Early Infection Transcriptome Analysis of *Globodera pallida* Infected in the Susceptible *Solanum tuberosum* and Resistant *Solanum sisymbriifolium*

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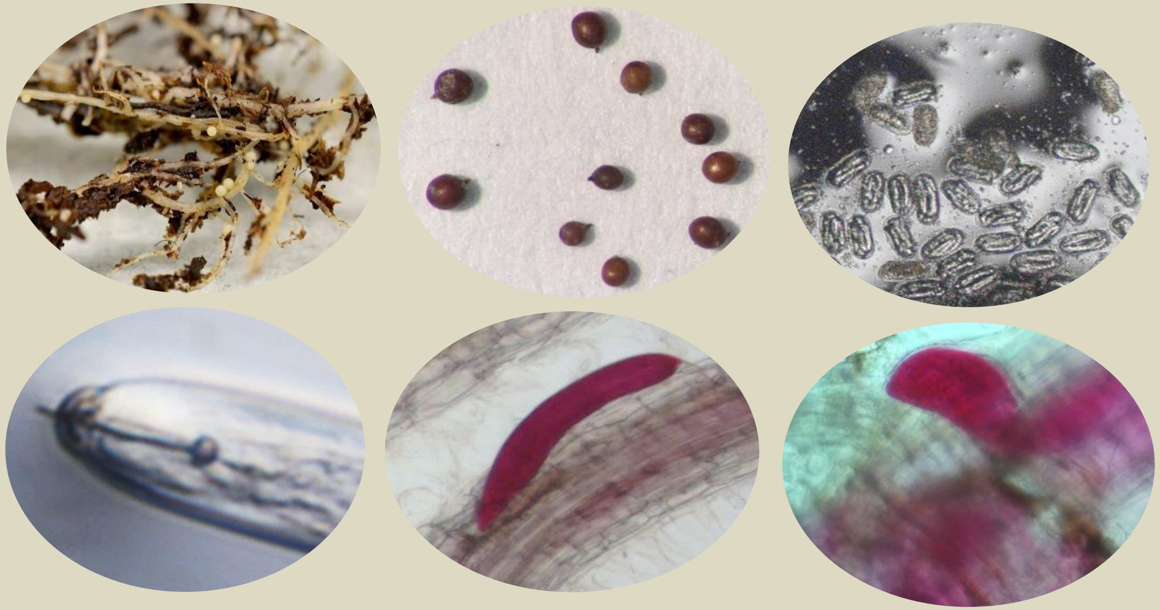
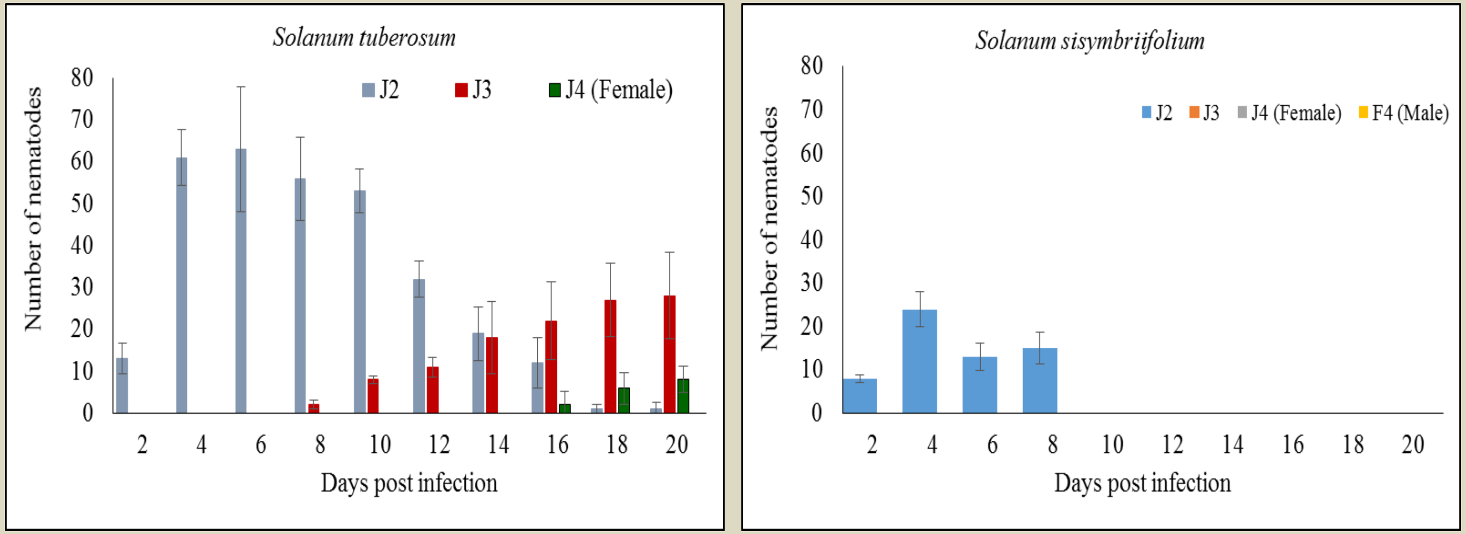
Globodera pallida (Pale Cyst Nematode, PCN)



