

TIC TOC: Targeting Improved Cotton Through Orbital Cultivation



ISS National Laboratory

CENTER FOR THE ADVANCEMENT OF SCIENCE IN SPACE

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Lucas Bauer
Sarah Swanson
Simon Gilroy

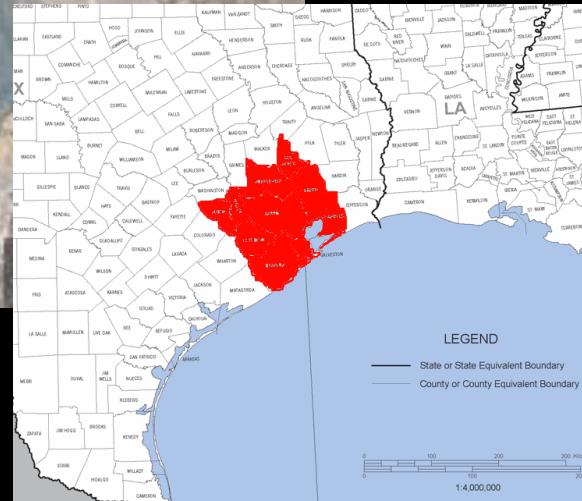
& all the awesome folk at KSC
Astronauts: Shane Kimbrough, Mark Vande Hei



Cotton is a thirsty crop.
1kg of cotton uses 10,000 liters of water



Greater Houston Area



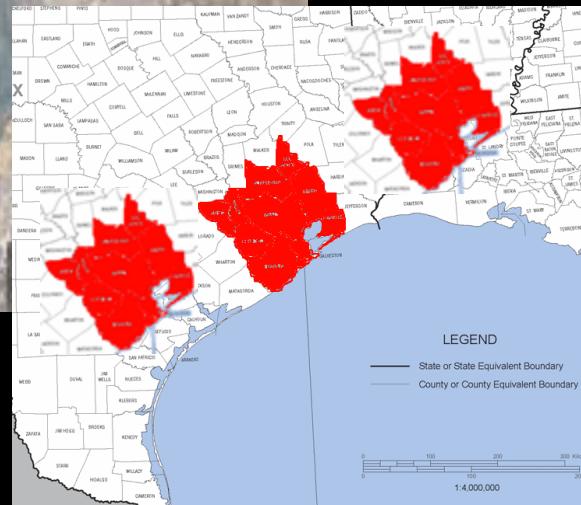
Known environmental impact

Cotton field irrigation caused the decline of the Aral sea (1993-present).

Cotton is a thirsty crop.
1kg of cotton uses 10,000 liters of water



3 x Houston = 1 Aral sea



Known environmental impact

Cotton field irrigation caused the decline of the Aral sea (1993-present).

So how can we adjust plant biology to reduce this environment impact?



Auxin can cause proton movement.
Allowing shoots and roots to change their morphology.

GMO Cotton plant that is drought and salt resistance



Wild-type
control

AVP1-expressing
cotton

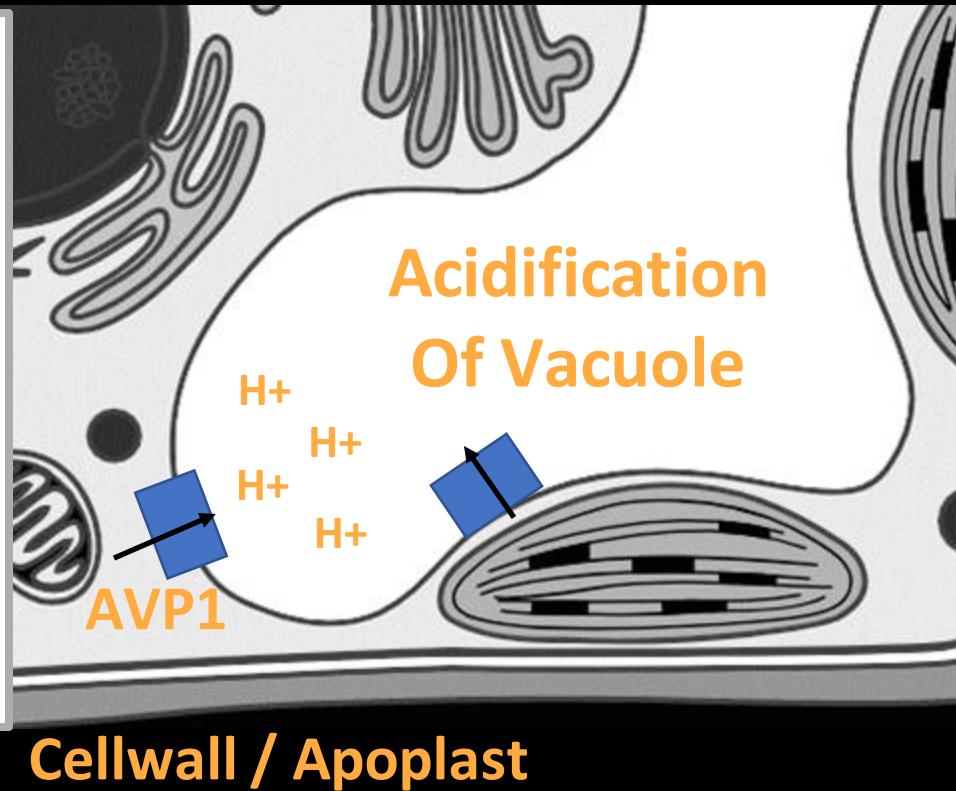
Arabidopsis Vacuolar Pyrophosphatase Over Expression AVP1-OX -> A68 & D130



Wild-type
control

AVP1-expressing
cotton

Zhang et al., 2011



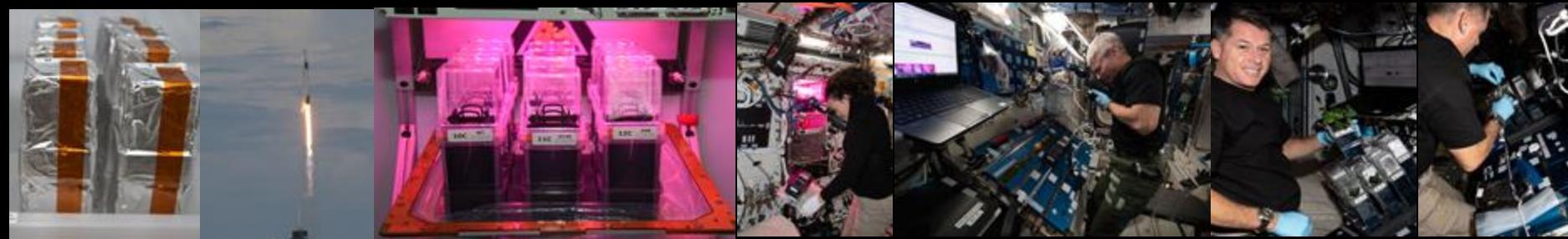
Hypothesis: AVP-over expressing plants could be resistant to spaceflight



Hypothesis: AVP-over expressing plants could be resistant to spaceflight?



WT and AVP1-ox lines (A68 & D130) grew on the ISS and photographed by astronauts.



Hypothesis: AVP-over expressing plants could be resistant to spaceflight?



WT and AVP1-ox lines (A68 & D130) grew on the ISS and photographed by astronauts.

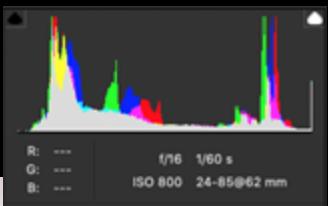


Samples were frozen and brought back to Earth for processing by Dr Arko Bakshi

Image analysis pipeline -> No spectral analysis possible

Original Image

- Rename
- geno_age_treatment
- RGB Color
- Tiif
- ~5-12Mb

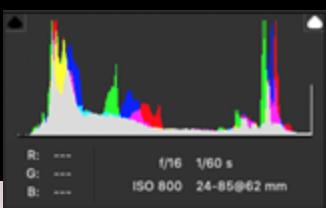


Note: Variation in the ambient light environment

Image analysis pipeline -> Morphological normalization

Original Image

- Rename
- geno_age_treatment
- RGB Color
- Tiff/JPG
- ~5Mb



Adobe bridge rename

- Image raw conversion
- -100% saturation (Grey scale)
- +100% clarity (sharpens edges)
- Crop (reduce size)
- Align parallel lines

