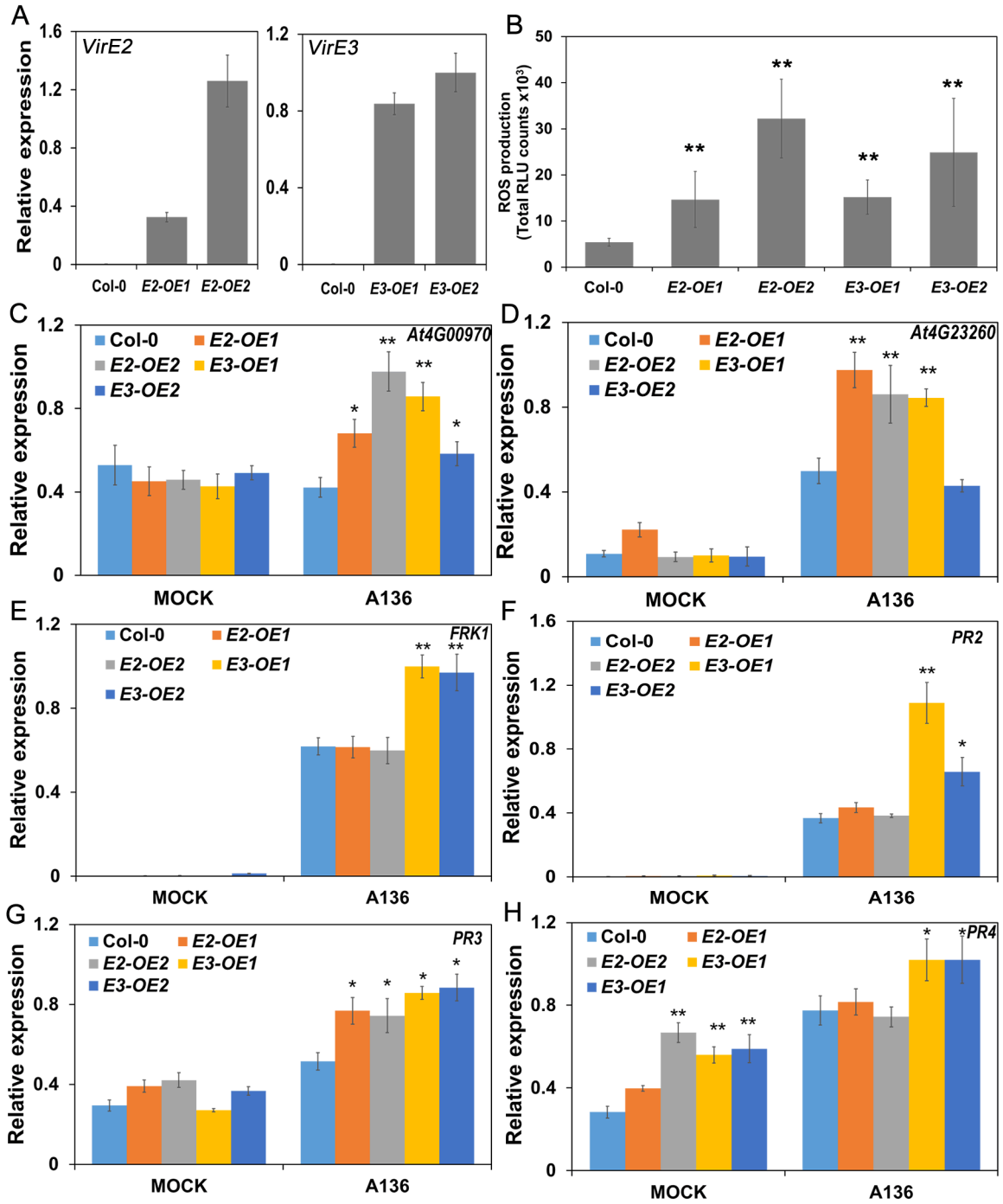
pEarleyGate202 for VirE2 and VirE3 Overexpression