- One of the most economically important pests of potato, causing in excess of 80% yield loss.
- First detected in the United States in 2006 in Idaho, and is regulated by both USDA-APHIS and the Idaho State Department of Agriculture.

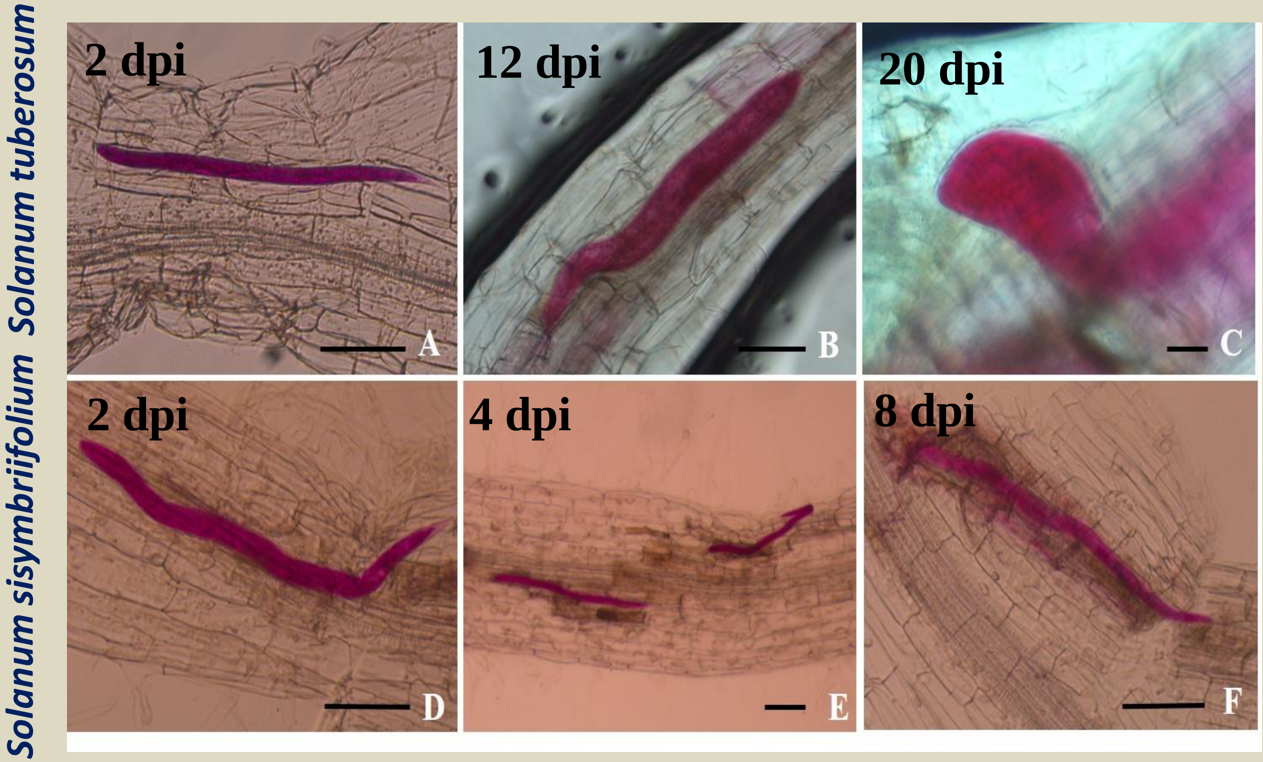
Solanum sisymbriifolium

- **Non-tuber-bearing solanaceous plant.**
- **Promising trap crop for the control of *G. pallida.***



Immune response in *S. sisymbriifolium*

Immune response in *S. sisymbriifolium*



Scale bar = 50 µM

Kooliyottil *et al.* (2016) Advances and Bioscience and Biotechnology 7, 311-318.