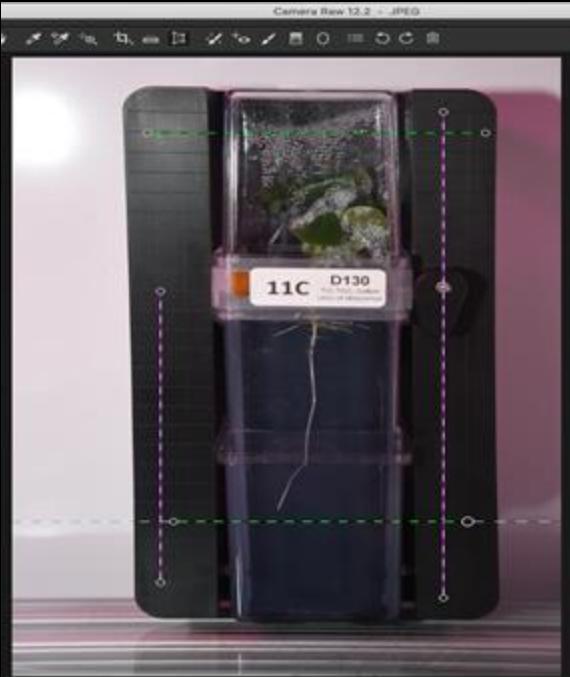


Image analysis pipeline -> Semi-automated root system detection

Original Image

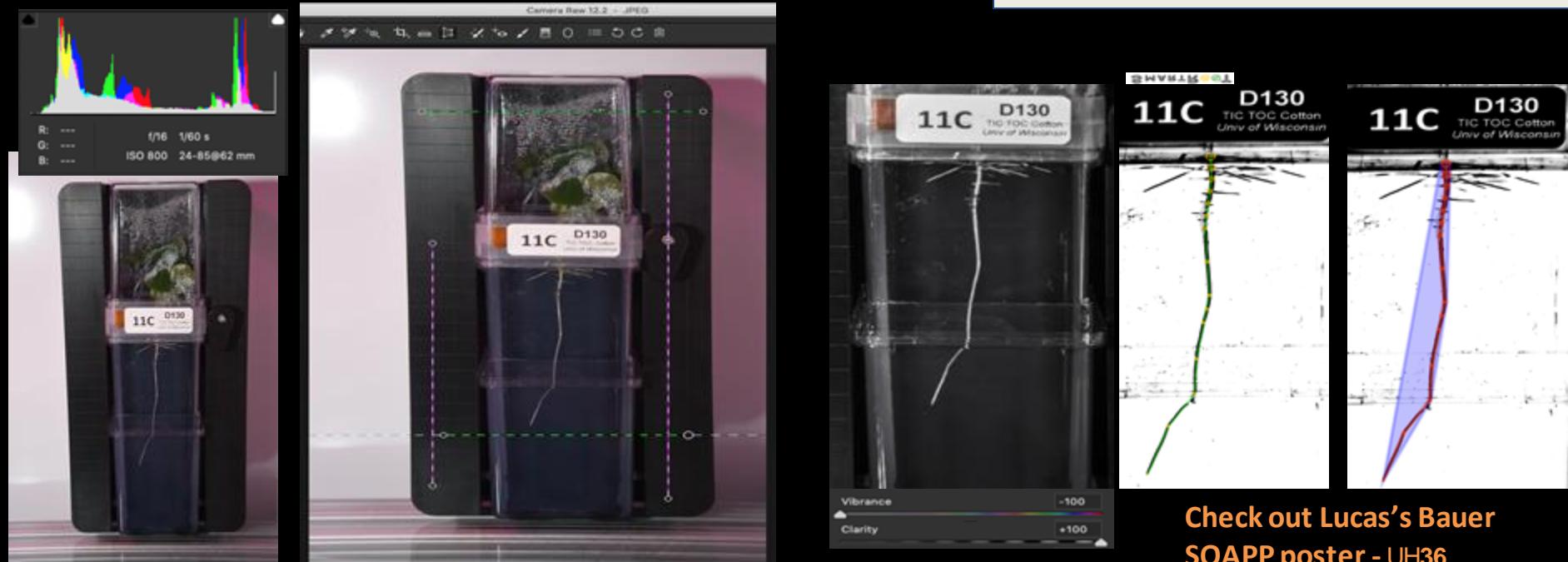
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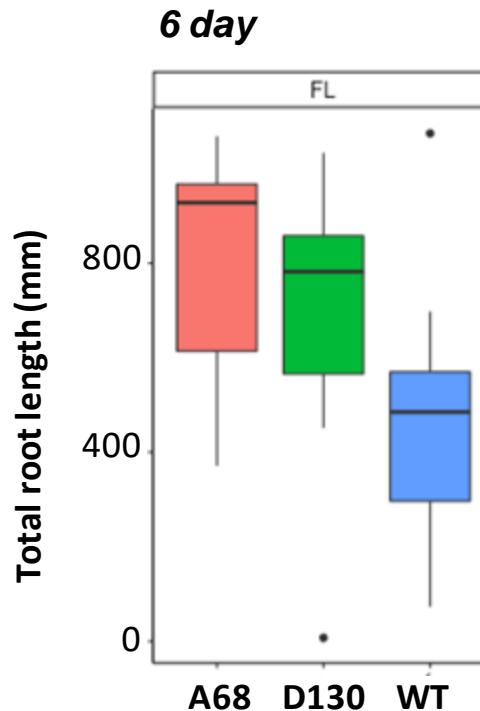
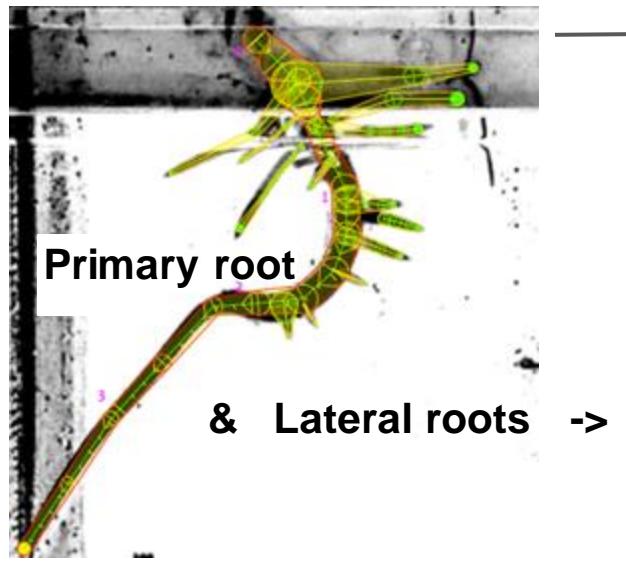
ImageJ & Smart Root Plugin

- Invert image
- User define scale conversion based on image marker
- User define root spine, lateral root autodetection.
- RSML output

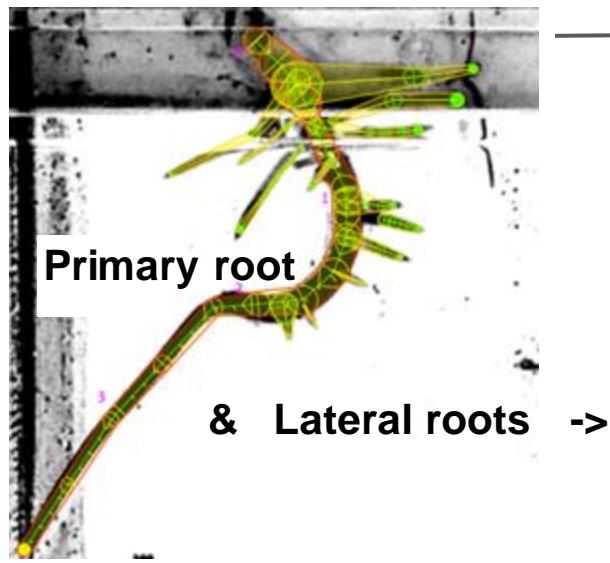


Check out Lucas's Bauer
SOAPP poster - UH36

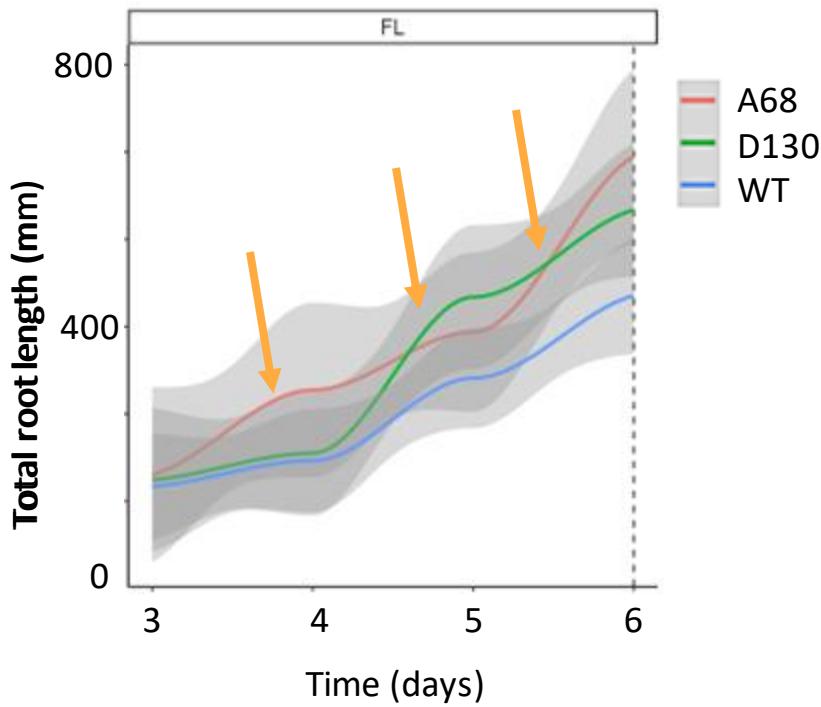
AVP-ox roots grew larger than WT during flight



AVP-ox lateral roots emerged earlier than WT



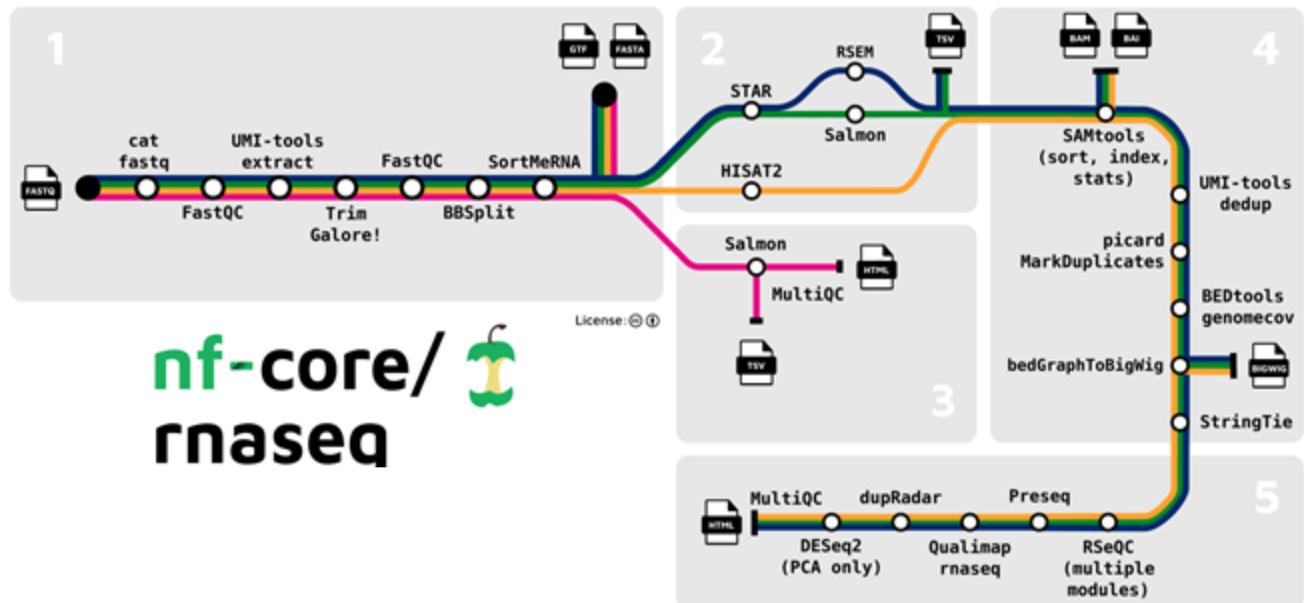
Note: *Lateral root emergence*





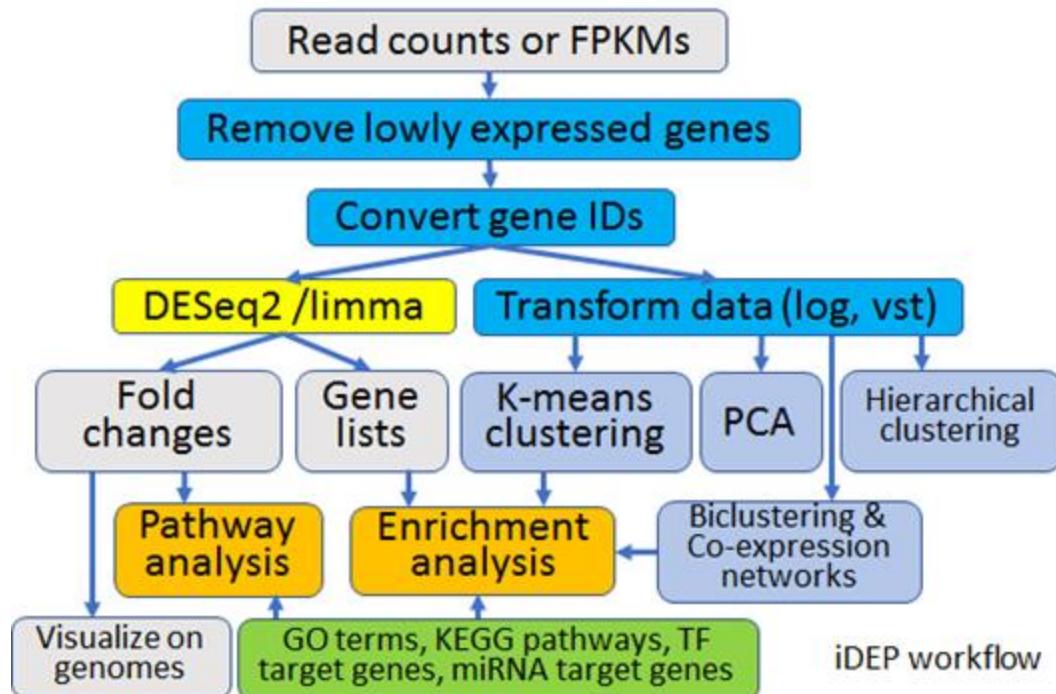
Gold line: nf-core was used HiSat2 for FASTQ alignment and transcript count performed with Dup-radar (*similar to BAM2count*).

Arko line: RNA extraction with SPECTRUM kit Illumina NOVAseq sequencing.

