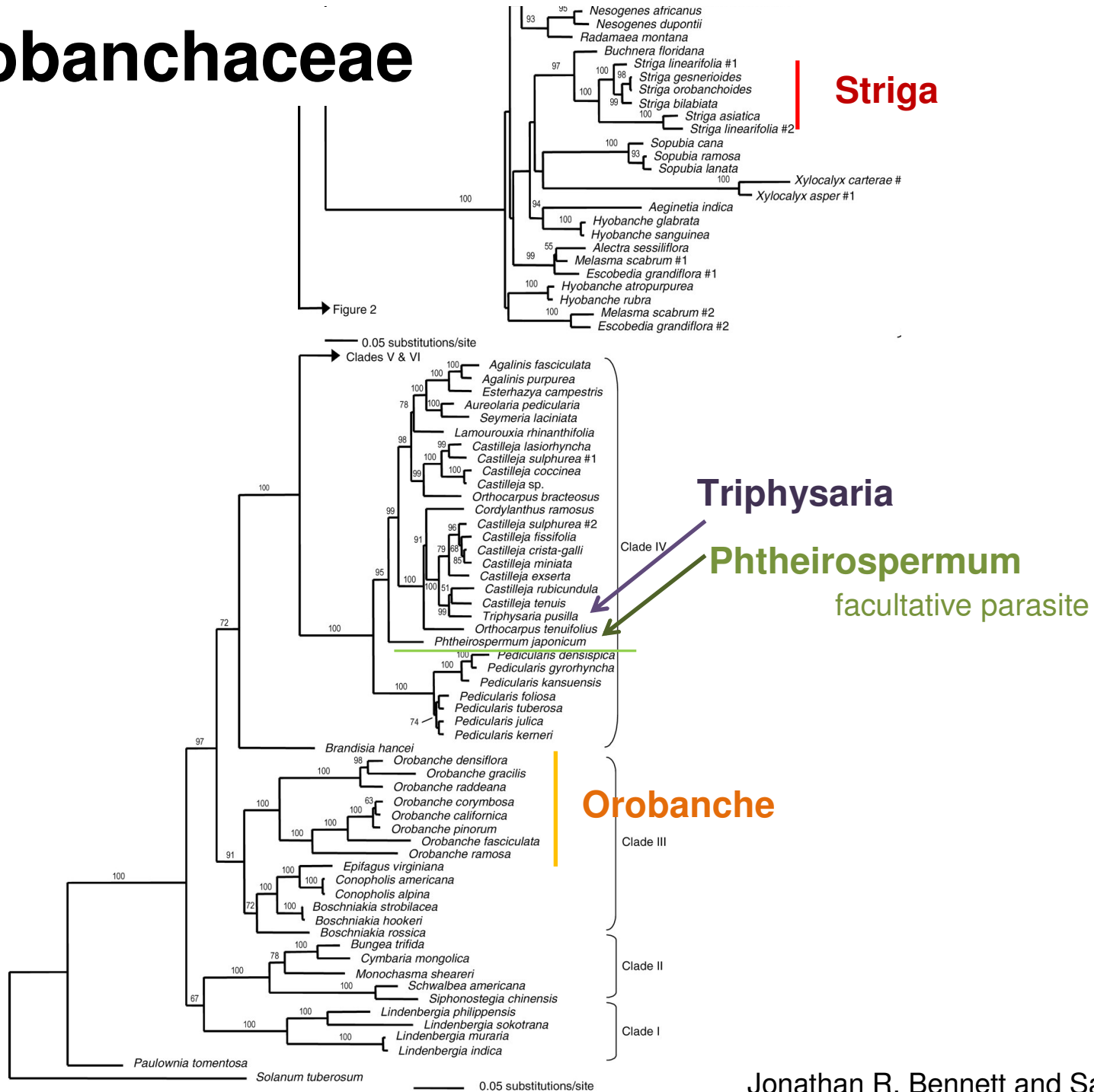


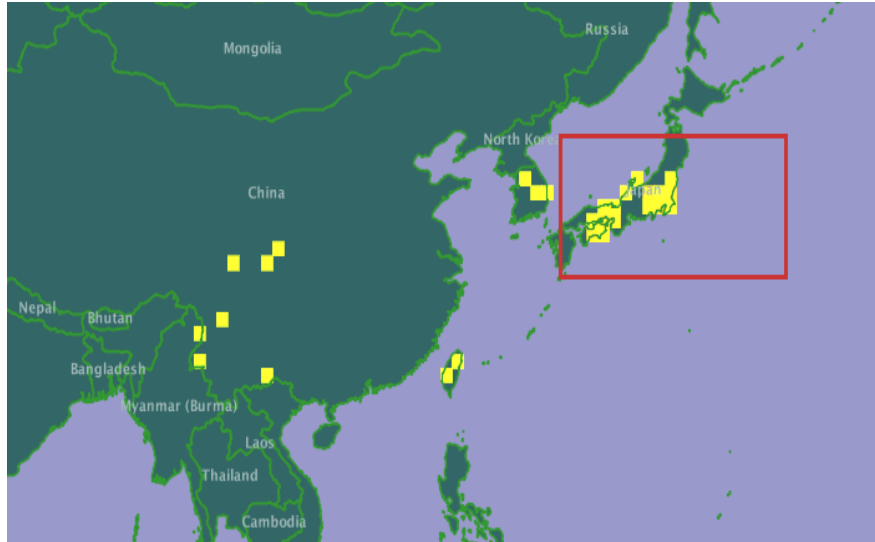


Transcriptome analysis of *Phtheirospermum japonicum*

Orobanchaceae



Diversity of *P. japonicum*

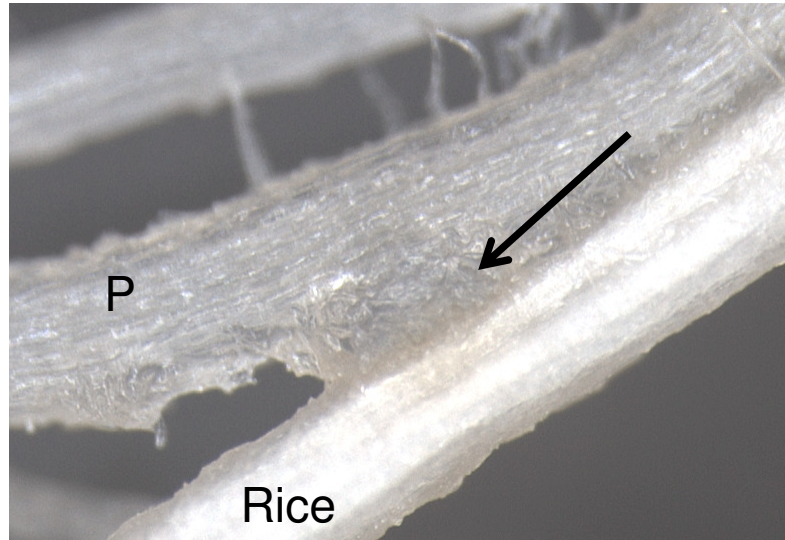


Beta: GBIF Open Geospatial Consortium services

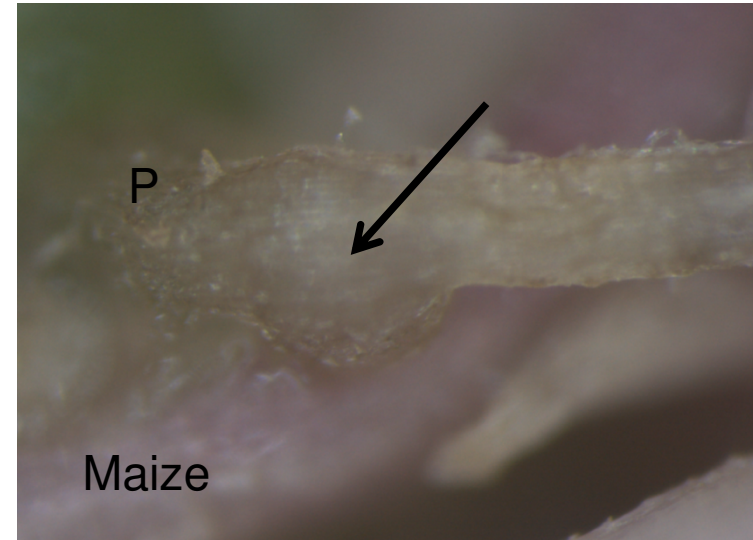


P. japonicum infects a wide range of plants

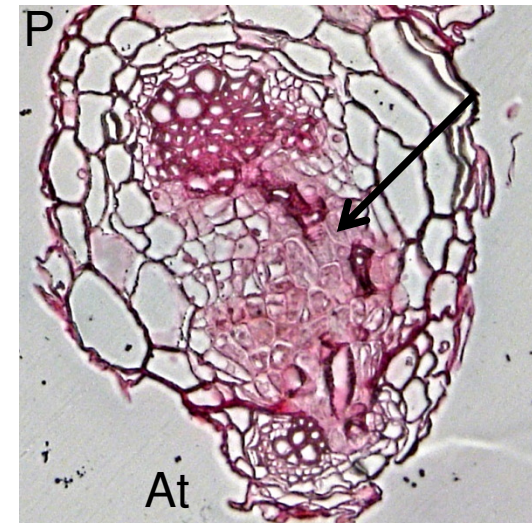
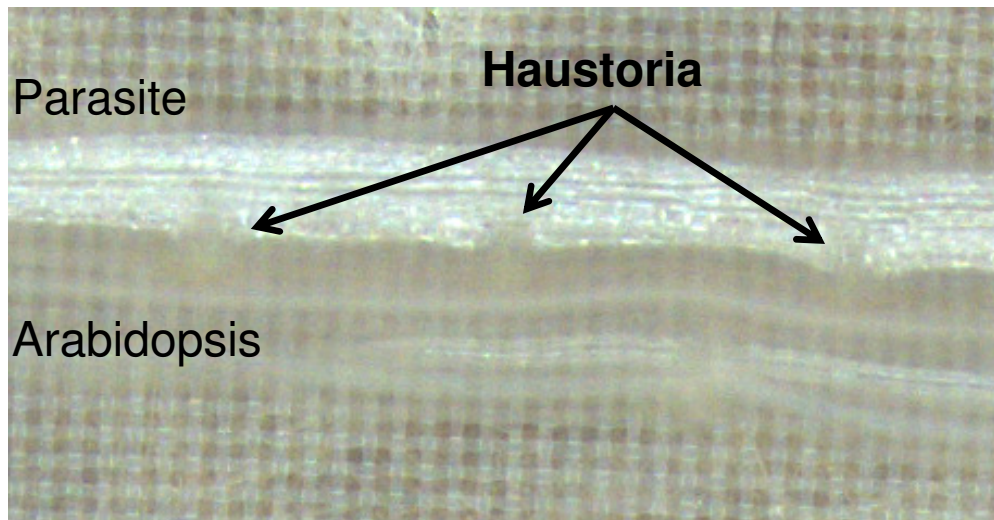
P. japonicum vs Rice



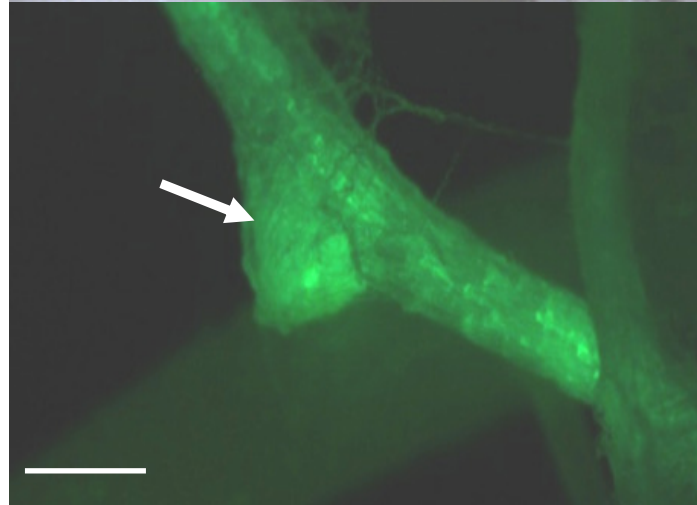
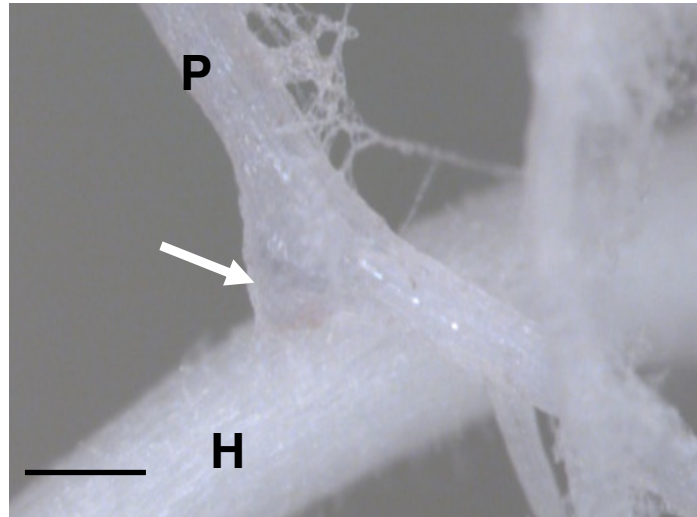
P. japonicum vs Maize