Objective: To study the transcriptome profile of *G. pallida* infected in *S. tuberosum* and *S. sisymbriifolium* at early infection stage

- **Infected nematodes were collected from *S. sisymbriifolium* and *S. tubersoum* roots at 24 hours post infestation and frozen in liquid nitrogen and stored at -80 °C.**

Single root inoculation prototype

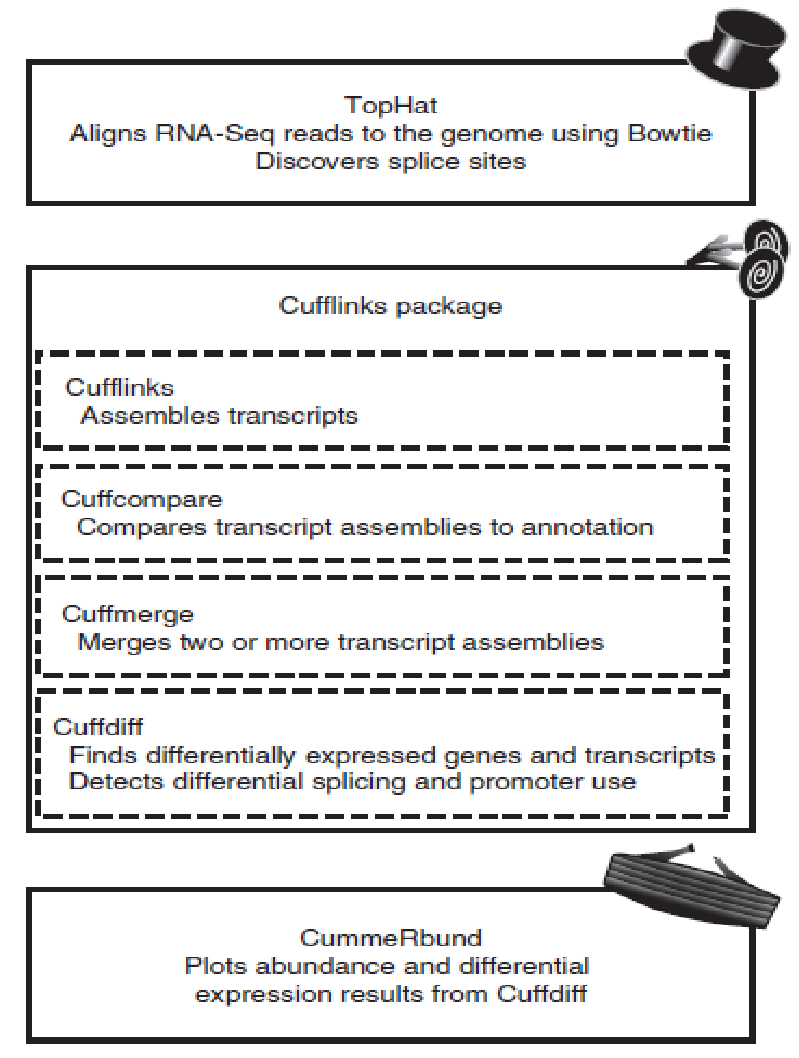
- **~ 150 J2s were collected from *S. tuberosum*, whereas, the number of J2s in *S. sisymbriifolium* were ~ 30-50.**

- **RNA was isolated and the RNA seq libraries were prepared using TruSeq® Stranded mRNA NeoPrep™ Kit (Illumina) and sequenced on Illumina HiSeq 4000 (100 bp, paired end) (UC Berkeley Sequencing facility).**

- **FAST QC for quality control**
- **Trimmomatic for read trimming to delete all the adapters and primer sequences**