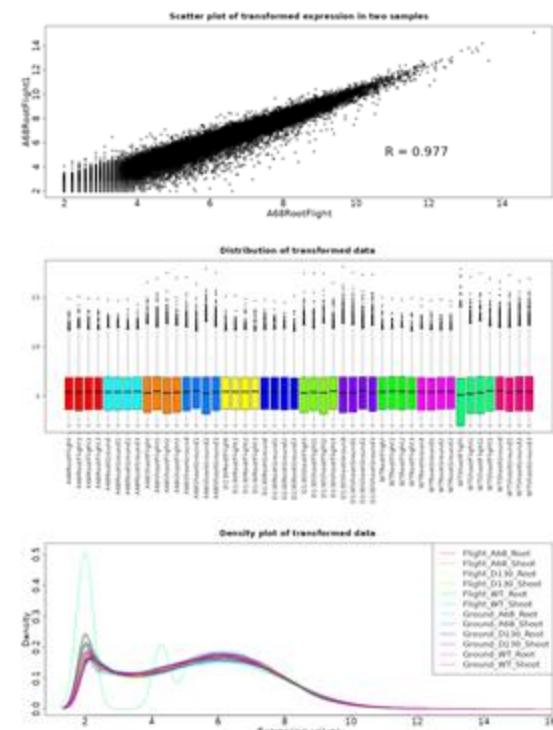




QC, normalization and differential expression calculation. Enabling ontological enrichment visualization.



iDEP workflow
Ge et al., 2022





RNAseq DEG count WT (FDR <=0.1 and logFC >=2)

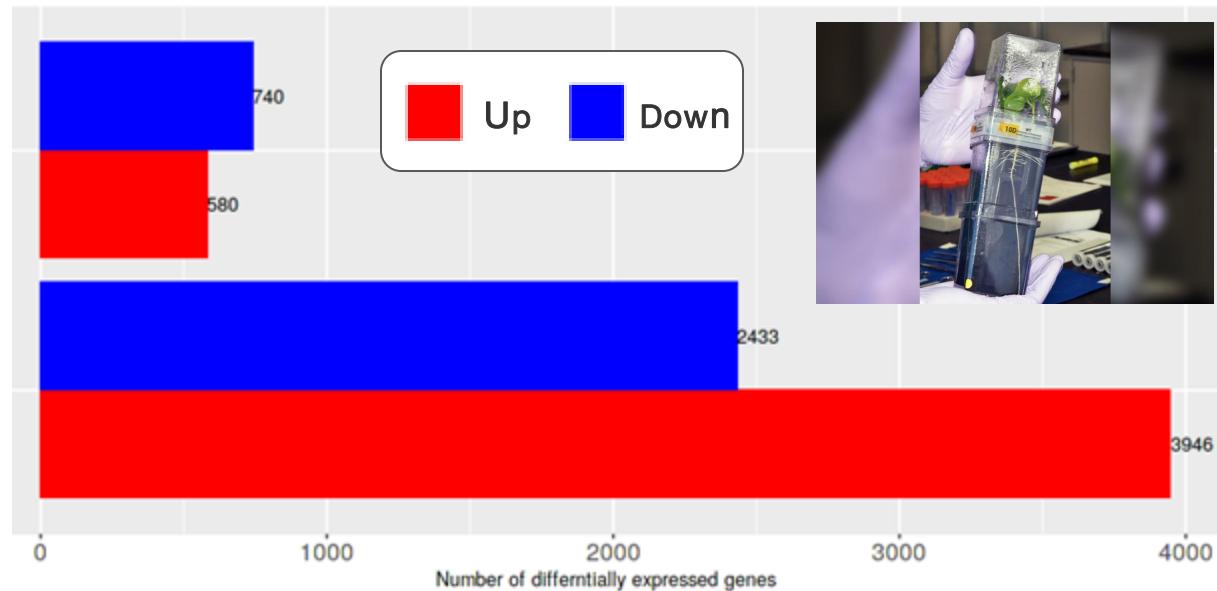
Comparisons	Up	Down
WT Shoot Flight -vs- WT Shoot Ground	580	740
WT Root Flight -vs- WT Root Ground	3946	2433

Differential Expressed Gene (DEG) Count

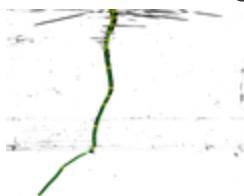
DESeq2 DEG Count Thresholds

Fold Change (FC) and FDR false discovery rate

WT Shoot Flight -vs- WT Shoot Ground



WT Root Flight -vs- WT Root Ground





RNAseq DEG count WT (FDR <=0.1 and logFC >=2)

Comparisons	Up	Down
WT Root Flight -vs- WT Root Ground	3946	2433

Differential Expressed Gene (DEG) Count

DESeq2 DEG Count Thresholds

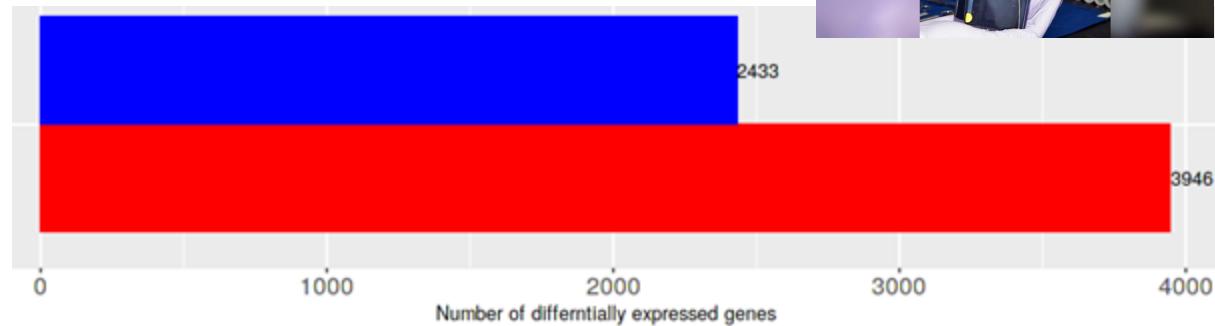
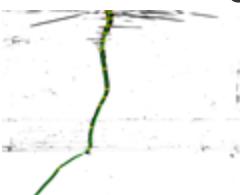
Fold Change (FC) and FDR false discovery rate

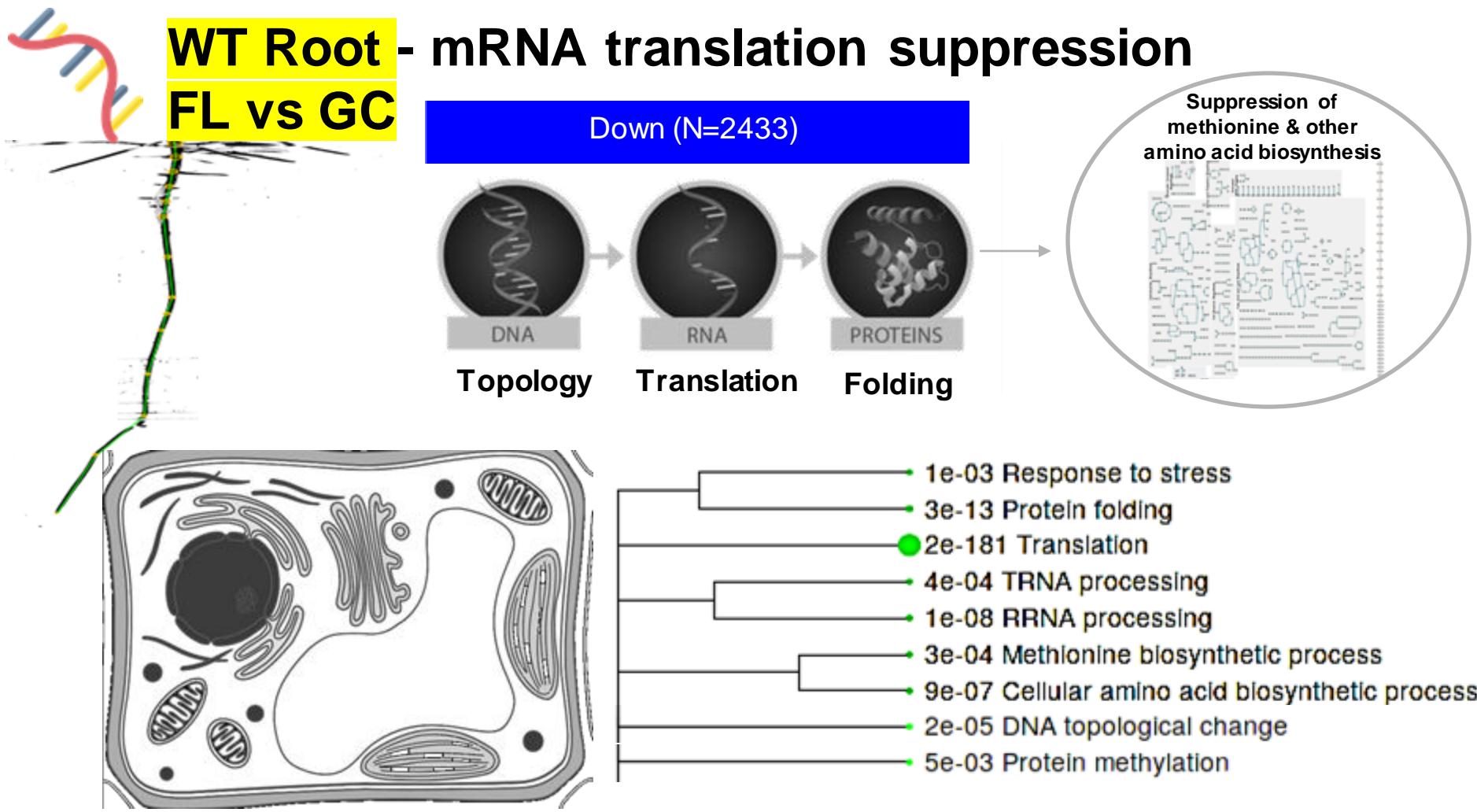
Focus on roots

Up Down



WT Root Flight -vs- WT Root Ground



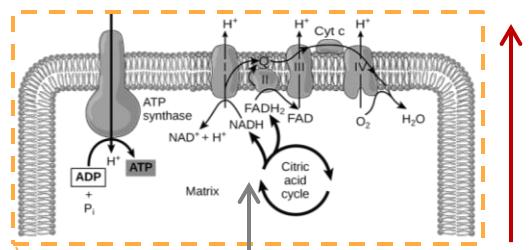
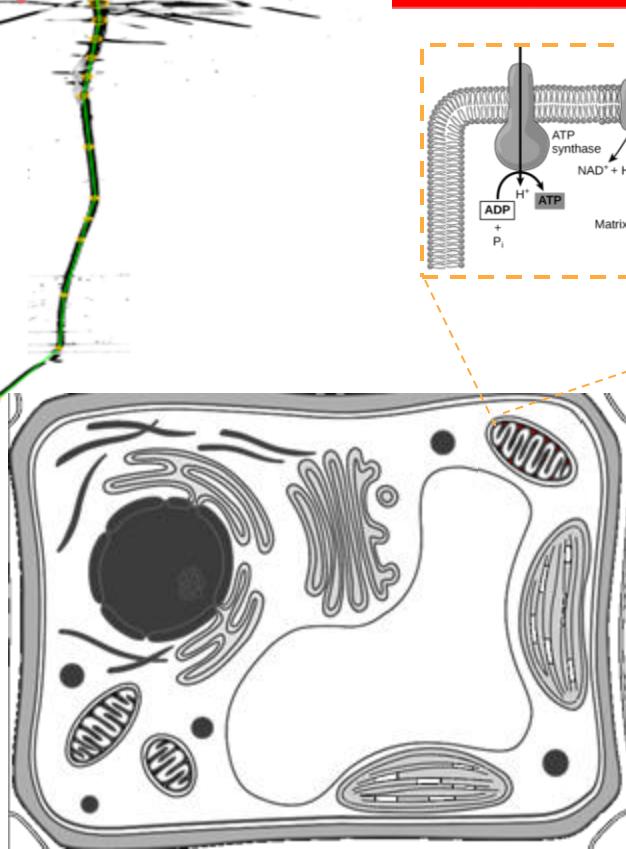




WT Root - Oxidative-Phosphorylation up-regulation

FL vs GC

Up (N=3946)



Carbohydrate : Lipid metabolism
Protein UBQ & ERAD pathway
Protein phosphorylation / dephosphorylation
Signaling / Exocytosis

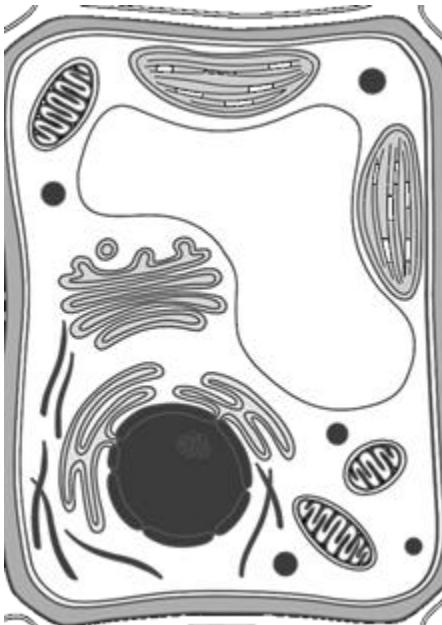
- 1e-06 Coenzyme A metabolic process
- 2e-12 Oxidation-reduction process
- 3e-03 Glycerol-3-phosphate catabolic process
- 6e-03 Isoprenoid biosynthetic process
- 2e-05 Lipid metabolic process
- 3e-13 Signal transduction
- 8e-46 Regulation of transcription-DNA-templated
- 2e-04 Protein dephosphorylation
- 2e-04 Exocytosis
- 8e-04 Protein kinase C-activating G-protein coupled receptor
- 3e-03 Carbohydrate derivative biosynthetic process