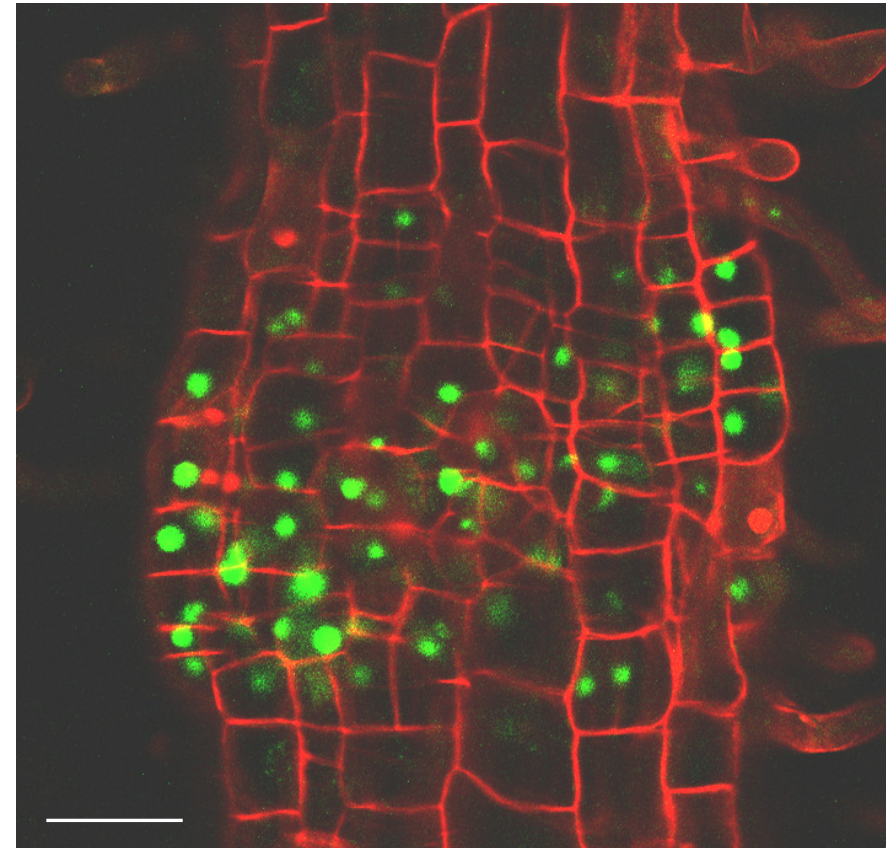
P. japonicum vs Arabidopsis



A. rhizogenes-mediated transformation of *Phtheirospermum japonicum*



Bar 250 μ m



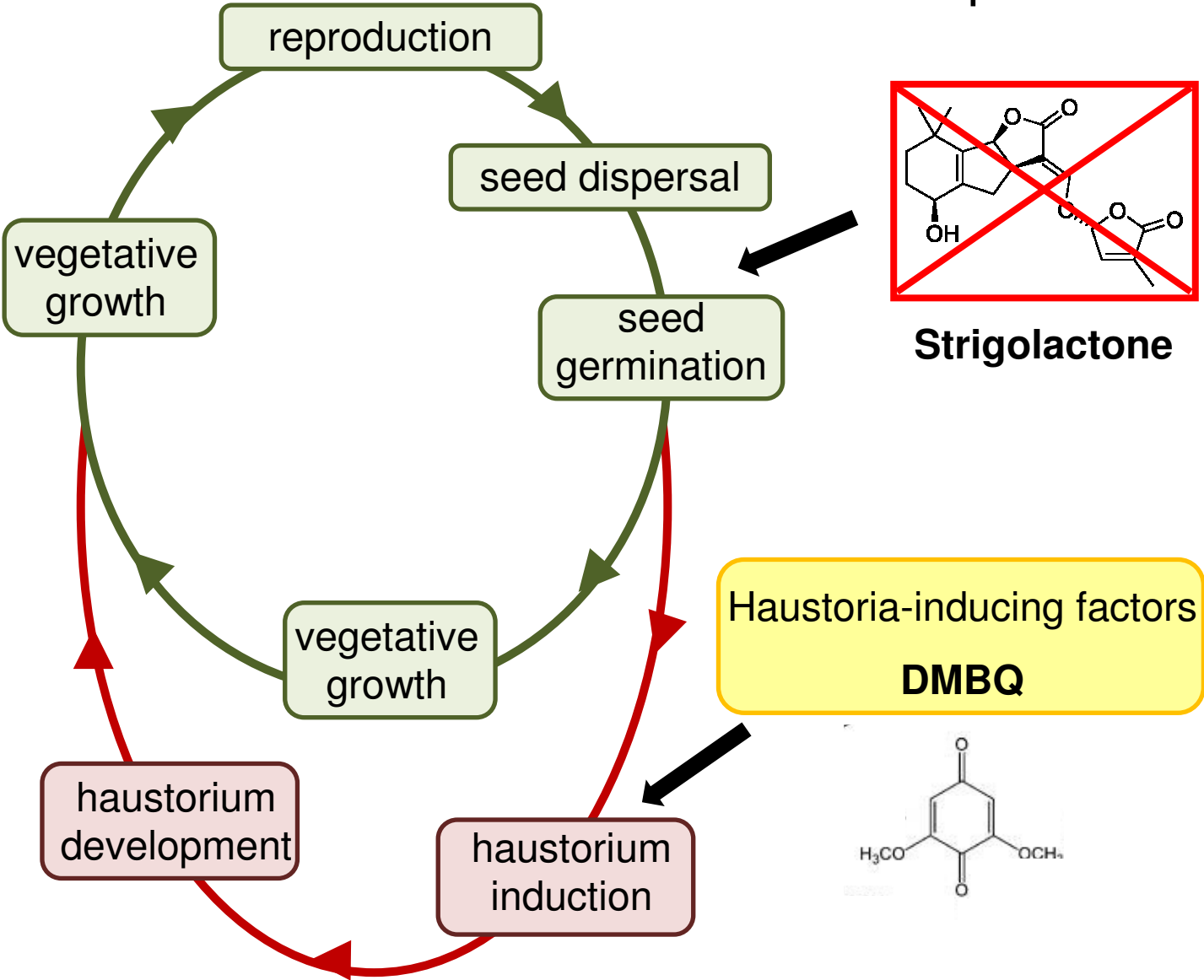
Bar 50 μ m



Phtheirospermum japonicum

Facultative parasite

Free living plant



Parasite

Modified from Westwood JH et al, 2010

GOAL

Identification of genes required for parasitism

RNA-seq

- Detection of parasite genes expressed during parasitism.

RNA - seq

Parasiting



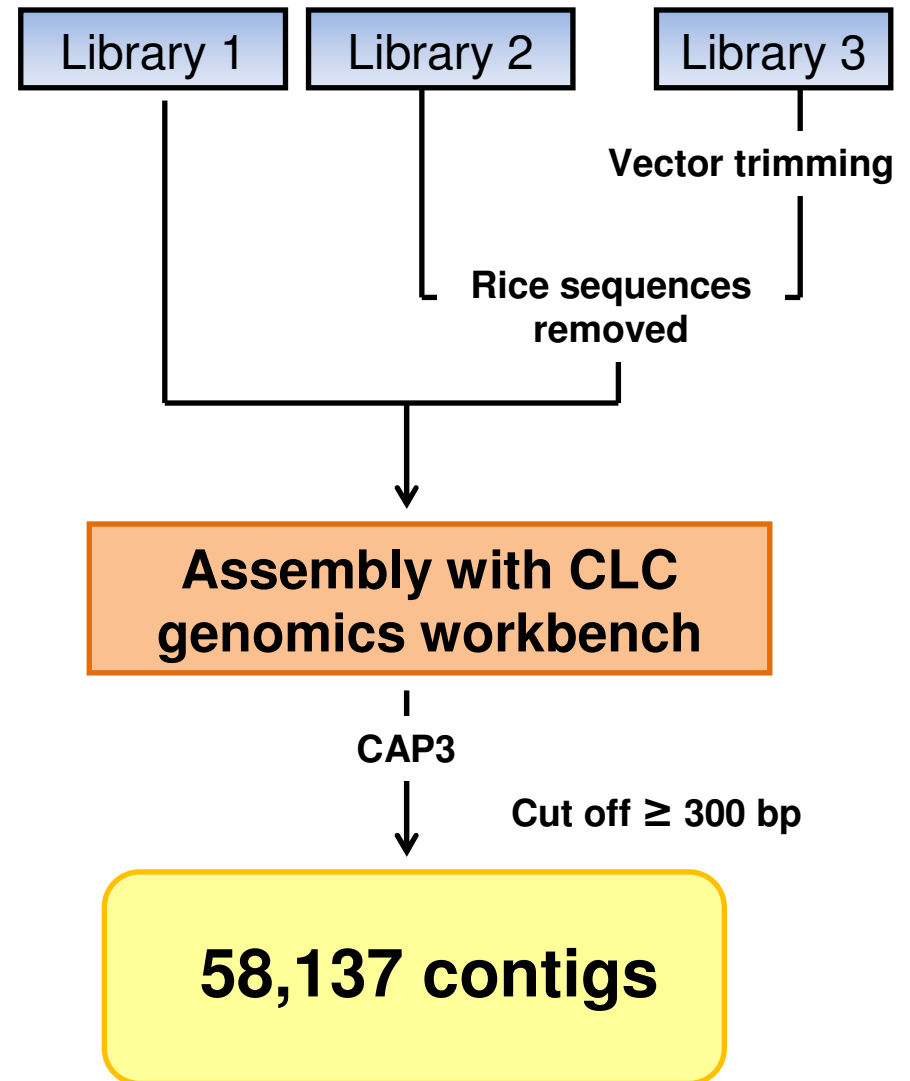
Free-living



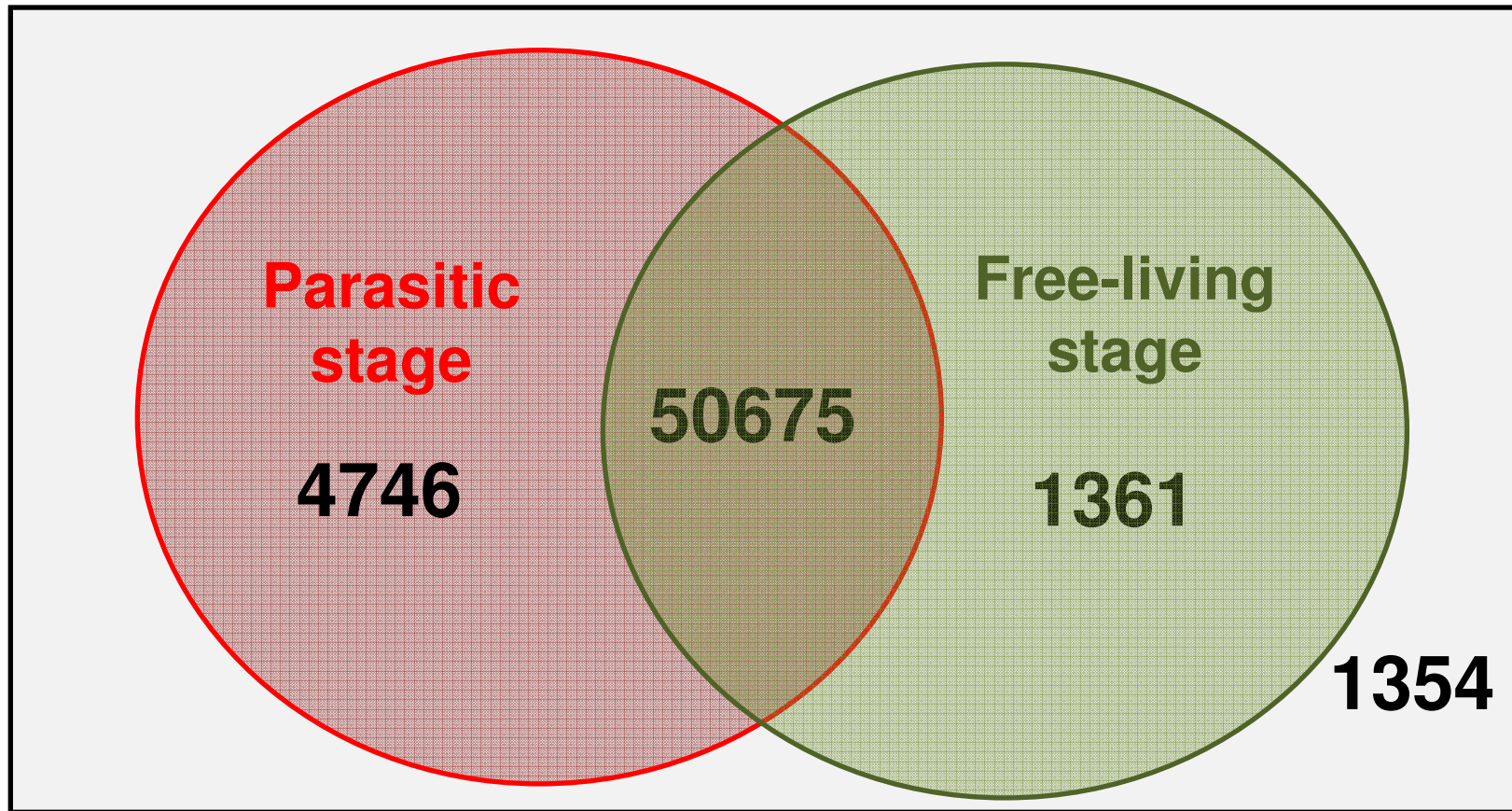
Library 3
Mixed/normalized
Roche 454 FLX