Tuxedo Suite

Trapnell *et al.* (2012). Nature Protocols 7, 562–578



TopHat
Aligns RNA-Seq reads to the genome using Bowtie
Discovers splice sites

Cufflinks package

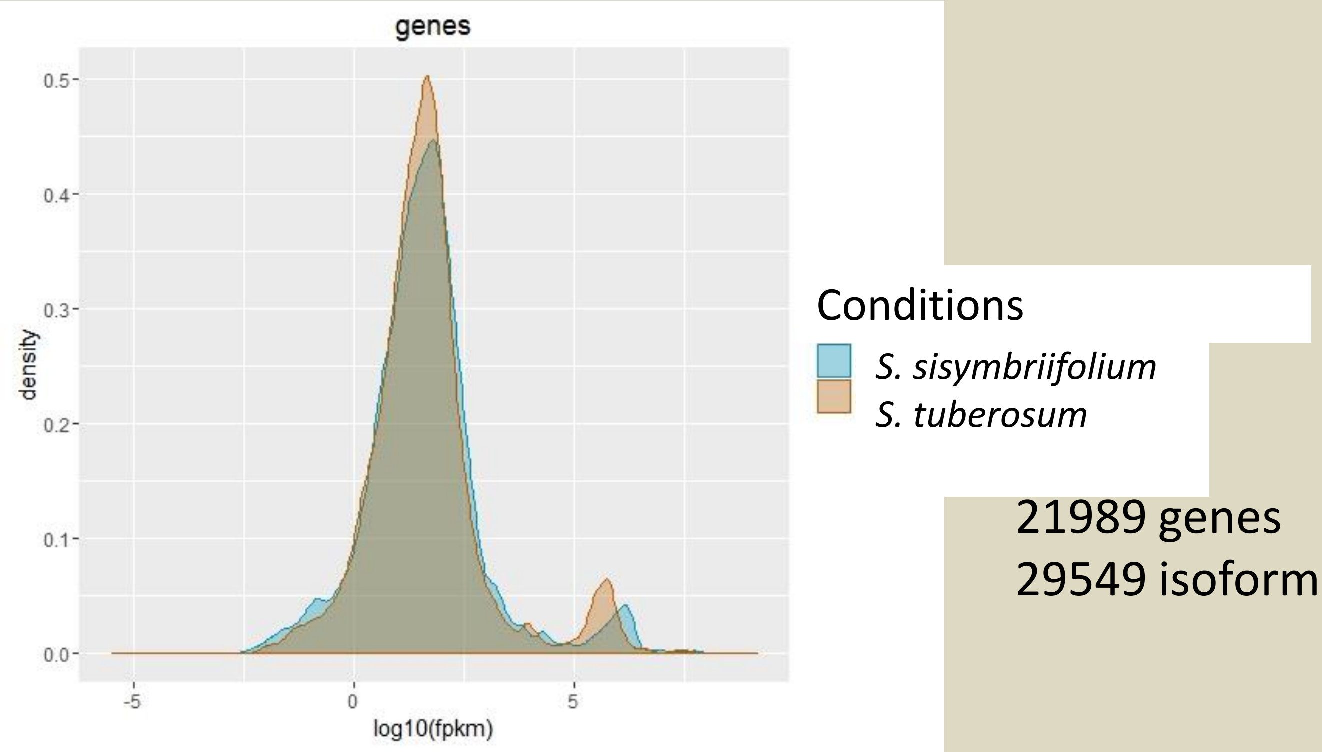
Cufflinks
Assembles transcripts

Cuffcompare
Compares transcript assemblies to annotation

Cuffmerge
Merges two or more transcript assemblies

Cuffdiff
Finds differentially expressed genes and transcripts
Detects differential splicing and promoter use