WT vs A68 – Root -> Pathways effected by AXP-1OX

FL vs FL

*Note: No significant difference
D130 vs A68 in Flight*

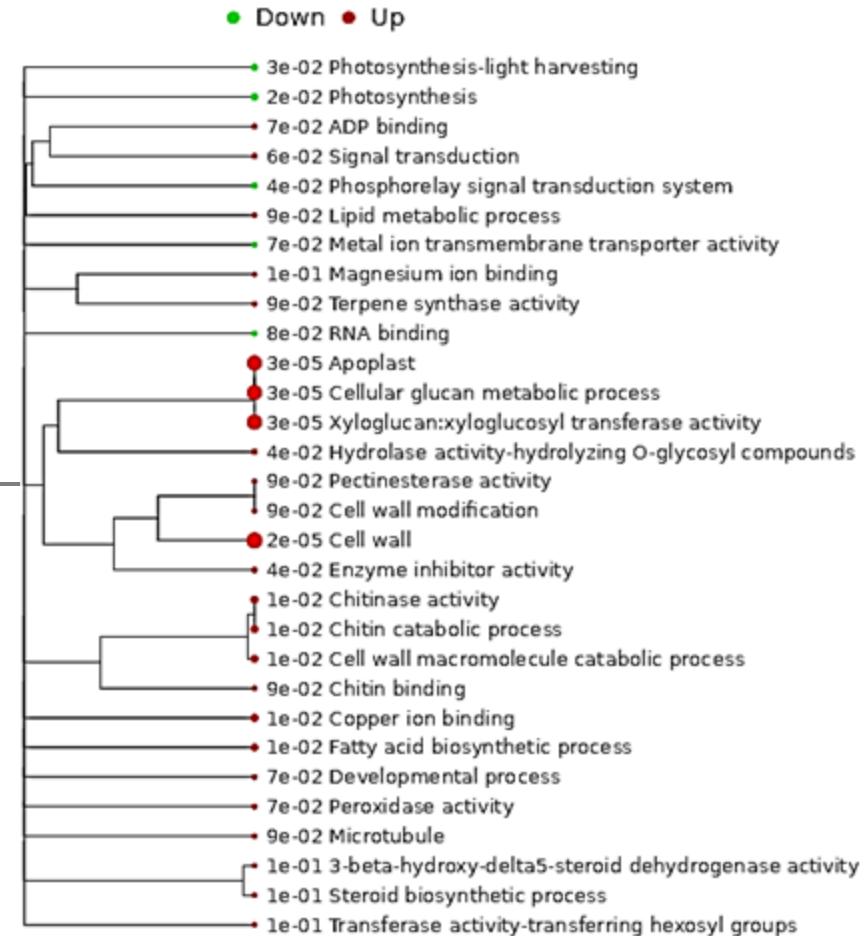
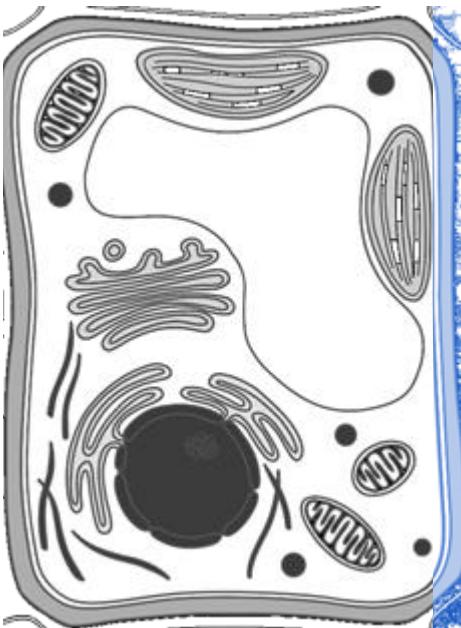


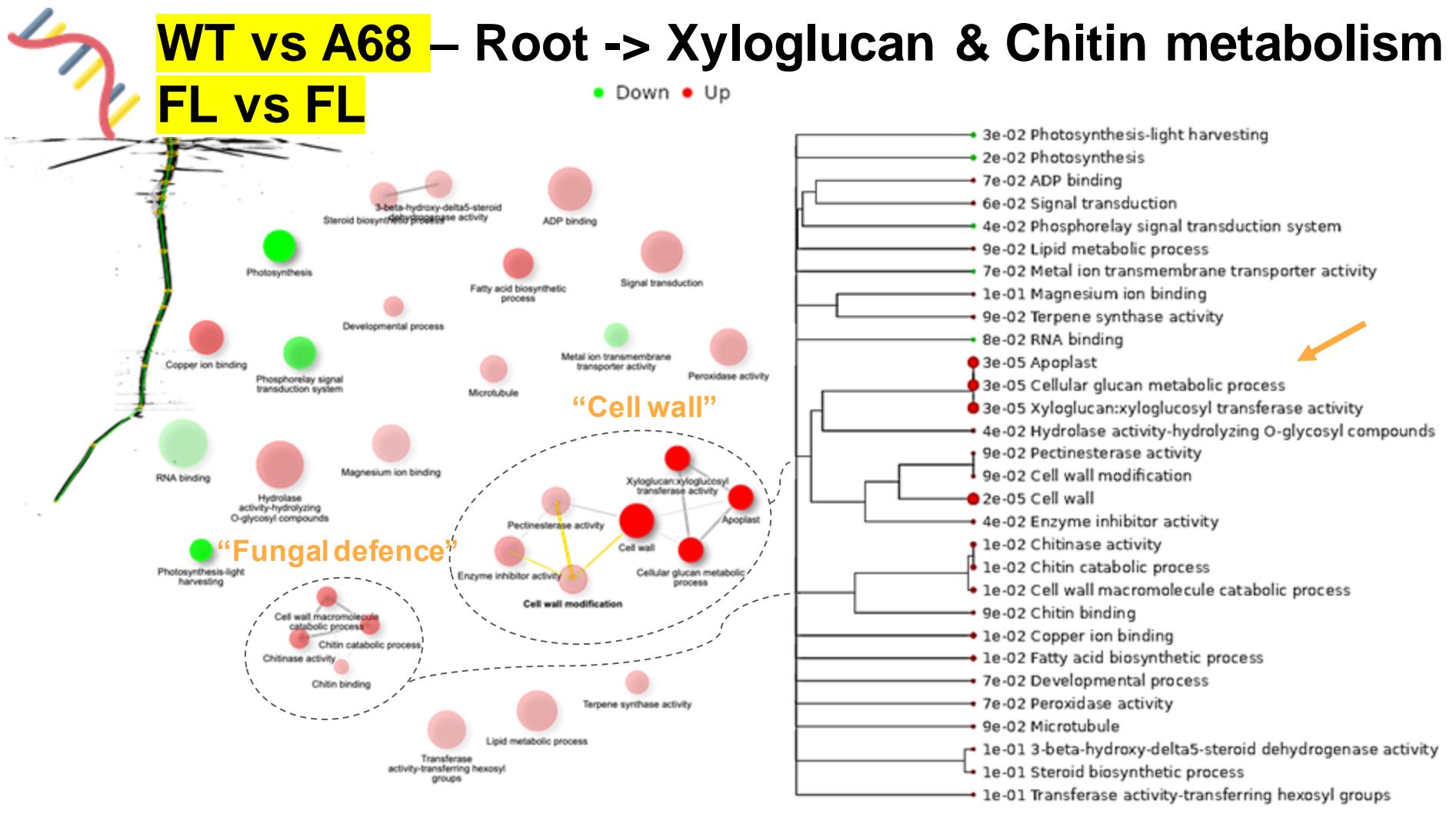


WT vs A68 – Root -> Pathways effected by AXP1-OX

FL vs FL

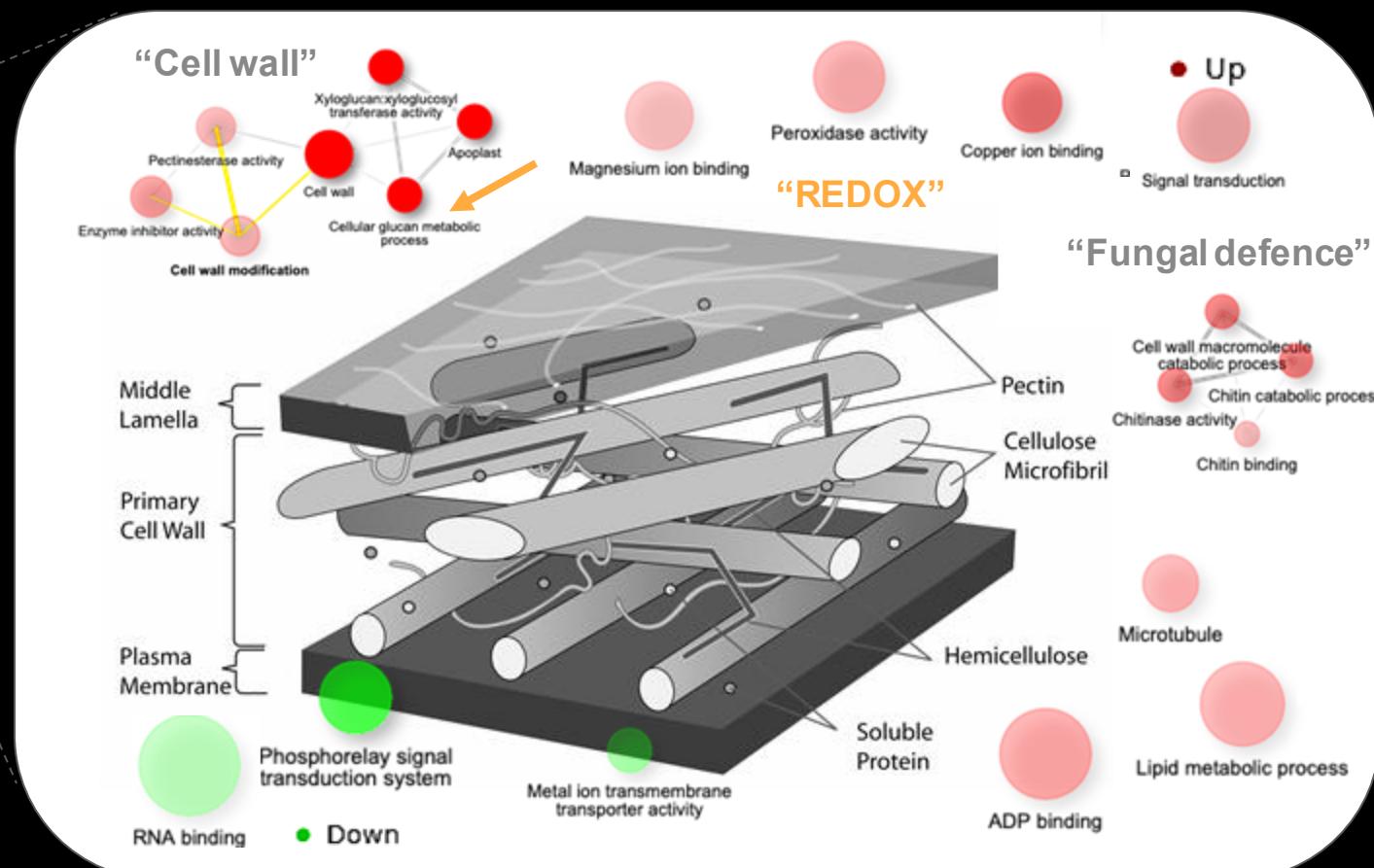
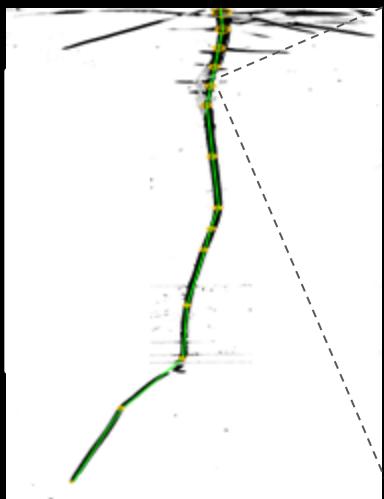
Let's break this down...