Library 1
Illumina HiSeq2000

Library 2
Illumina HiSeq2000



4746 sequences were exclusively found in parasitic stage

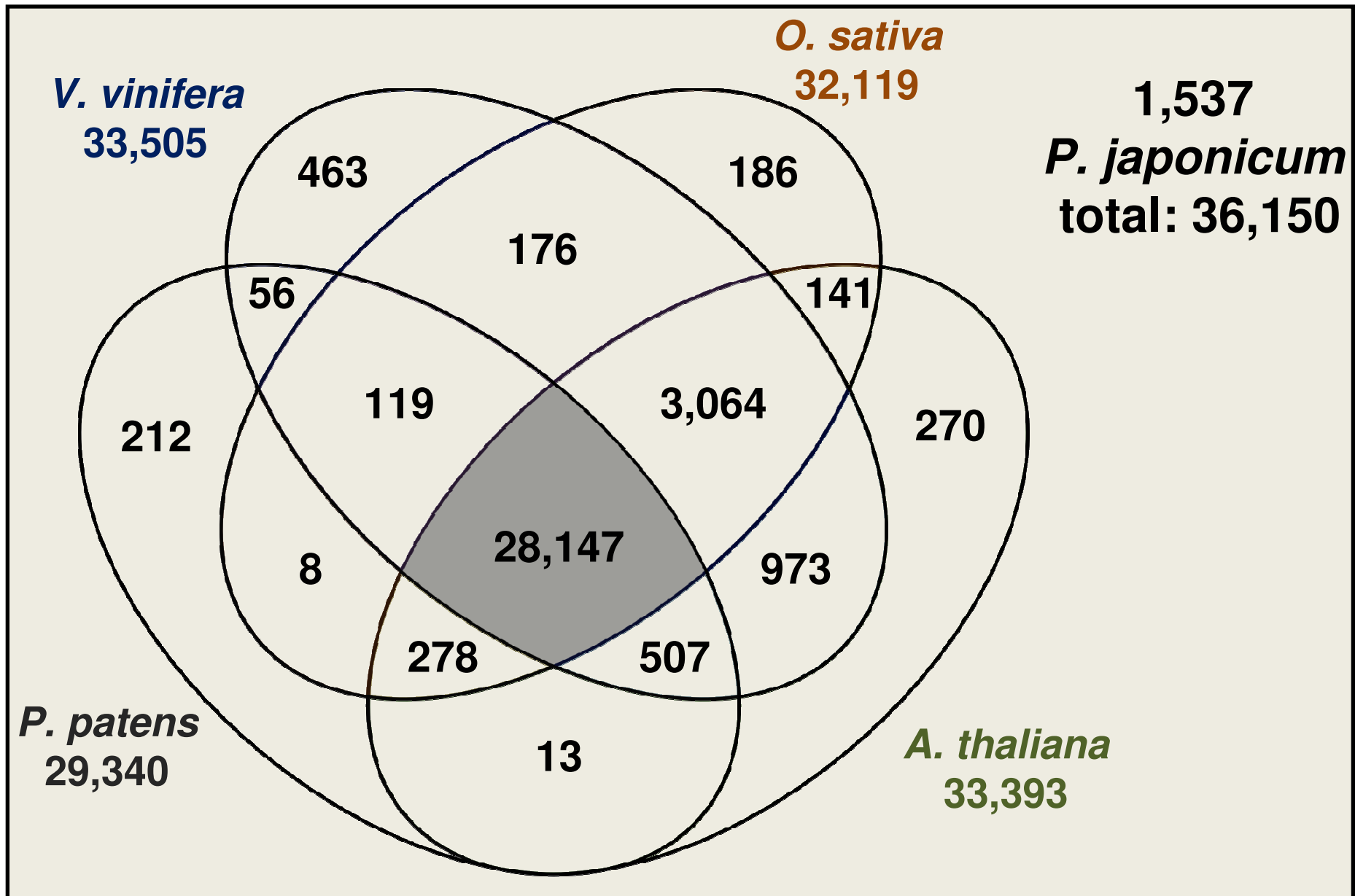


Total 58137

Highly expressed genes in parasitic tissues

Expression level (RPKM)	Description
247.54	Subtilisin-like serine protease
119.301	***** No hits found *****
105.672	Cucumisin, putative
95.456	Subtilisin-like serine protease
91.787	Subtilisin-type protease
64.026	Disease resistance response protein
57.016	Putative pre-pro-subtilisin
52.314	GTP-binding protein EsdC
47.635	Subtilisin-type protease
46.53	Subtilisin-like serine protease
43.422	Isoform 2 of Serine carboxypeptidase-like
42.091	***** No hits found *****
36.016	Serine carboxypeptidase, putative

Orthologous



GOAL

RNA-seq

- Identify parasite genes expressed during parasitism.

Microarray

- Identify parasite genes expressed in the beginning of haustorium development.