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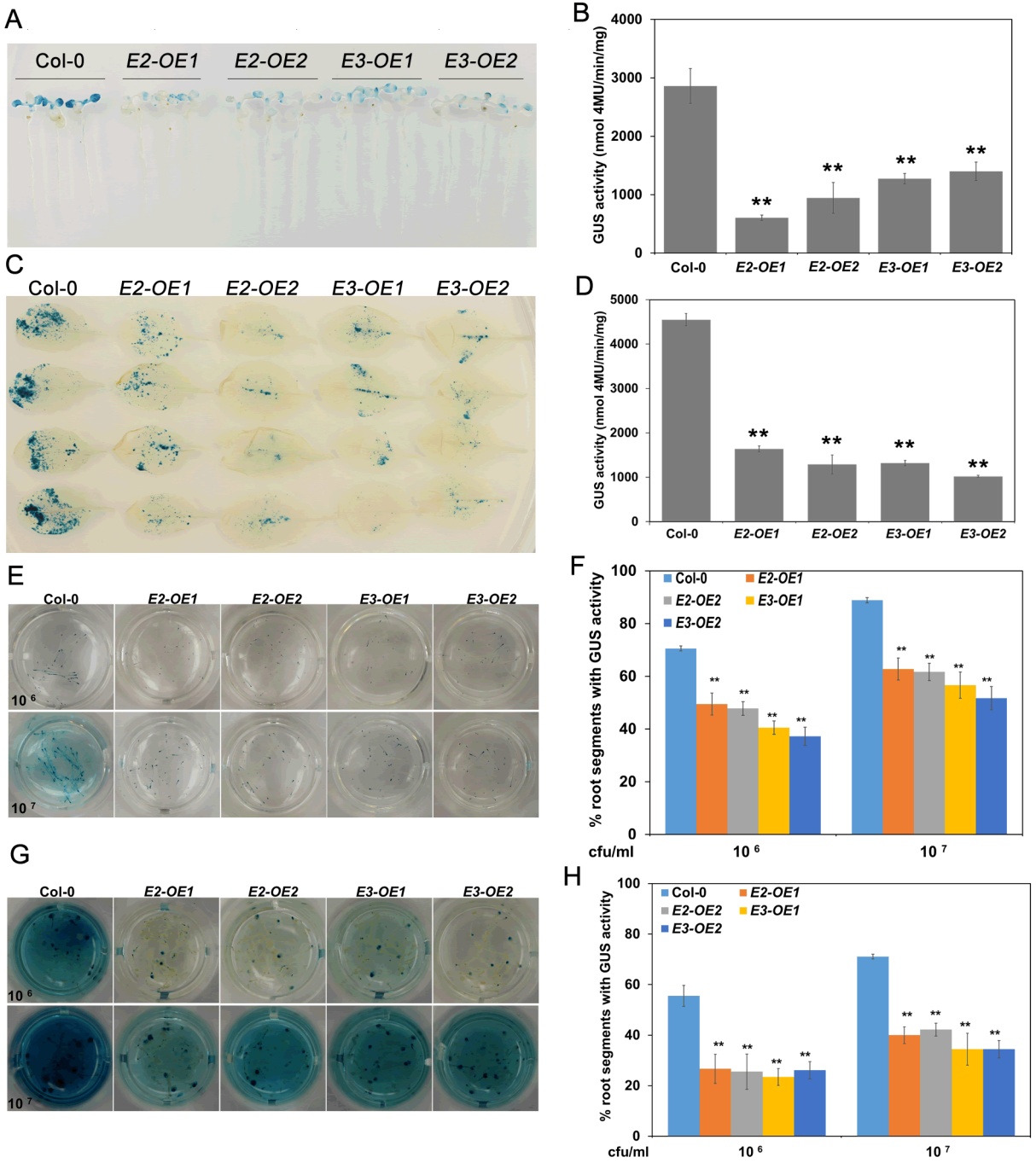
AGROBEST

Leaf infiltration

Root assay

Root stable transformation



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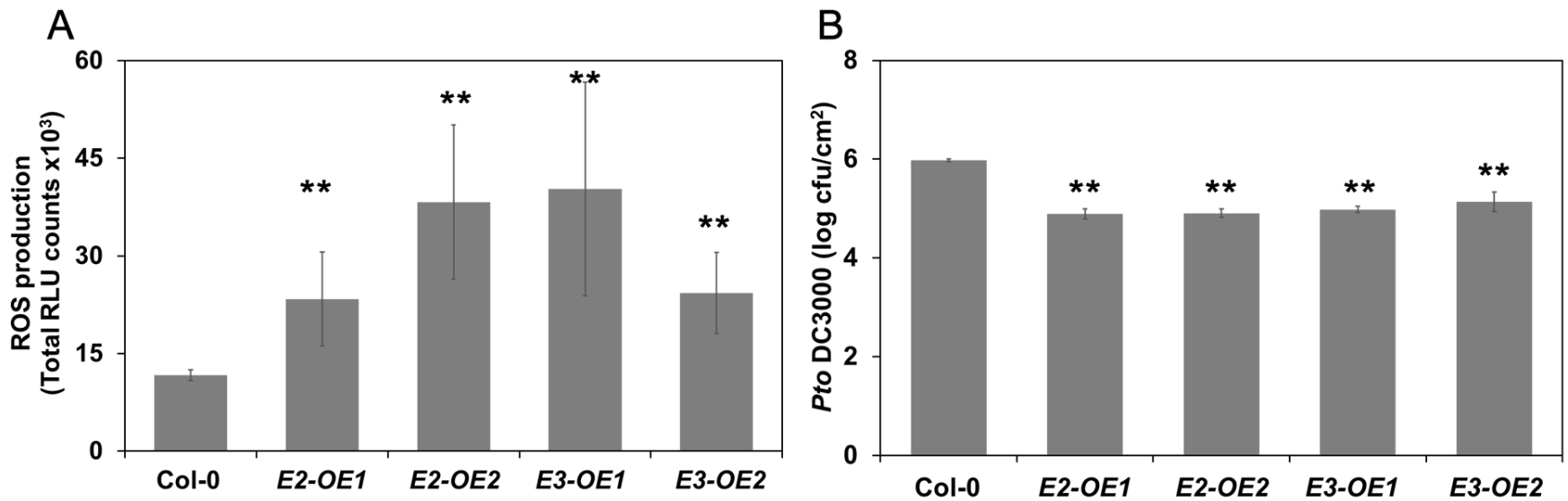
Responses of VirE2 and VirE3 overexpression lines to bacteria