WT Root vs AVP1-Ox -> Cell wall REDOX

FL vs FL



Change ion transport and phosphorelay signaling.



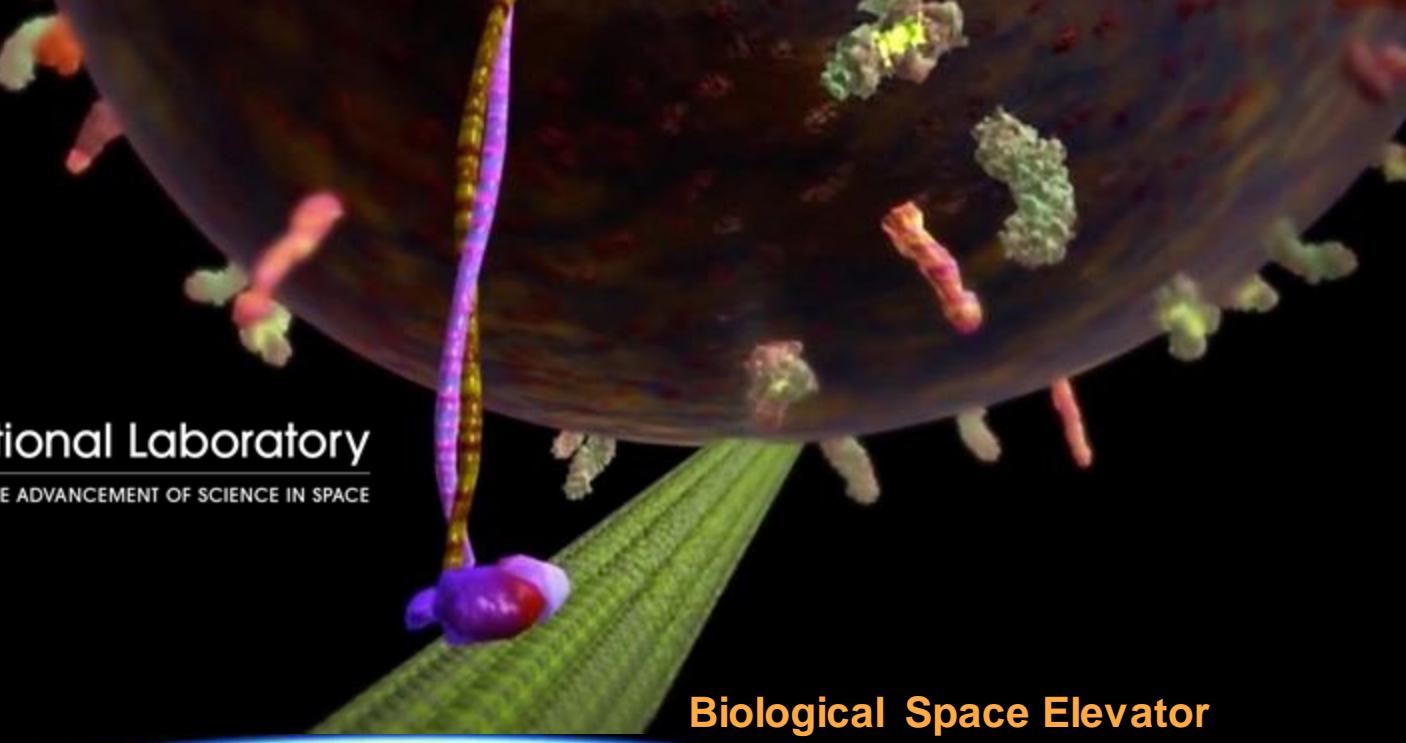
TICTOC Conclusion AGSR 2022



- WT Cotton RNAseq “functionally resembles WT Col-0 *Arabidopsis thaliana* response to space flight.”
- The AVP-OX lines exhibited greater growth in flight relative to WT controls.
- AVP-OX may changes vesicle trafficking system influencing cation ion transporters and cell wall modifying enzymes.



Y U R I



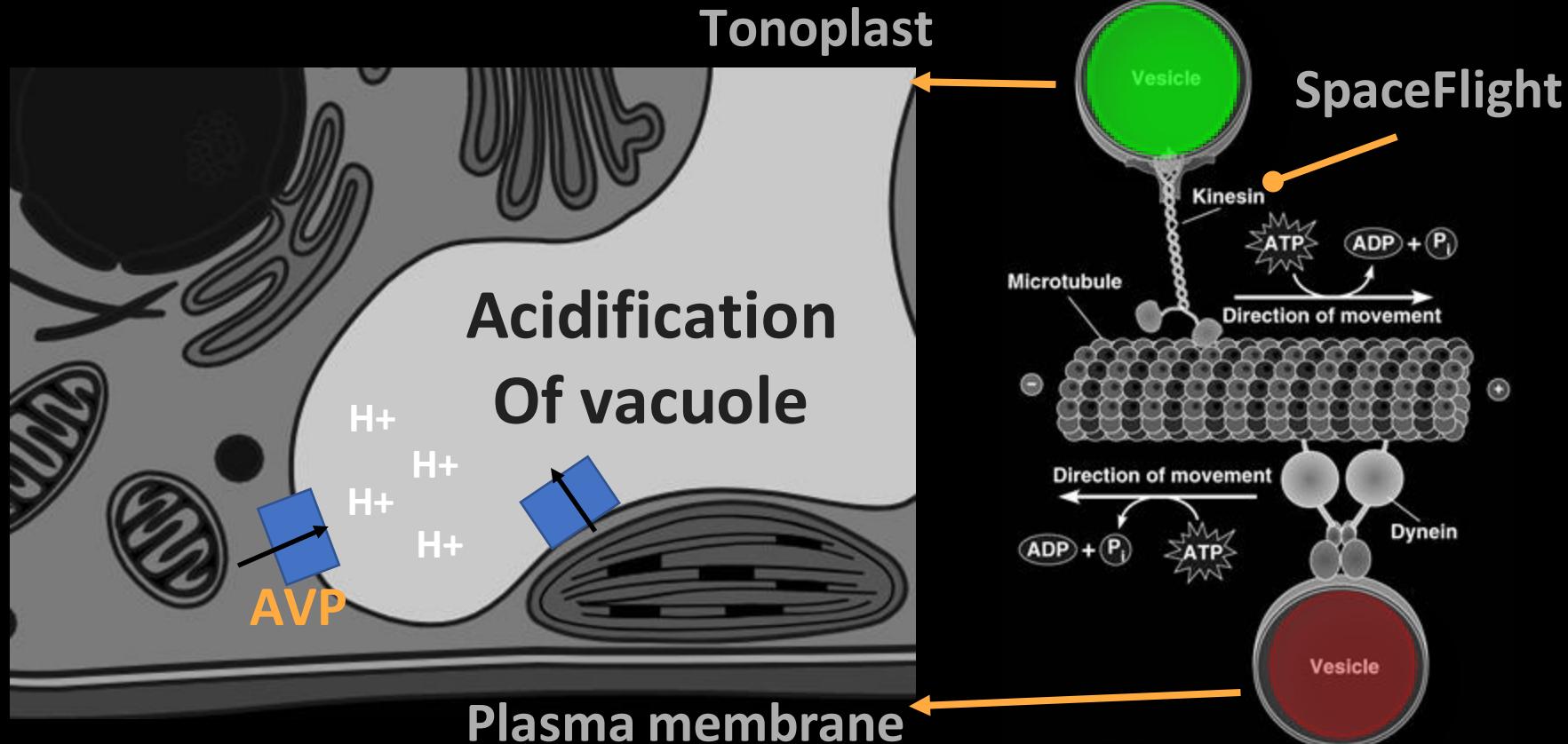
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Biological Space Elevator

AVP-ox might alter the orientation of vesicle movement?

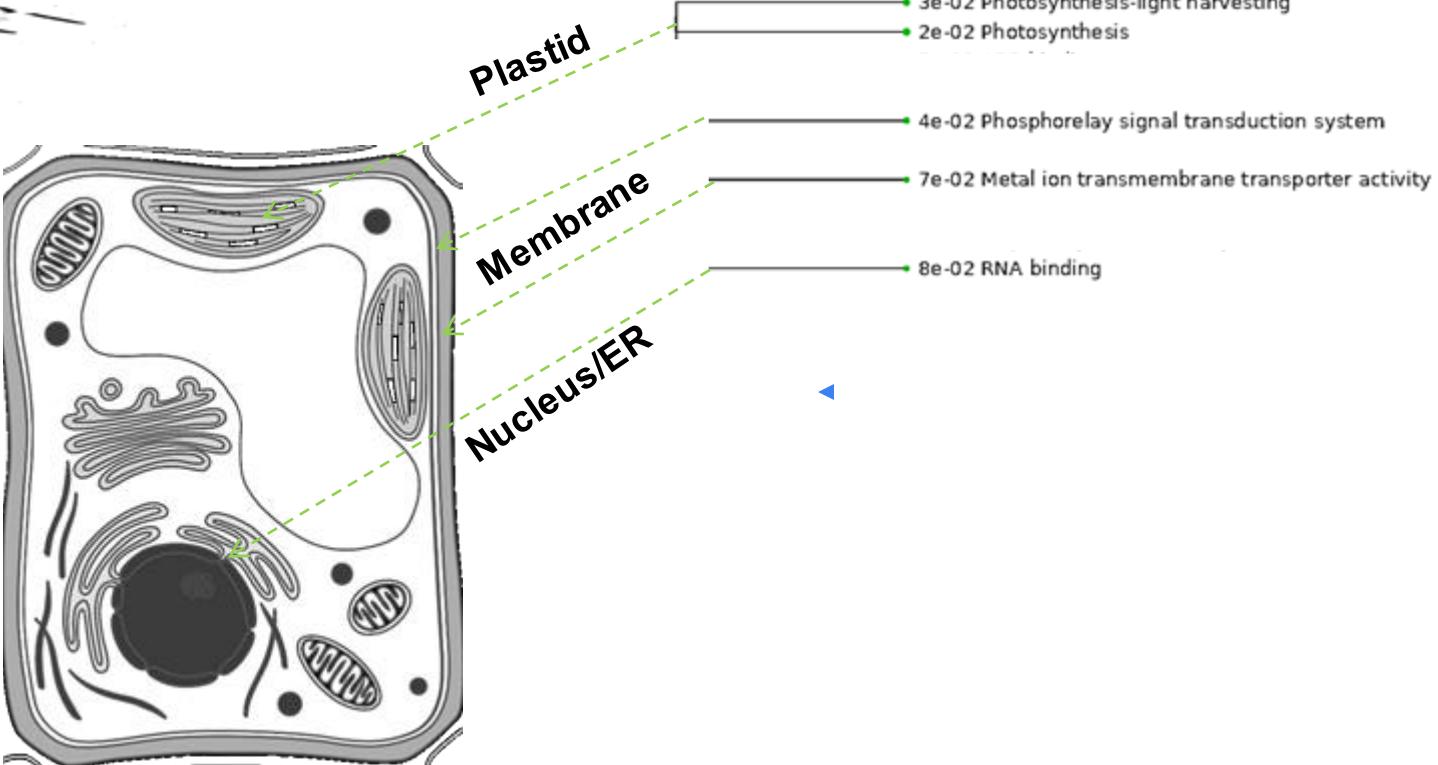


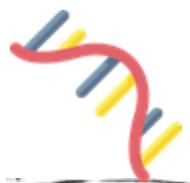
&/Or how does this change what is delivered to vacuole or apoplast?