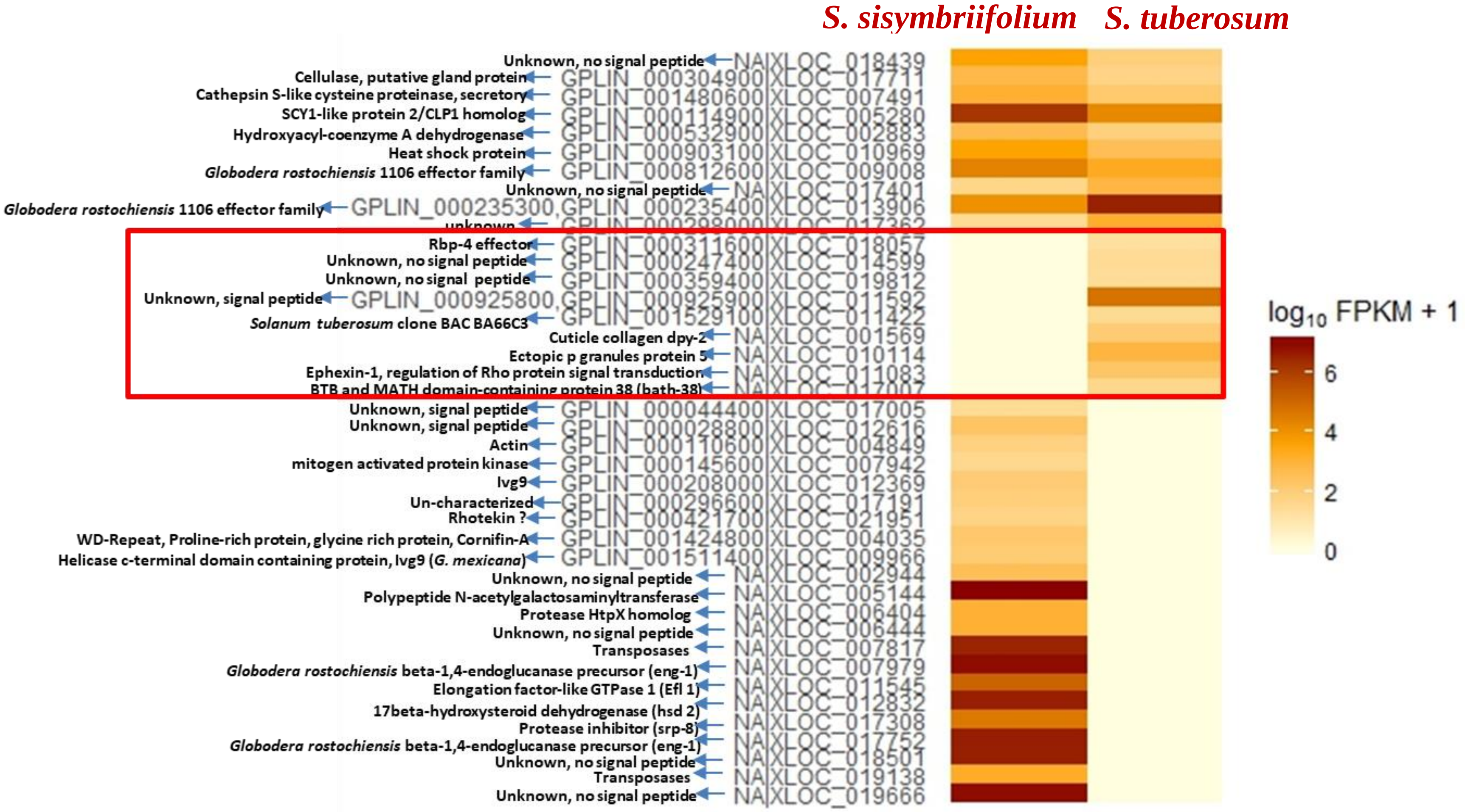
CummeRbund
Plots abundance and differential expression results from Cuffdiff