Identify parasite genes transcriptionally regulated by DMBQ

58 137 contigs
length > 300bp

+

15 560 contigs
250bp < length < 300bp



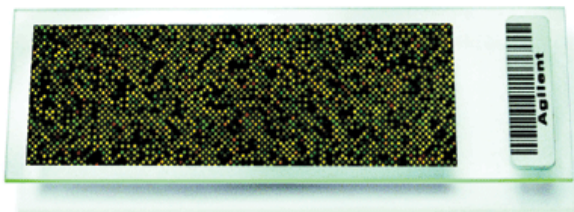
ESTScan
to detect coding region

65 163

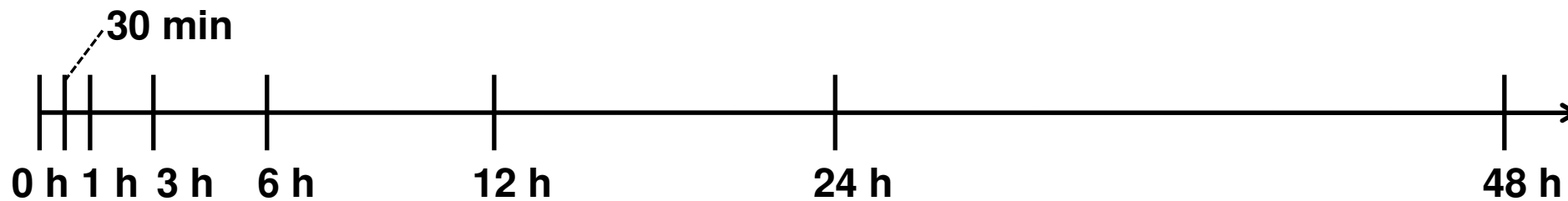


putative translated region
with a minimum size of 50 aa

58 363



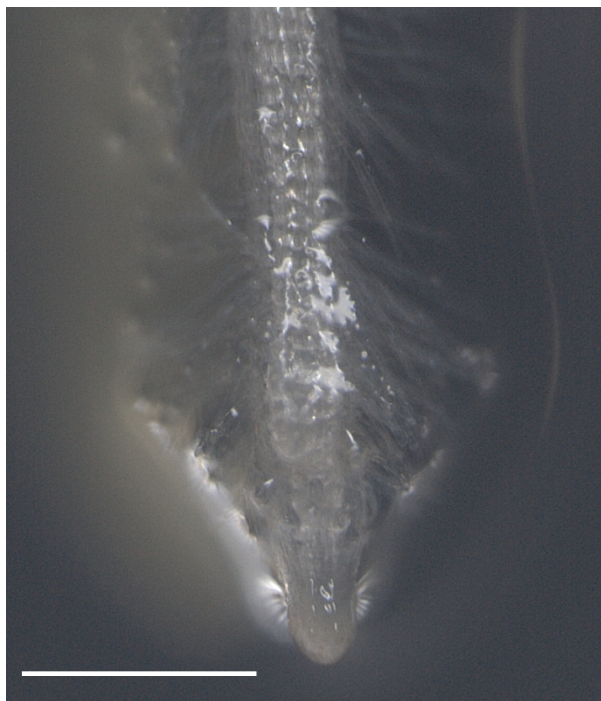
DMBQ treatment



0 h



12 h

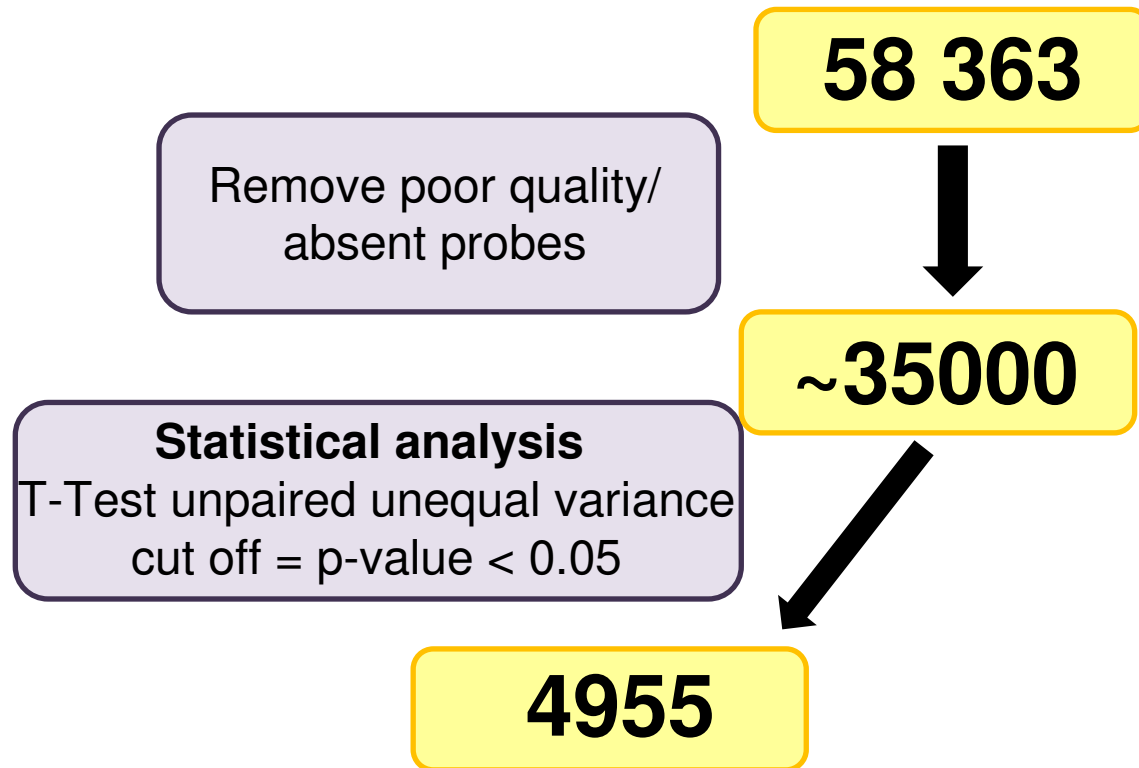