WT vs A68 – Root -> Down regulated pathways

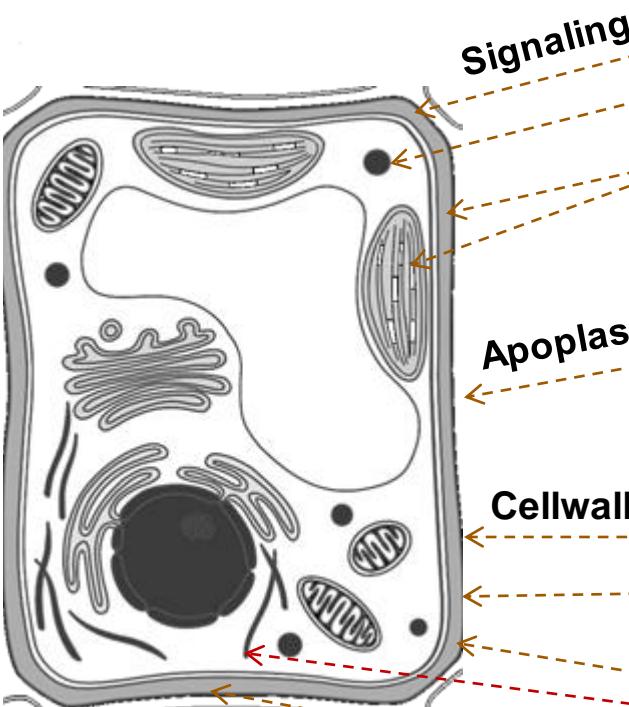
FL vs FL



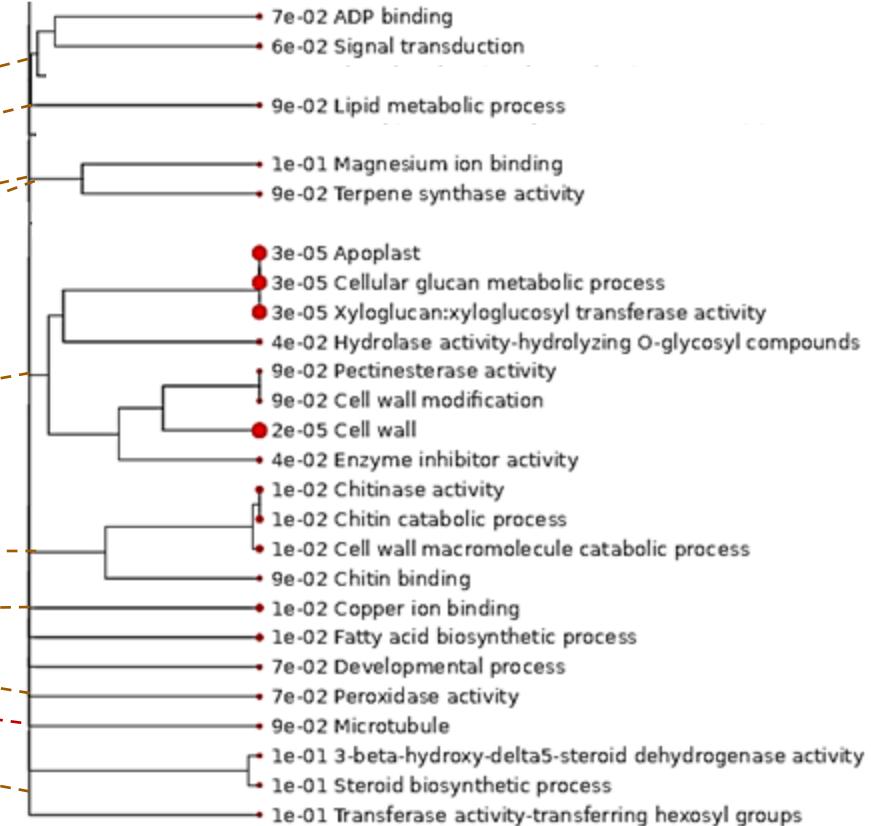


WT vs A68 – Root -> Apoplast & Cell wall

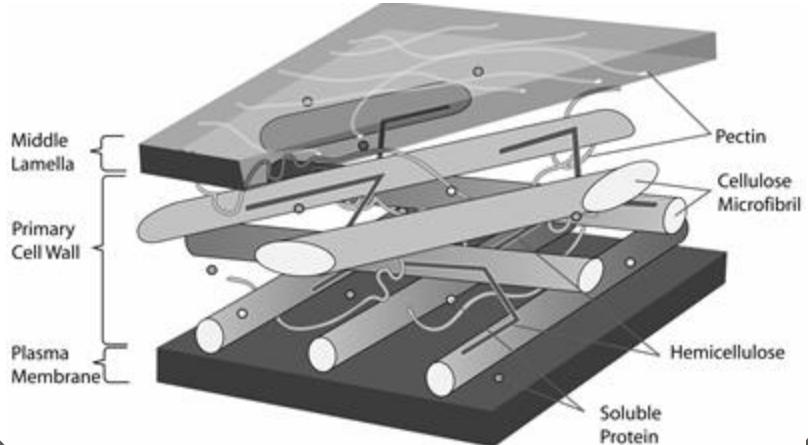
FL vs FL



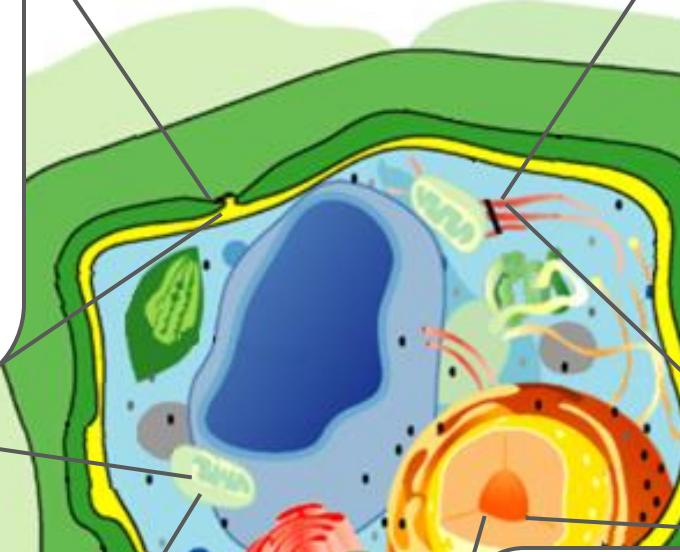
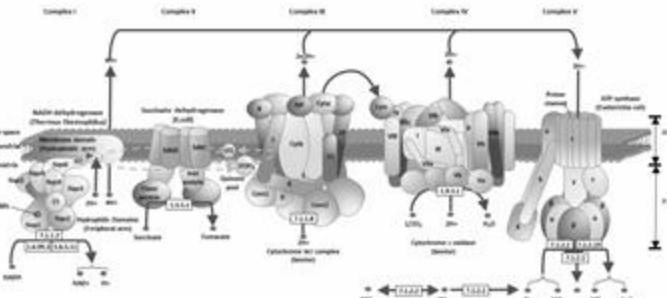
● Up



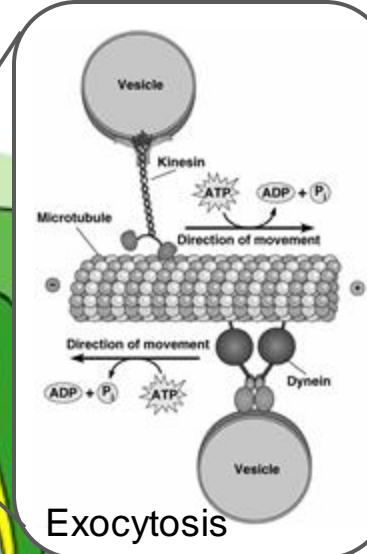
Apoplast/ Cell wall



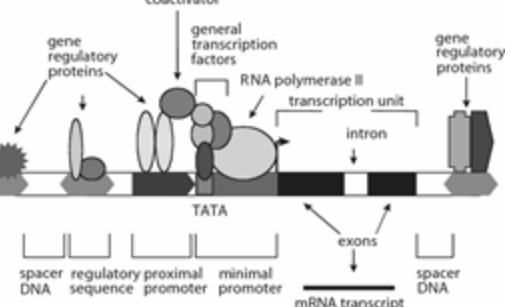
Oxidative phosphorylation

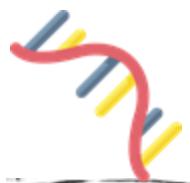


Exocytosis

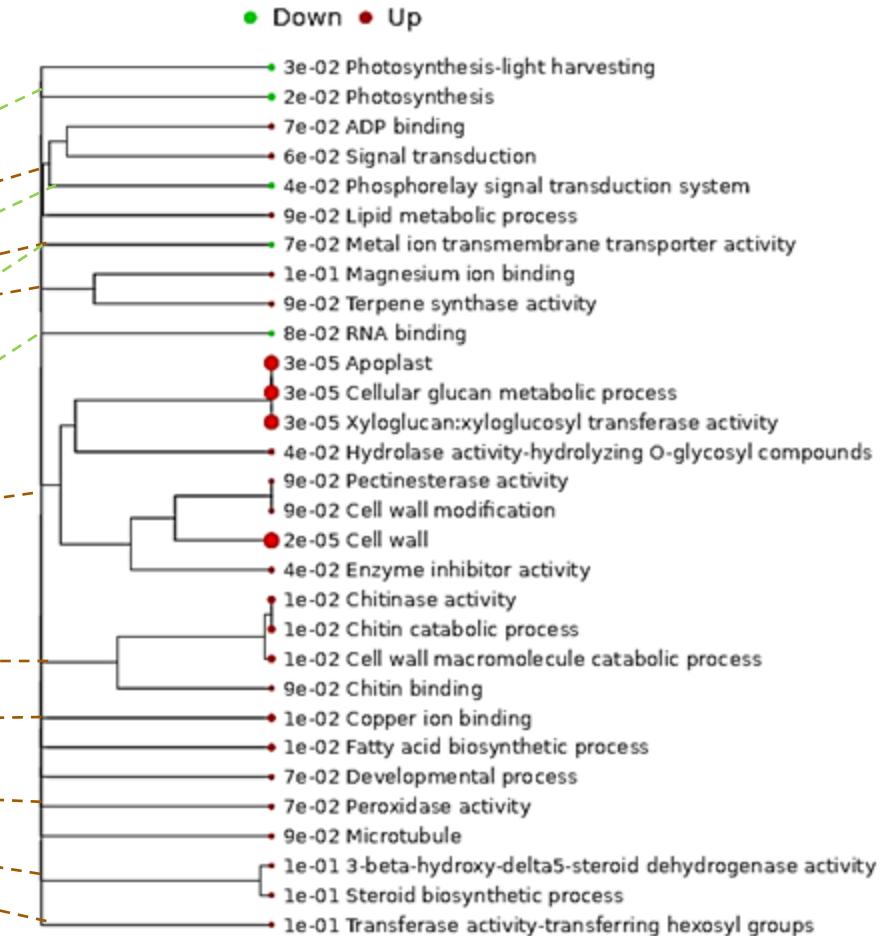
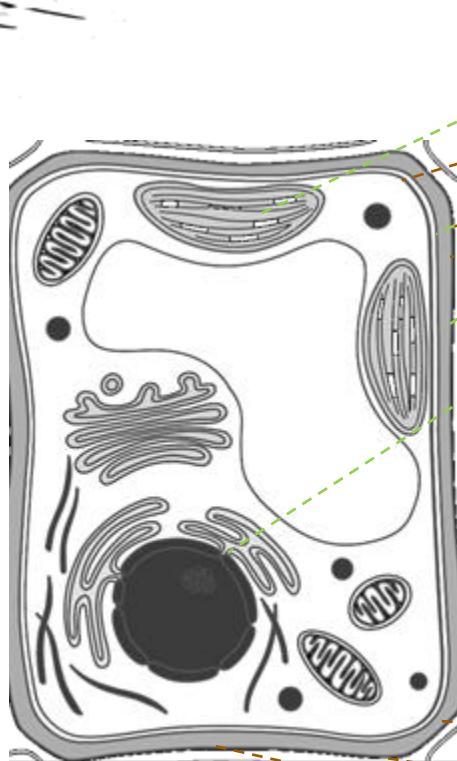


Transcription





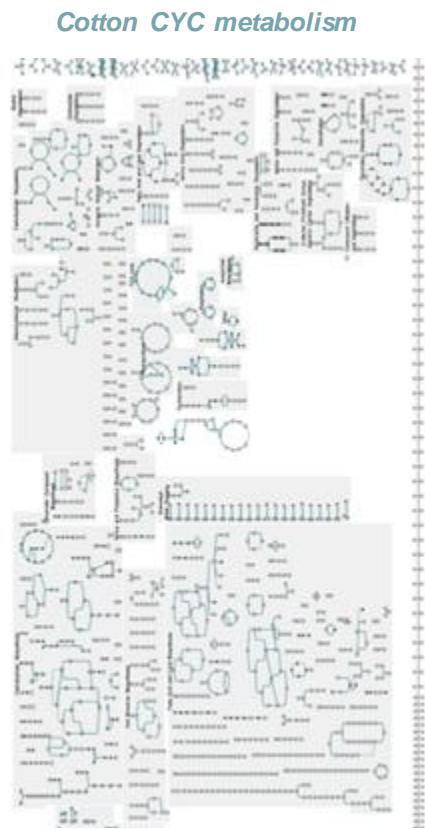
WT vs A68 – Root -> Pathways changes -> FL vs FL



Genome ontology analysis of WT and AVP-ox ?