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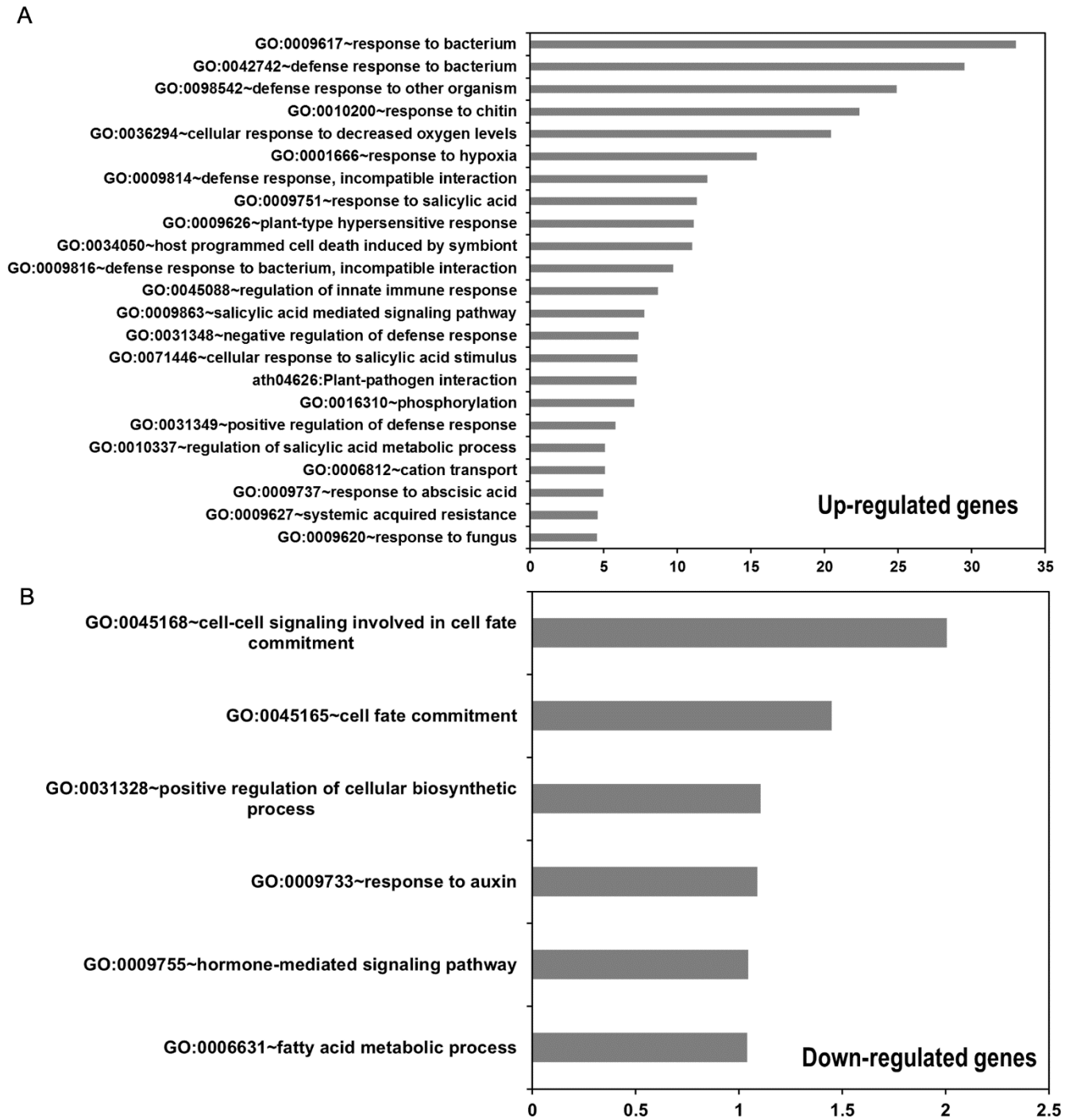
Responses of VirE3 Overexpression lines (Courtesy raw data from Niu et al., 2015)