48 h

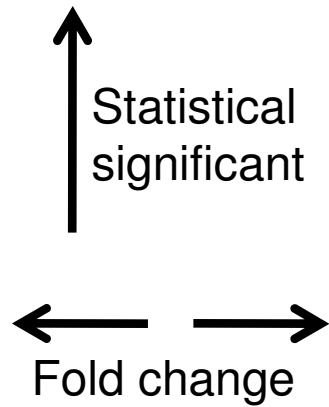


Bar = 500 μ m

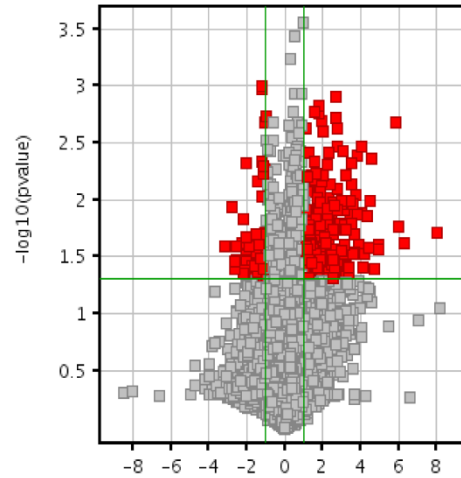
Identification of parasite genes



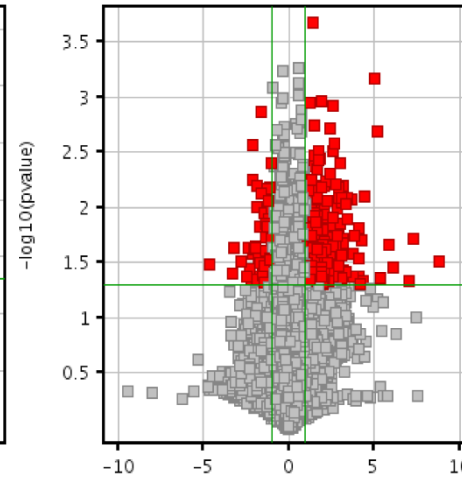
Differentially expressed genes



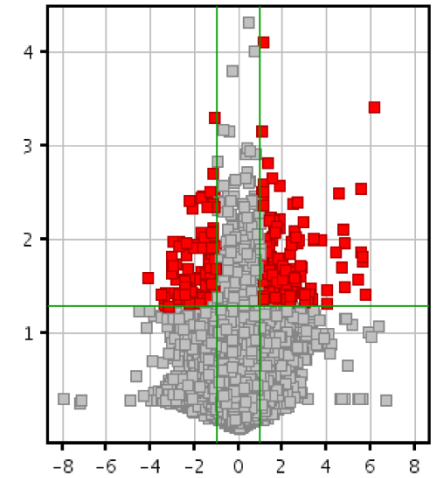
30 min vs 0 h



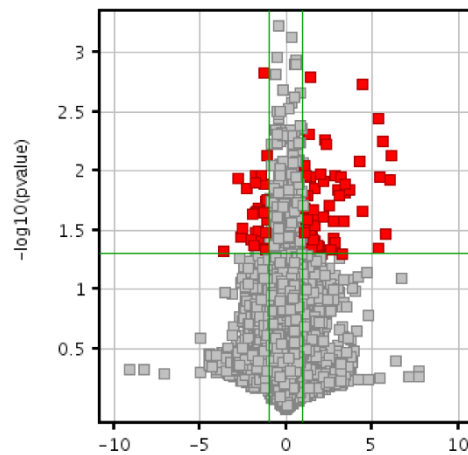
1 h vs 0 h



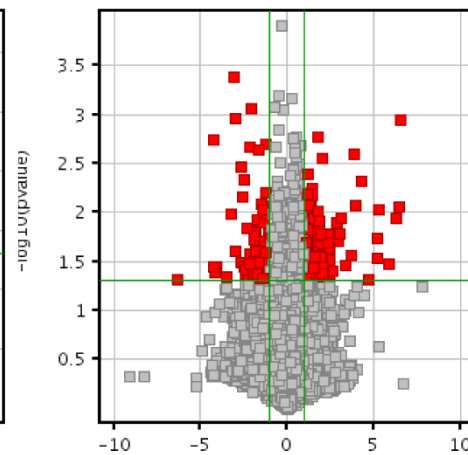
3 h vs 0 h



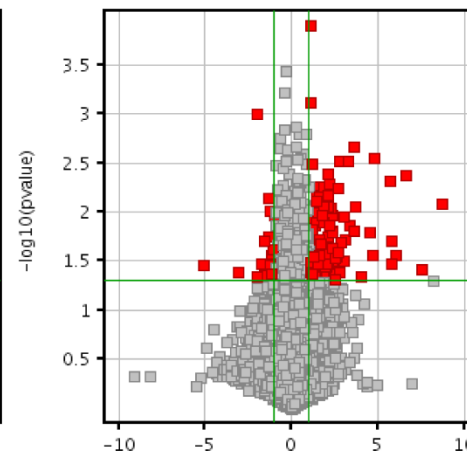
6 h vs 0 h