D130 vs WT

Ref: Roots & Ground control



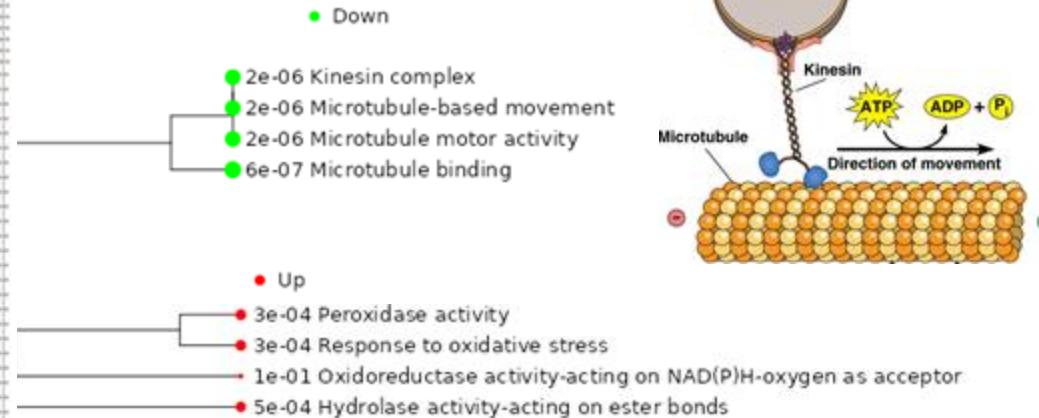
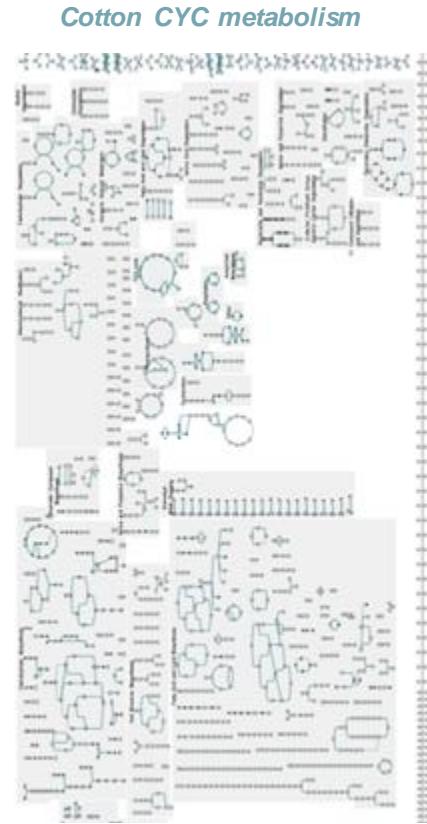
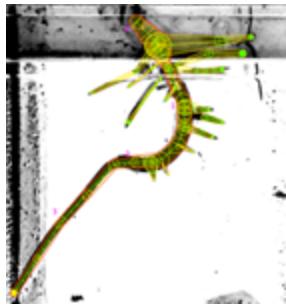
● Down ● Up

- 5e-02 DNA-directed RNA polymerase activity
- 4e-02 Transcription-DNA-templated
- 1e-02 Developmental process
- 9e-02 Regulation of cell cycle
- 9e-02 DNA-directed DNA polymerase activity
- 3e-04 DNA replication
- 2e-06 Kinesin complex
- 2e-06 Microtubule-based movement
- 2e-06 Microtubule motor activity
- 6e-07 Microtubule binding
- 1e-01 RNA processing
- 2e-03 RNA binding
- 1e-01 RRNA processing
- 9e-02 Nucleosome assembly
- 4e-02 Nucleosome
- 6e-02 Ribosome biogenesis
- 3e-04 Peroxidase activity
- 3e-04 Response to oxidative stress
- 1e-01 Oxidoreductase activity-acting on NAD(P)H-oxygen as acceptor
- 5e-04 Hydrolase activity-acting on ester bonds
- 7e-02 Photosystem II
- 4e-03 Photosynthesis
- 2e-01 Photosystem I
- 9e-02 Transferase activity-transferring acyl groups other than amino-acyl groups
- 4e-02 ATP hydrolysis coupled proton transport
- 1e-01 Transporter activity
- 1e-01 Vesicle-mediated transport
- 1e-01 Transferase activity-transferring glycosyl groups
- 2e-01 Oxidoreductase activity-acting on the CH-OH group of donors-NAD or NADP
- 2e-01 Response to biotic stimulus

Microtubule mediated vesicle trafficking of proton, divalent cation, anion and amino acid transporters

D130 vs WT

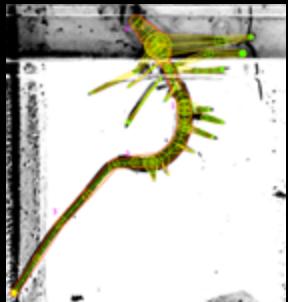
Ref: Roots & Ground control



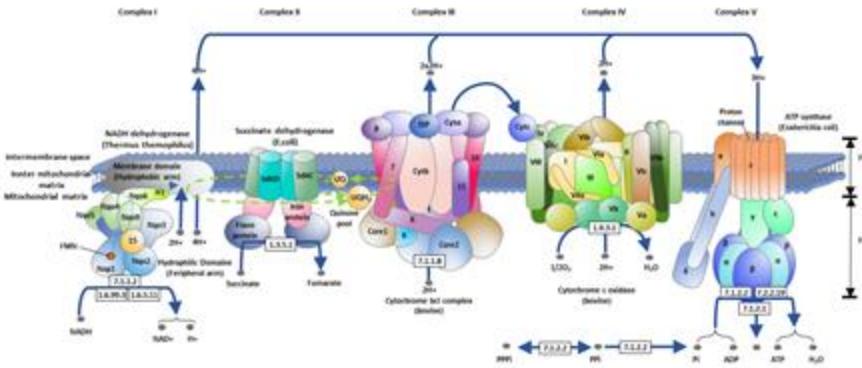
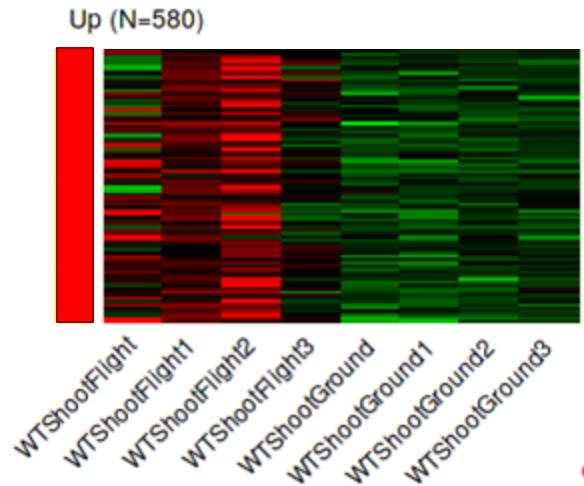
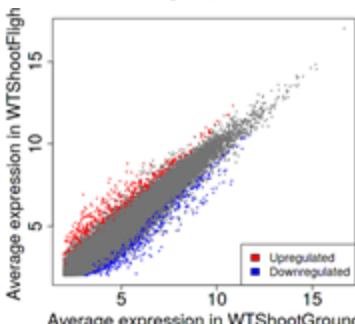
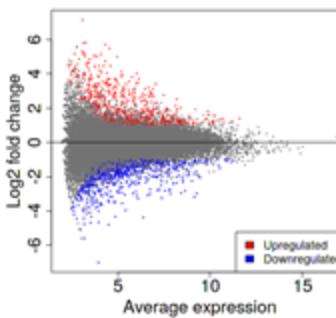
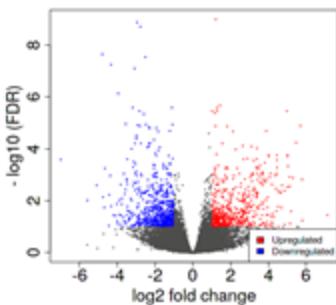
So what is difference between WT and AVP-ox on Earth?

D130 vs WT

Ref: Roots & Ground control



WT Shoot - FL vs GC - Protein recycling and OxPHOS Activation



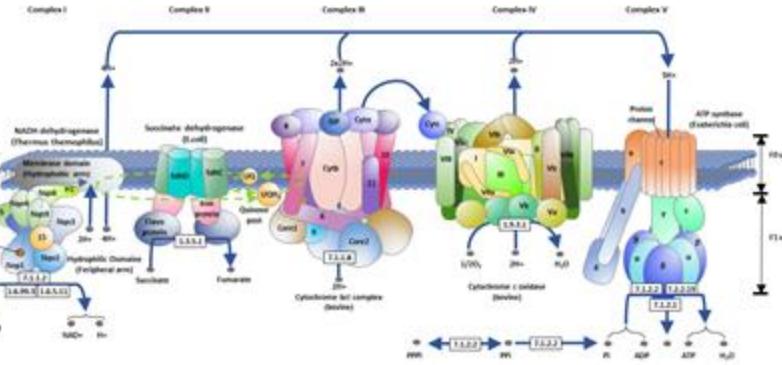
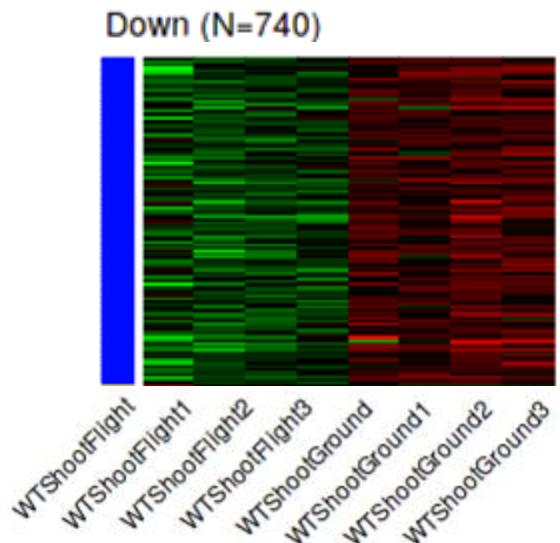
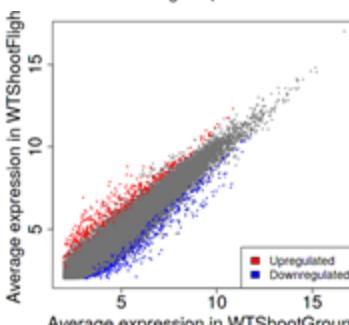
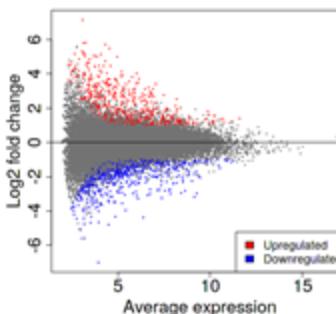
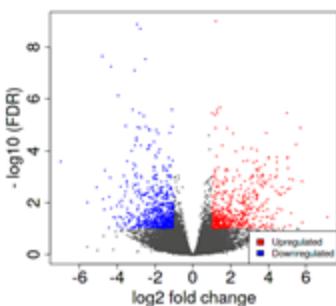
● Up

- 8e-04 Oxidation-reduction process
- 5e-06 Coenzyme A metabolic process
- 2e-05 Protein ubiquitination
- 1e-04 RNA processing
- 1e-04 Protein folding
- 1e-04 Regulation of transcription-DNA-templated
- 5e-04 Protein dephosphorylation
- 7e-03 Proteolysis involved in cellular protein catabolic process