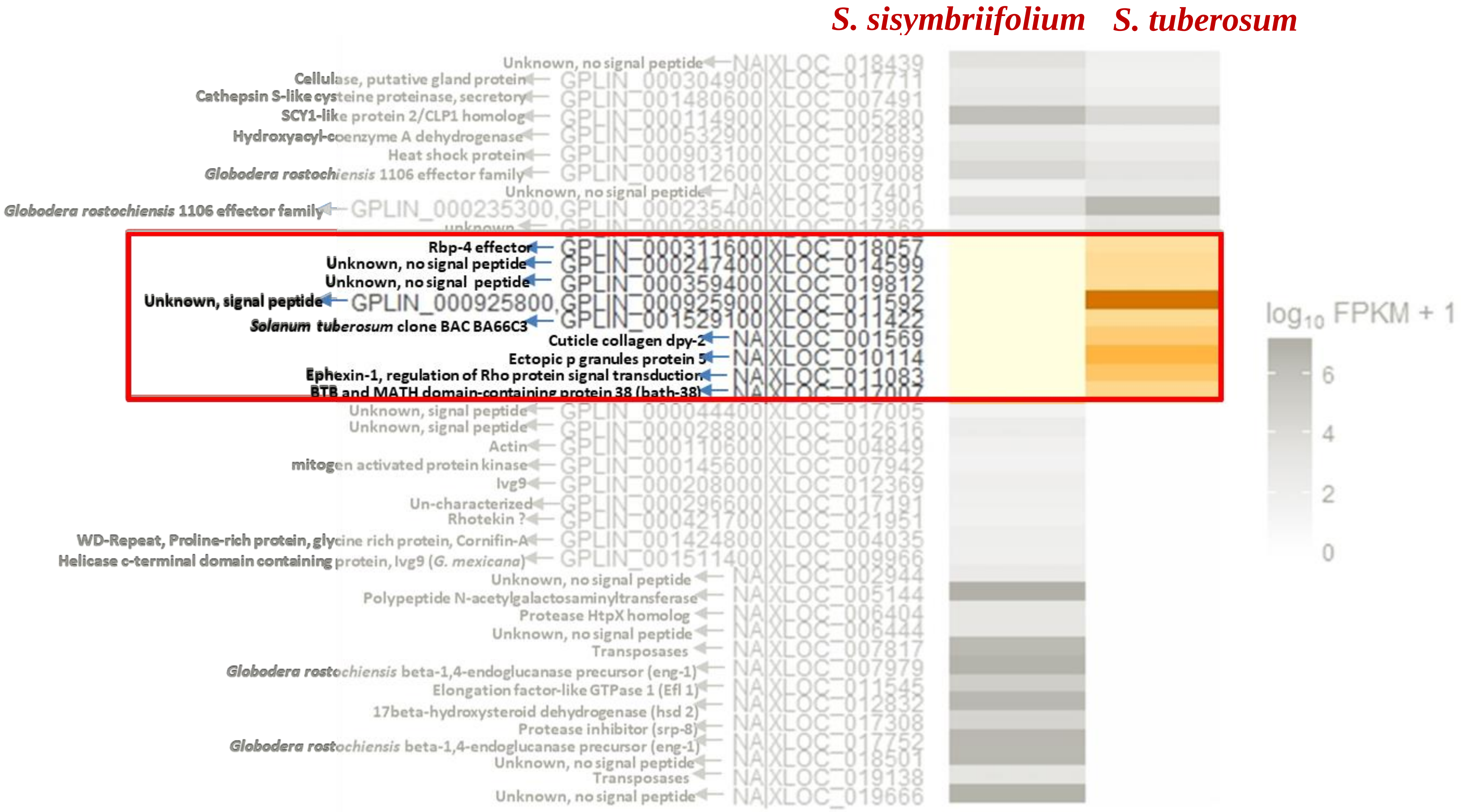
21989 genes
29549 isoforms

Density plot: Distribution of expression values for the transcripts
* FPKM: Fragments per kilobase of exons per million fragments mapped