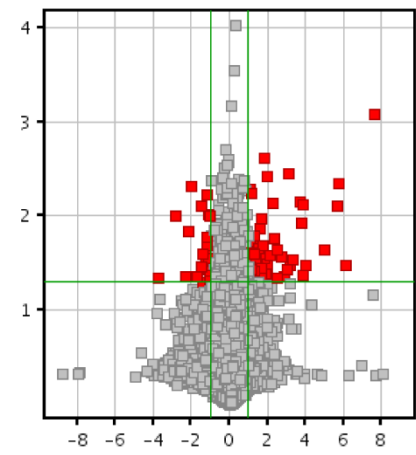
12 h vs 0 h



24 h vs 0 h



48 h vs 0 h



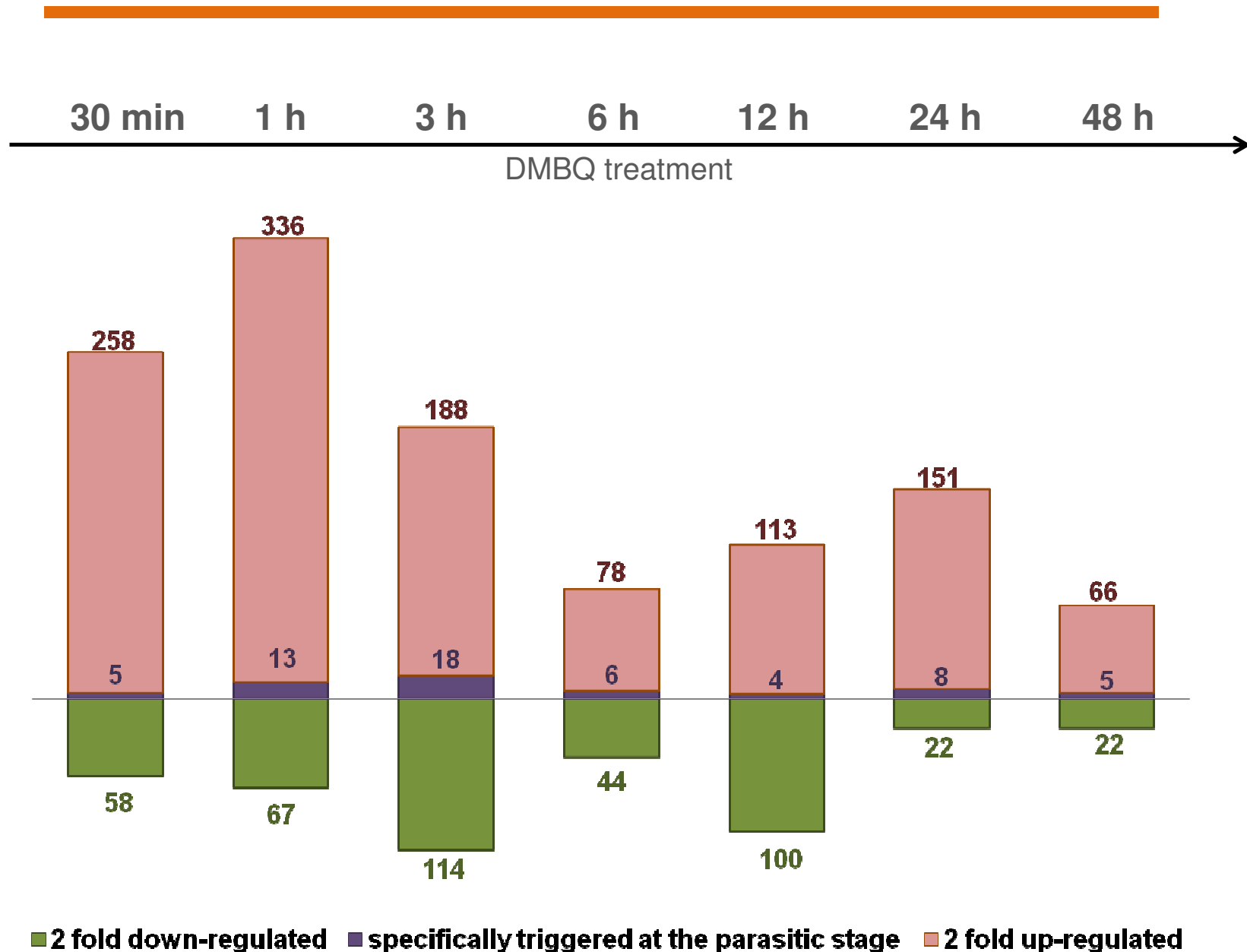
$\log_2(\text{Fold change})$

$\log_2(\text{Fold change})$

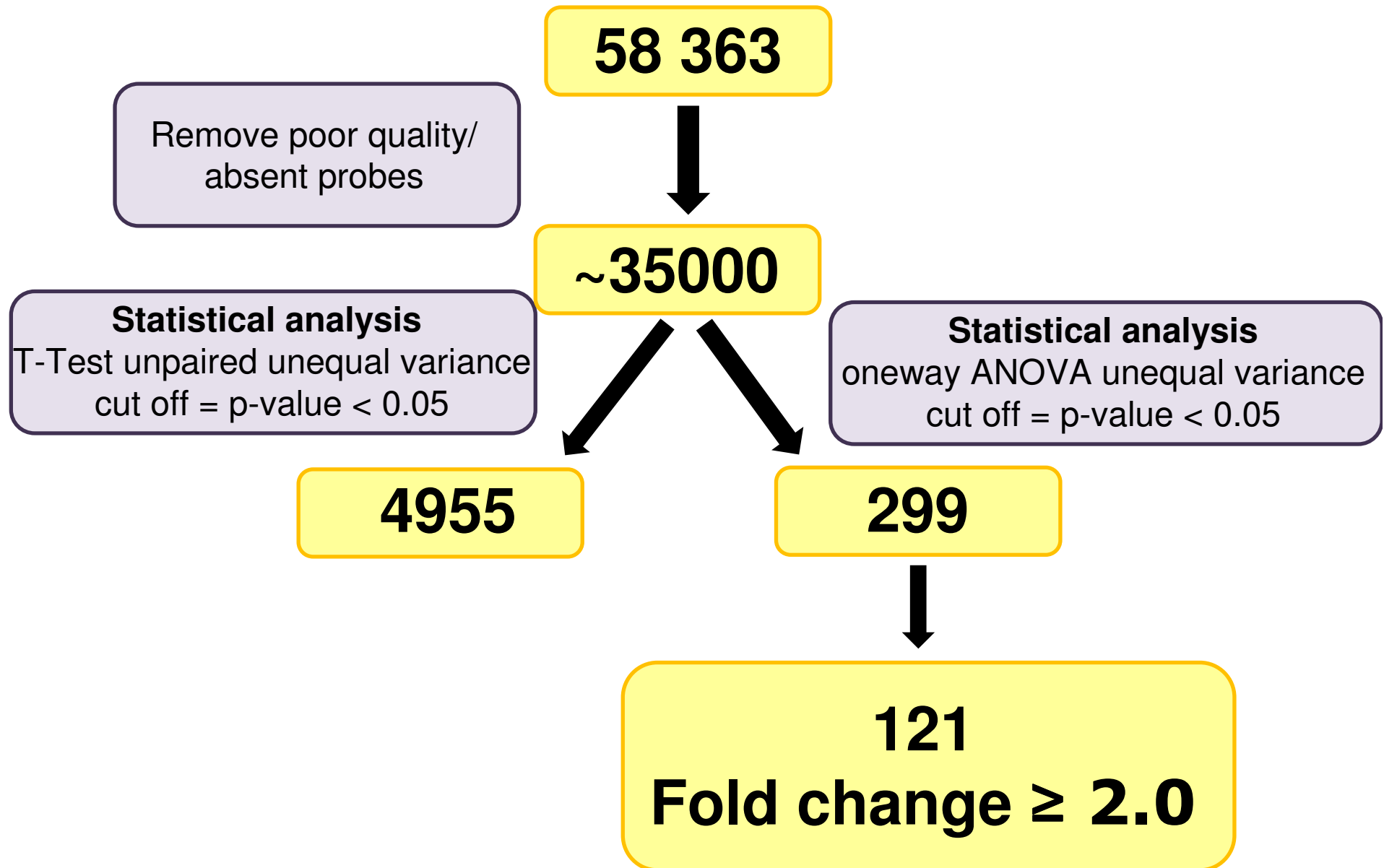
$\log_2(\text{Fold change})$

$\log_2(\text{Fold change})$

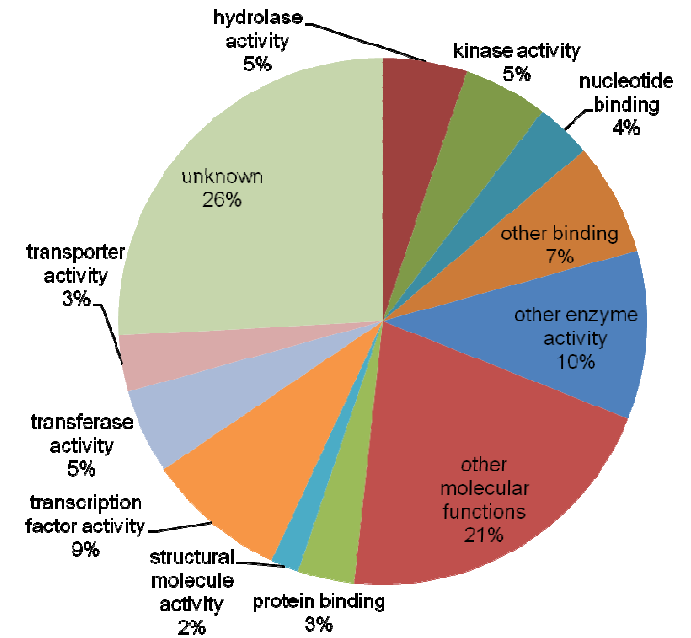
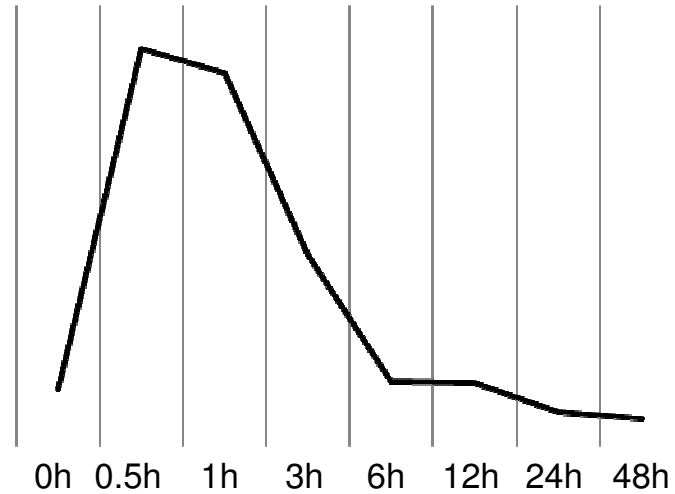
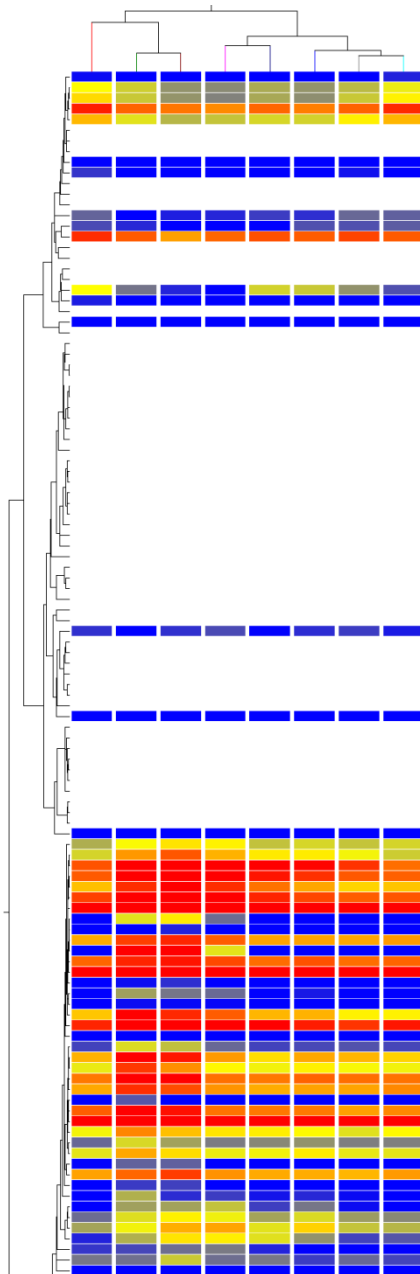
Gene activation during the DMBQ treatment