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Conclusions

- We found a large number of plant genes in response to T-DNA transfer
- Growth and development gene expressions were repressed
- Defense response gene expressions were induced
- Vir proteins VirE2 and VirE3 may have additional role in enhancing defense
- Further study on individual genes and associated pathways are needed

Acknowledgements

MU Plant Transformation Core Facility

- Kaixuan Duan, Ph.D.
- Christopher Willig, Ph.D. student

MU Informatics Research Core Facility

- William G. Spollen, Ph.D.

MU DNA Core Facility

- Library Prep and RNA-Seq

Funding

- MU Research Board
- MU Life Sciences Fellowhsips

Plant Transformation Core Facility

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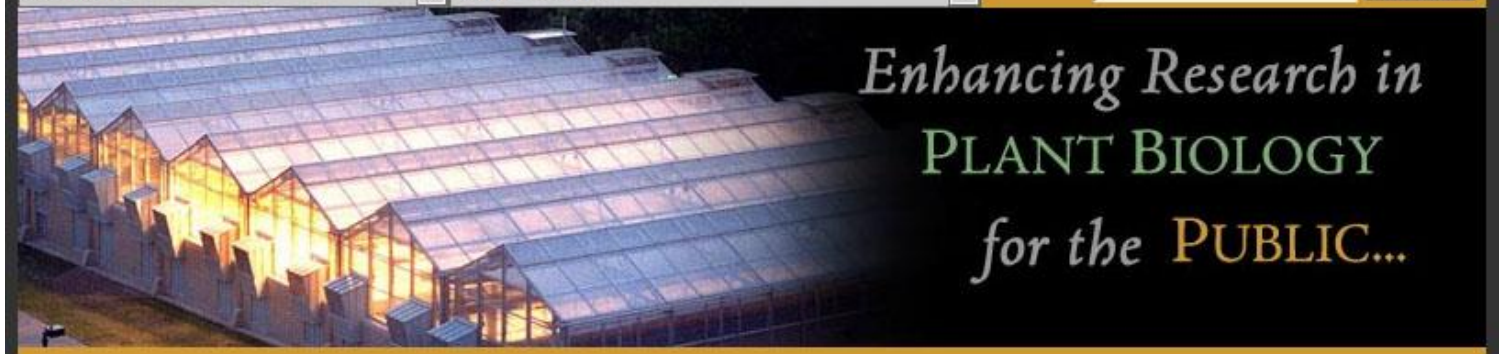

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Mission

The goal of the Plant Transformation Core Facility at the University of Missouri is to enhance both basic and applied research in plant biology for the public by providing plant transformation services.

The facility is also expected to foster funding opportunities and national prominence for various research groups through our efforts in generation of transgenic events. The Core's staff assists in developing transgenic approaches to address research questions.

We are making continued efforts in advancing transgenic technology to satisfy the needs of crop improvement and gene discovery.

As part of an educational role, the staff provides technical training in plant tissue culture and transformation for undergraduate and graduate students, post-doctoral researchers and other researchers.

Revised: January 17, 2014

Published by Division of Plant Sciences, 52 Agriculture Lab, Columbia, MO 65211 | zhangzh@missouri.edu | 573-882-6922.

Service Pricing

Stable Transformation Service:

Plant Species	Internal	External	Number of events
Glycine max	$1,560	$2,600	3-5
Zea mays inbred B104	$2,830	$4,065	3-5
Zea mays Hi-II	$2,240	$3,200	5-10
Sorghum bicolor	$2,420	$4,010	5-10
Triticum aestivum	$2,400	$3,660	5-10
Panicum virgatum	$1,650	$2,925	5-10

Listed price is for per construct. The number of independent transgenic events produced with each construct is indicated in the last column. Each event will be carried to T1 seeds except for switchgrass which is carried to plantlets in culture vessels. Events that are carried to T0 seeding or earlier culture stages will be charged at a lower price.

For soybean (*Glycine max*) and maize inbred B104, a minimum of 3 events will be required for the service.

For remaining crop species, at least 5 events will be required for the service.

To request more events than 3-5 or 5-10, simply multiply the price by number of events needed to get an estimate of the cost.

Please contact Zhanyuan Zhang if you have questions regarding our pricing.

Revised: September 9, 2016

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Thanks for Your Attention!