Heatmap of significantly differentially expressed genes



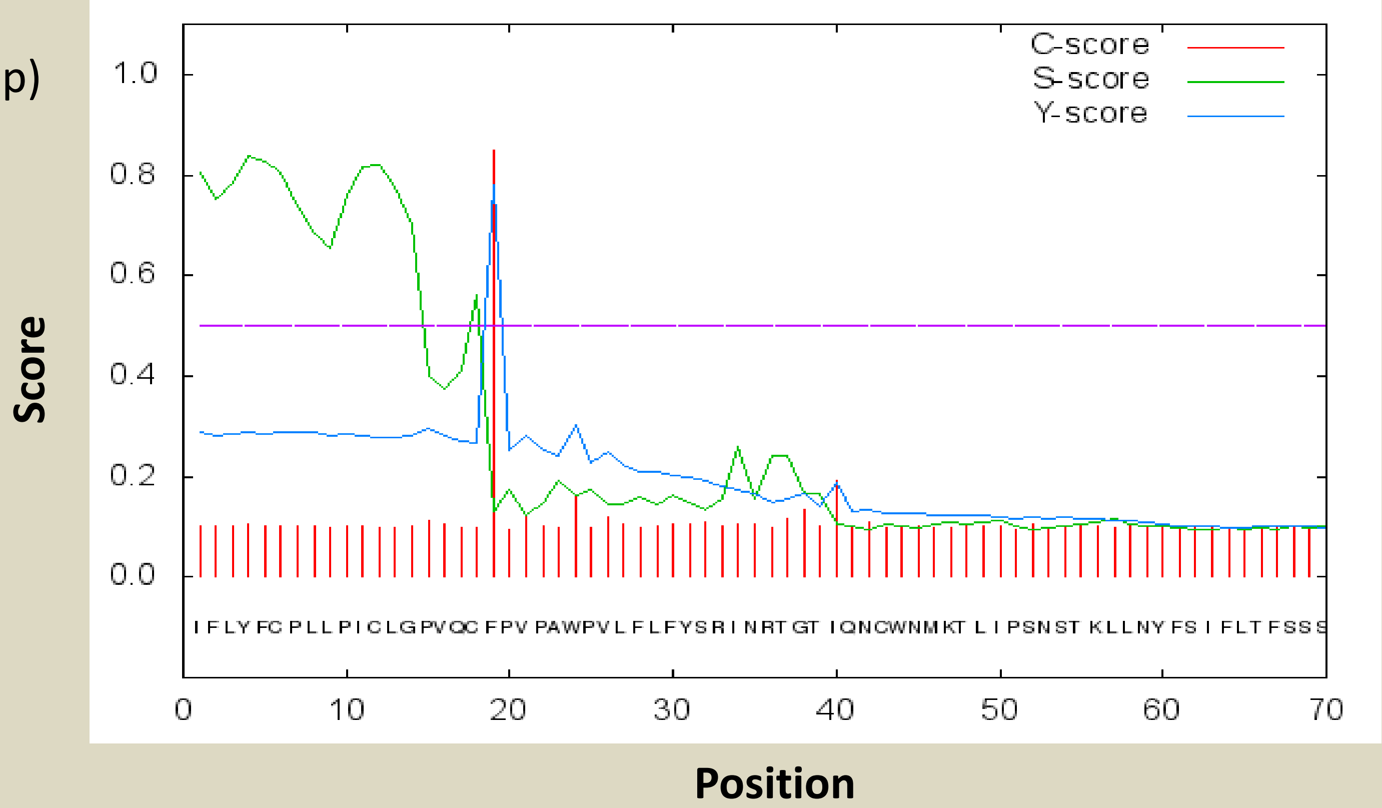
Heatmap of significantly differentially expressed genes



Rbp-4 effector

Retinol binding proteins (Rbp)

SignalP-4.1 prediction (euk networks): Sequence



Postma *et al.* (2012) Plant Physiol 160, 944-954.
Jones et al. (2009 Mol Plant Pathol. 10 (6) 815-828.
Sacco *et al.* (2009) PLoS Pathogens 5 (8) e1000564.

Cuticle collagen dpy-2

Mutations in individual cuticle collagen genes can cause exoskeletal defects that alter the shape of the animal