Identification of parasite genes

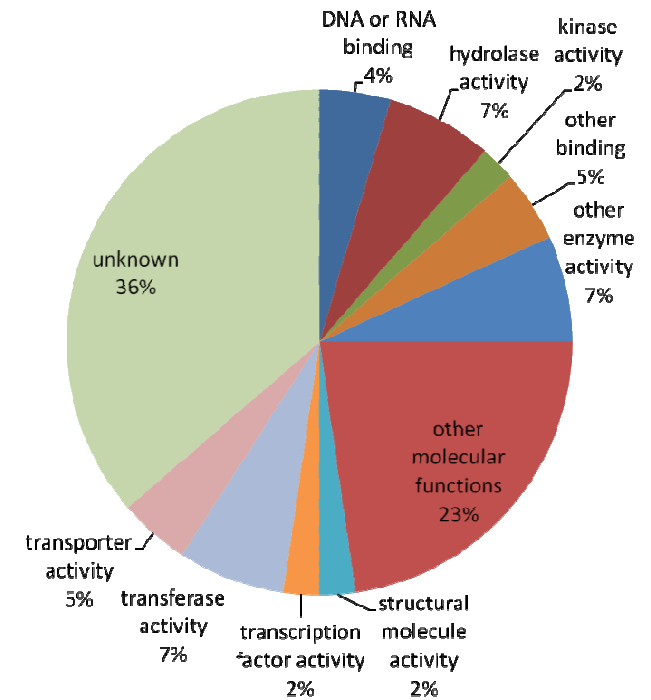
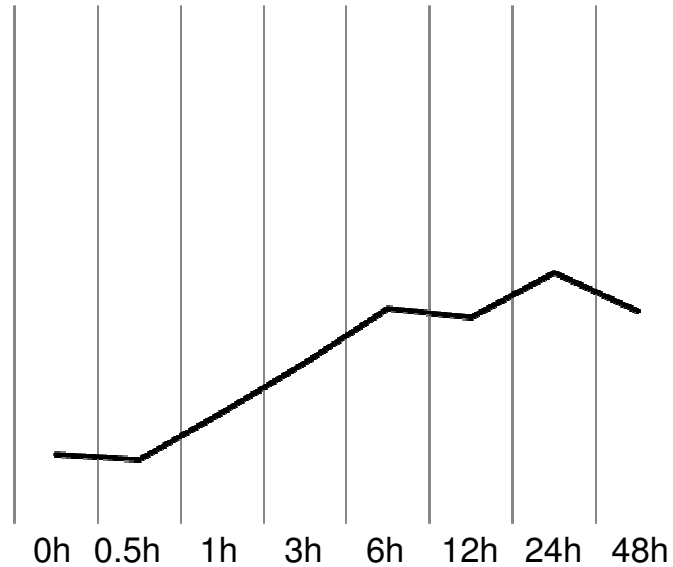
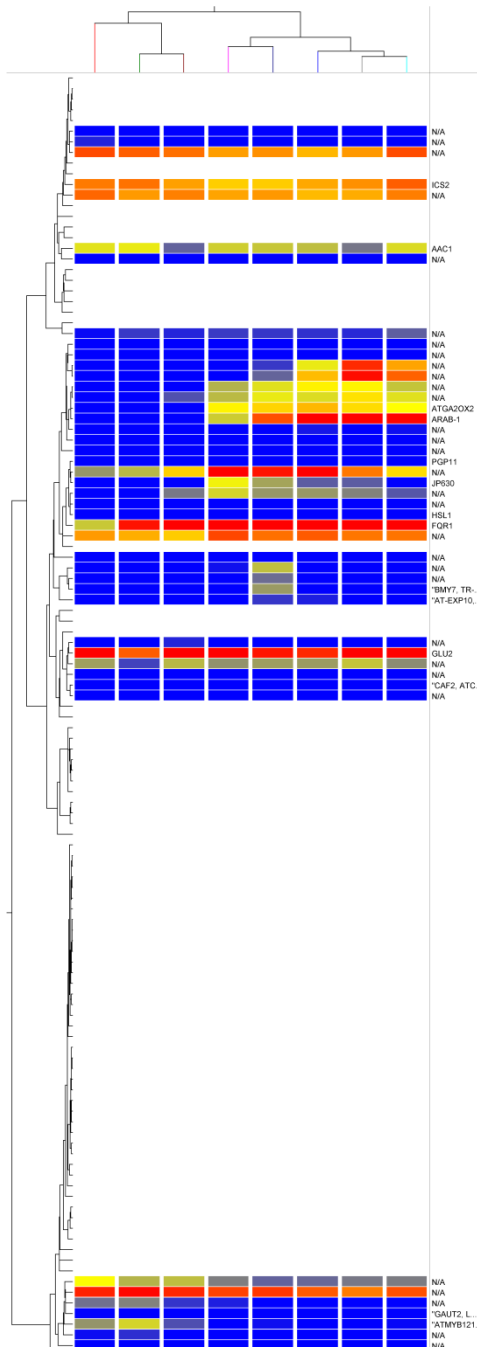


GROUP 1 (58 members)