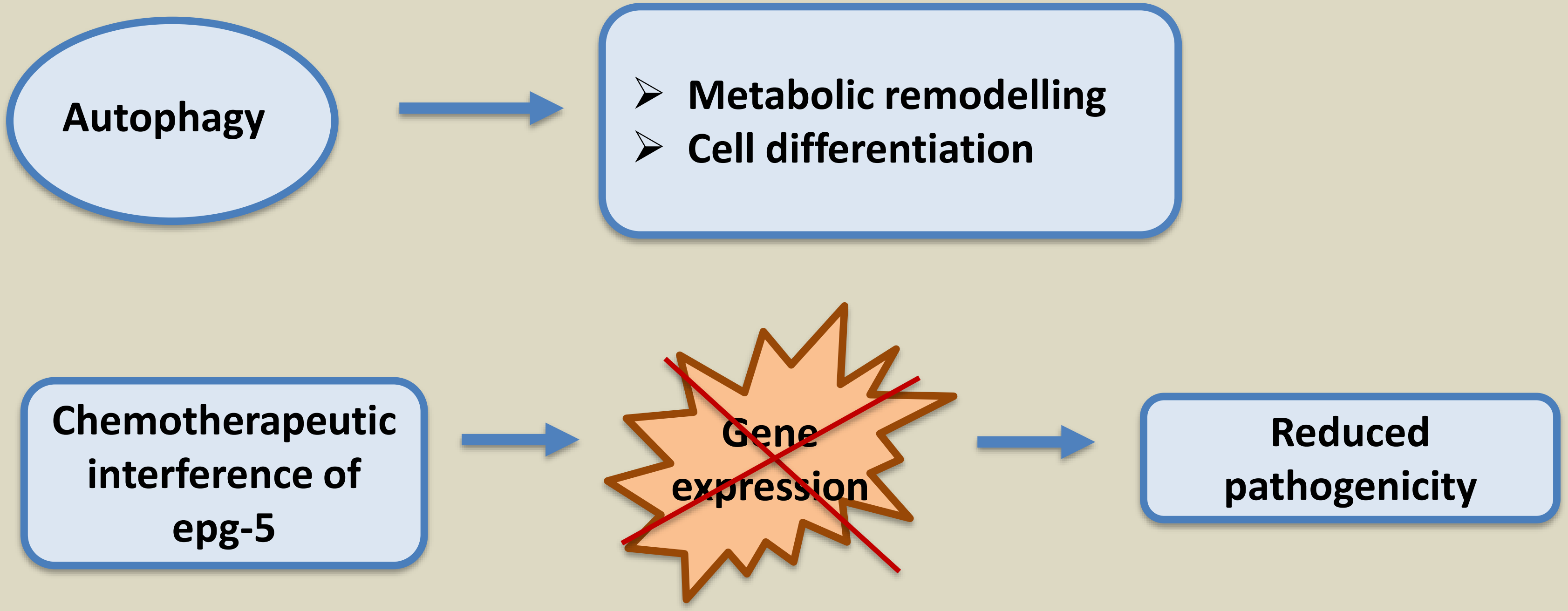
Cuticle collagen dpy-2

Encodes collagen

Important in morphogenesis and other developmental events

Johnstone IL (2000) Trends in Genetics16(1):21-7.

Ectopic p granules protein-5 (epg-5)



Autophagy

- ➢ Metabolic remodelling
- ➢ Cell differentiation

Chemotherapeutic interference of epg-5

~~Gene expression~~

Reduced pathogenicity

Brennand *et al.* (2011). Molecular and Biochemical Parasitology 177 (2) 83–99.

BTB and MATH domain-containing protein 38 (bath-38)

- **Mediate protein–protein interactions.**
- **Associated with signal transduction, cell-cycle regulation.**
- **Play an important role in nematode immunity.**

Srinivasan *et al.* (2013) Genetics 193(4): 1279–1295.

Metabolic pathways associated with expressed genes

Secretory proteins		Uncharacterized proteins with secretory functions		Immunity and defense	
Cellulase, putative gland protein	▲🔻	GPLIN_000044400	▲🔻	Clp-1	▲🔻
Cathepsin S-like cysteine protease	▲🔻	GPLIN_000028880	▲🔻	CTSS	▲🔻
Eng-1	▲🔻	GPLIN_000925800	🔺▼	Exn-1	▲🔻
Ivg-9	▲🔻			bath-38	▲🔻
G. rostochiensis 1106 effector	🔺▼				
Rbp-4	🔺▼				

▲ Upregulated in *S. sisymbriifolium* ▼ Downregulated in *S. sisymbriifolium*
🔺 Upregulated in *S. tuberosum* 🔻 Downregulated in *S. tuberosum*

Conclusion

- ***Globodera pallida*** **infected in** ***S. sisymbriifolium*** **have 12 genes completely turned off.**

- **Downregulation of parasitism related genes including effector genes namely, Rbp-4 homologue and** ***Globodera rostochiensis*** **1106 effector family ortholog in the** ***G. pallida*** **infected** ***S. sisymbriifolium.***

- **High expression of immunity and defense genes when** ***G. pallida*** **was infected in** ***S. sisymbriifolium*****. This attributes towards the unsuccessful effort of** ***G. pallida*** **to overcome the resistance of the plant.**

Acknowledgements

- USDA-NIFA
- USDA-APHIS
- Northwest Potato research Consortium
- Idaho Potato Commission

PCN Team, University of Idaho

Thank you