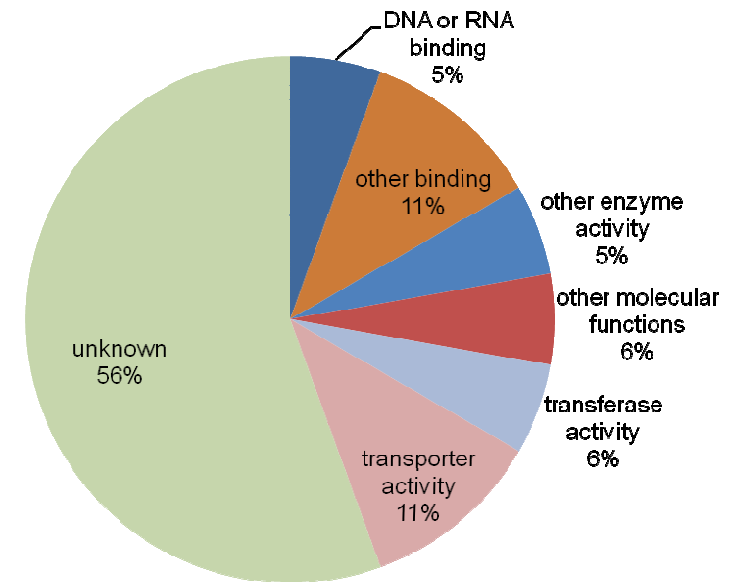
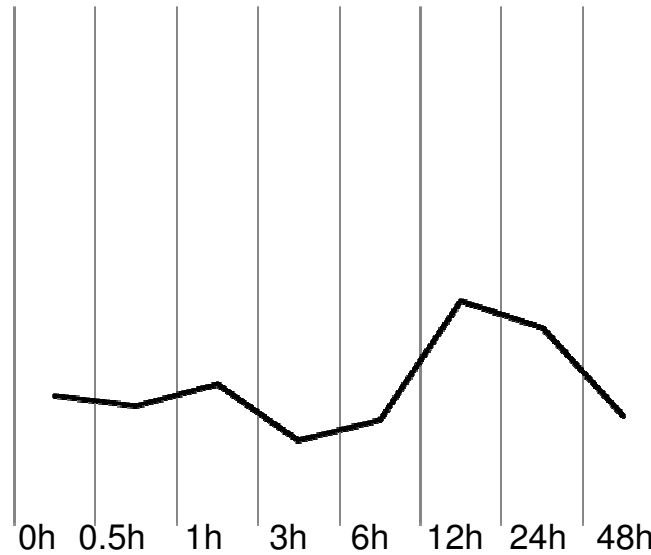
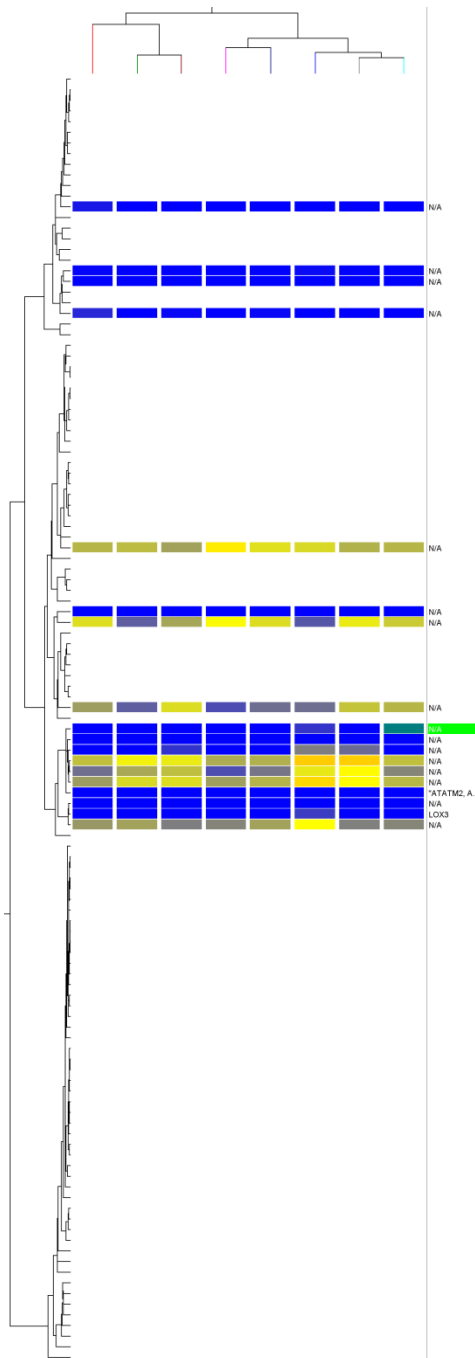
Description
AtSerat2;1 (SERINE ACETYLTRANSFERASE 1)
ATERF10/ERF10 (ERF domain protein 10)transcription factor
pyridine nucleotide-disulphide oxidoreductase family protein
WRKY40 (WRKY DNA-binding protein 40); transcription factor
DDF1 (DWARF AND DELAYED FLOWERING 1); DNA binding / transcription factor
auxin-responsive family protein
calcium ion binding

GROUP 2 (45 members)



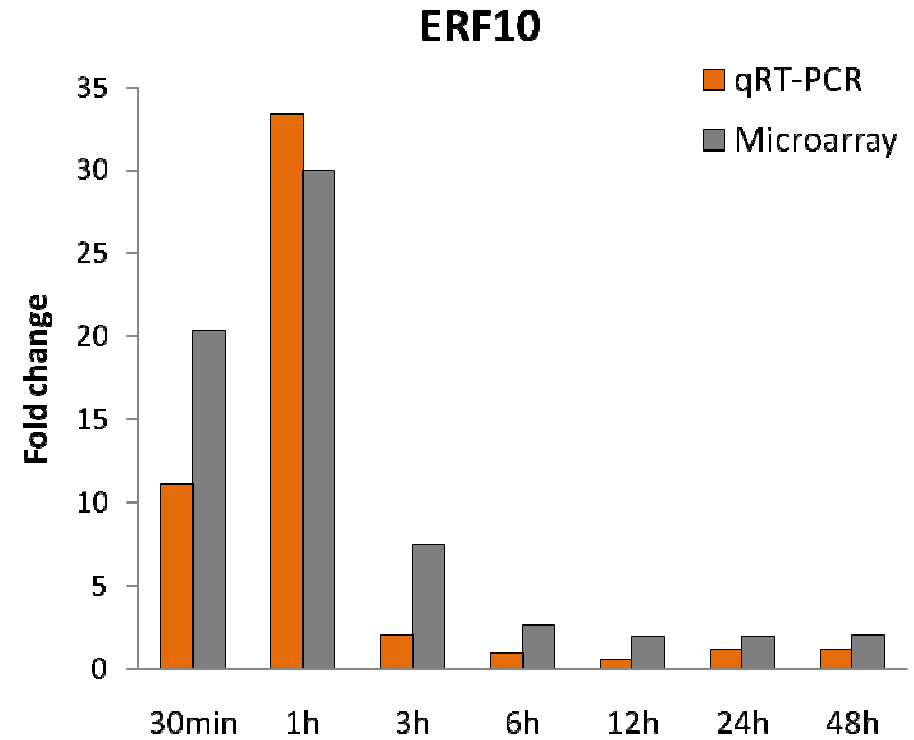
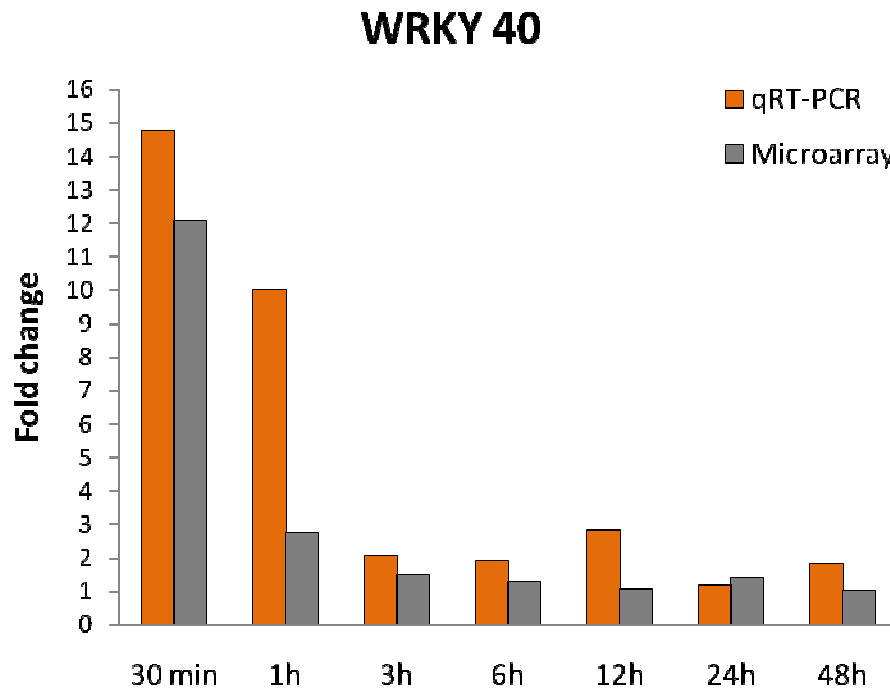
Description
peroxidase, putative
ATEXPA10 (ARABIDOPSIS THALIANA EXPANSIN A10)
similar to disease resistance-responsive family protein
UDP-glucuronosyl and UDP-glucosyl transferase family protein
FQR1 (FLAVODOXIN-LIKE QUINONE REDUCTASE 1)
GLU2 (Glutamate synthase)

GROUP 3 (18 members)



Description
proline-rich family protein
proton-dependent oligopeptide transport (POT) family protein
LOX3 (Lipoxygenase 3); iron ion binding / lipoxygenase
ATM2 (Arabidopsis thaliana ABC transported of the mitochondria 2); ATPase
RNA recognition motif (RRM)-containing protein
glycosyltransferase family protein

Confirmation of microarray by qRT-PCR



Summary

RNA-seq

- Subtilisin-like protease families are highly expressed in parasitic tissues.
- 1537 *P. japonicum* genes do not have orthologs in rice, arabidopsis, grape and moss.

Microarray

- Large number of genes are functionally unknown
- Few transcriptional factors are early induced in response to DMBQ

Acknowledgments

Ken Shirasu (RIKEN)

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Shigetou Namba (University of Tokyo)

All members of Plant Immunity Group

Assembly and Annotation data

Eric Wafula (Penn State University)

Claude W. dePamphilis (Penn State University)

All the members of Claude`s laboratory

***P. japonicum* seeds**

T. Enomoto, T. Yahara, T. Yaeno, K. Yoshimoto, S. Tsuchinaga