? UP

► “ERAD” pathway on ER/Golgi

WT Shoot- FL vs GC - suppress OxPhos and ion transport

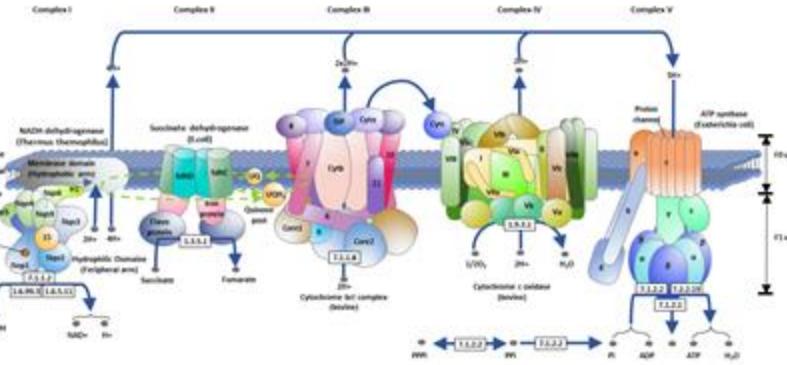
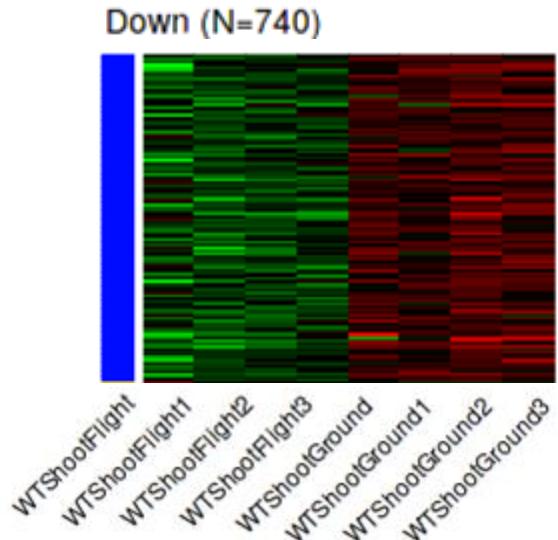
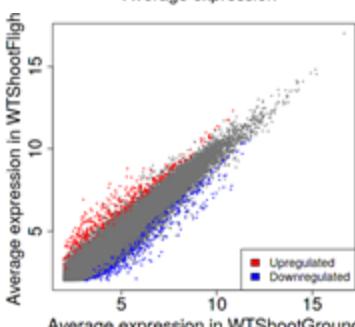
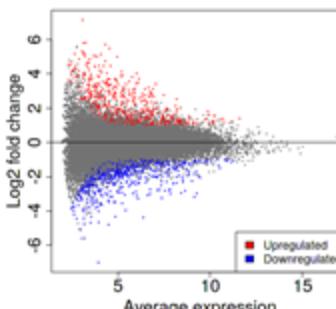
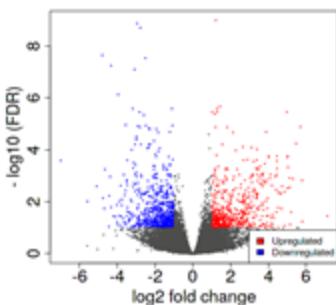


● Down

- 9e-03 Steroid biosynthetic process
- 2e-03 Oxidation-reduction process
- 5e-03 Protein repair
- 9e-03 Fatty acid biosynthetic process
- 4e-03 Drug transmembrane transport
- 1e-04 Transmembrane transport
- 9e-03 Transport
- 9e-03 Ion transport
- 4e-03 Lipid biosynthetic process
- 4e-03 Phospholipid transport
- 9e-03 Anion transport
- 9e-03 Carbohydrate metabolic process

? & down

WT Shoot suppress OxPhos and ion transport



● Down

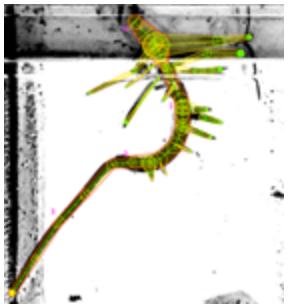
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- 1e-04 Transmembrane transport
- 9e-03 Transport
- 9e-03 Ion transport
- 4e-03 Lipid biosynthetic process
- 4e-03 Phospholipid transport
- 9e-03 Anion transport
- 9e-03 Carbohydrate metabolic process

? & down

On Earth D130 have a suppression of Kinesin complex relative to WT.

D130 vs WT

Ref: Roots & Ground control

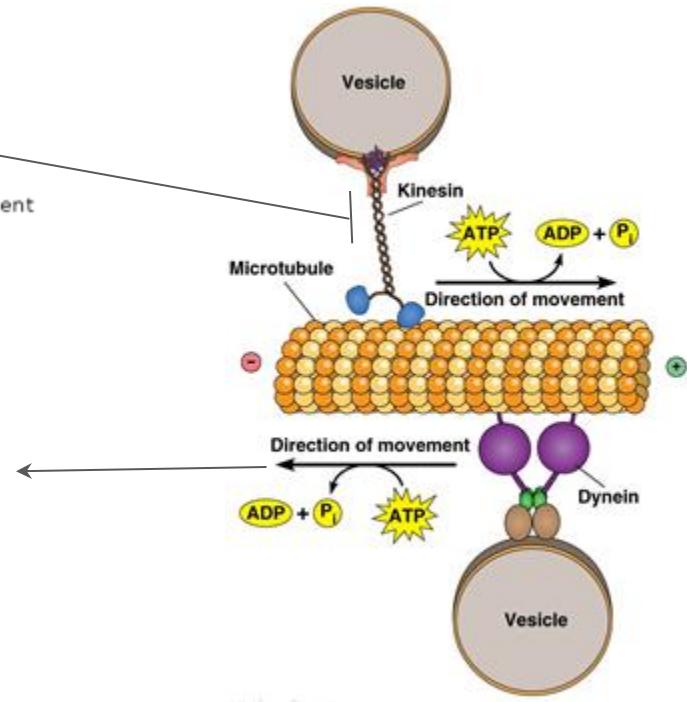


Down in D130 vs WT roots



Cotton CYC metabolism?

- Down
 - 2e-06 Kinesin complex
 - 2e-06 Microtubule-based movement
 - 2e-06 Microtubule motor activity
 - 6e-07 Microtubule binding



Does AVP-ox causes changes to vesicle mediated transport?

D130 vs WT

Ref: Roots & Ground Control

• Up

• 3e-04 Peroxidase activity

• 3e-04 Response to oxidative stress

• 1e-01 Oxidoreductase activity-acting on NAD(P)H-oxygen as acceptor

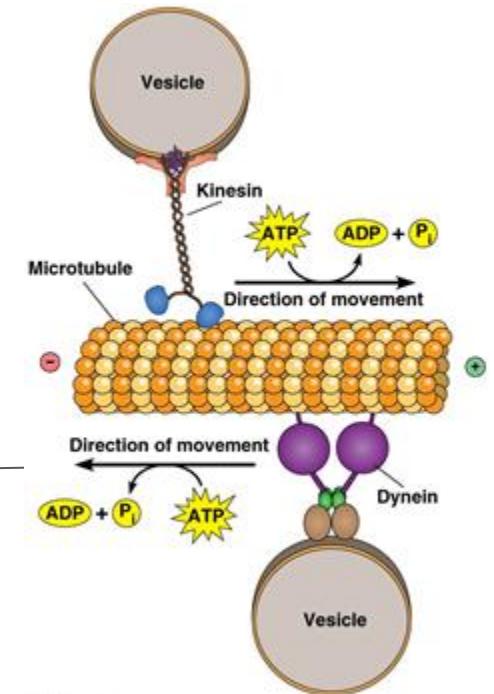
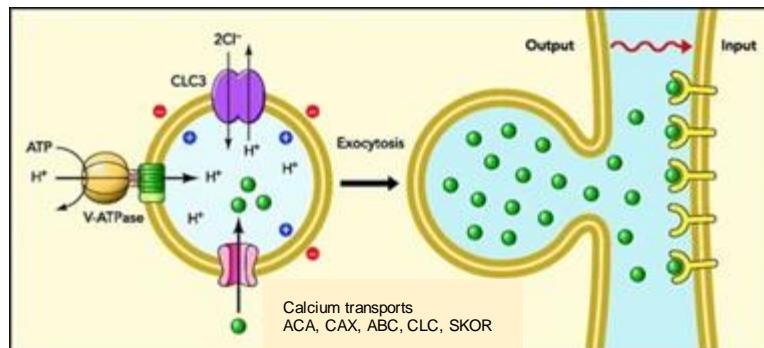
• Up

• 9e-02 Transferase activity-transferring acyl groups other than amino-acyl

• 4e-02 ATP hydrolysis coupled proton transport

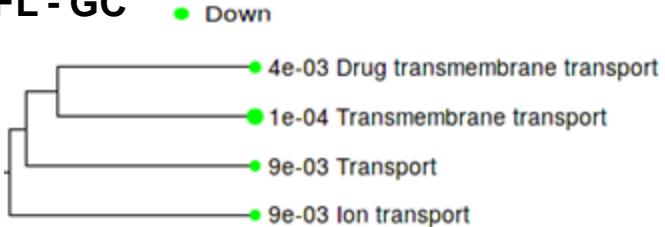
• 1e-01 Transporter activity

• 1e-01 Vesicle-mediated transport



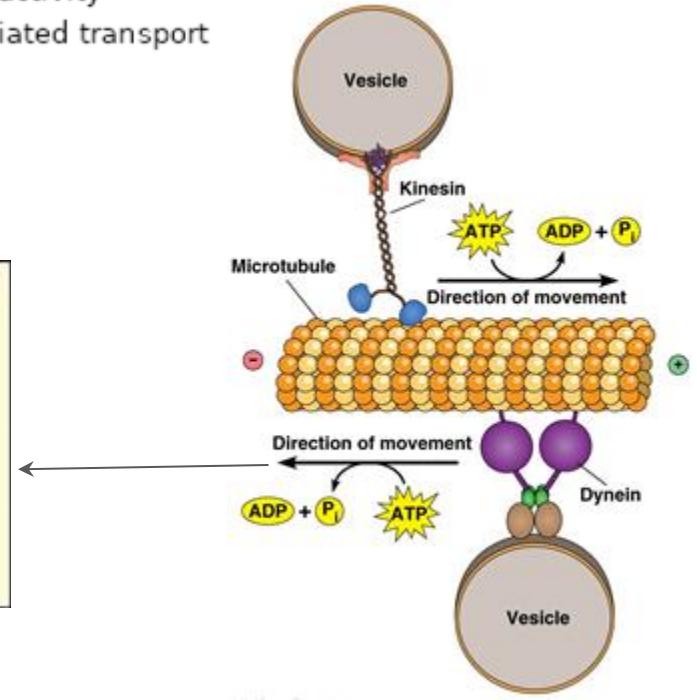
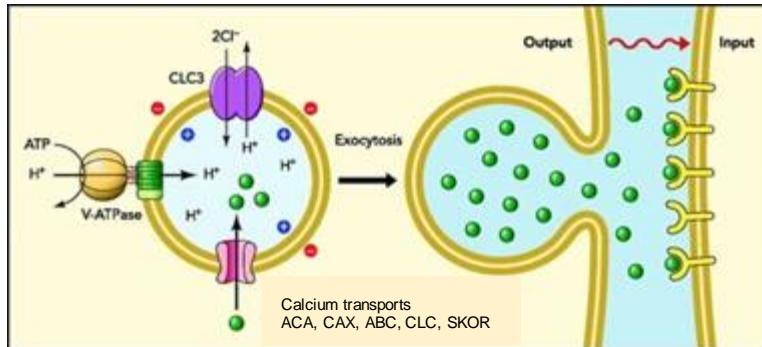
Could increased transporter activity due to increase ATP hydrolysis coupled proton transport this influence lateral root emergence and growth rates?

Anion ion transporters are suppressed in WT FL - GC

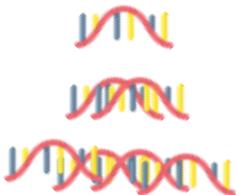
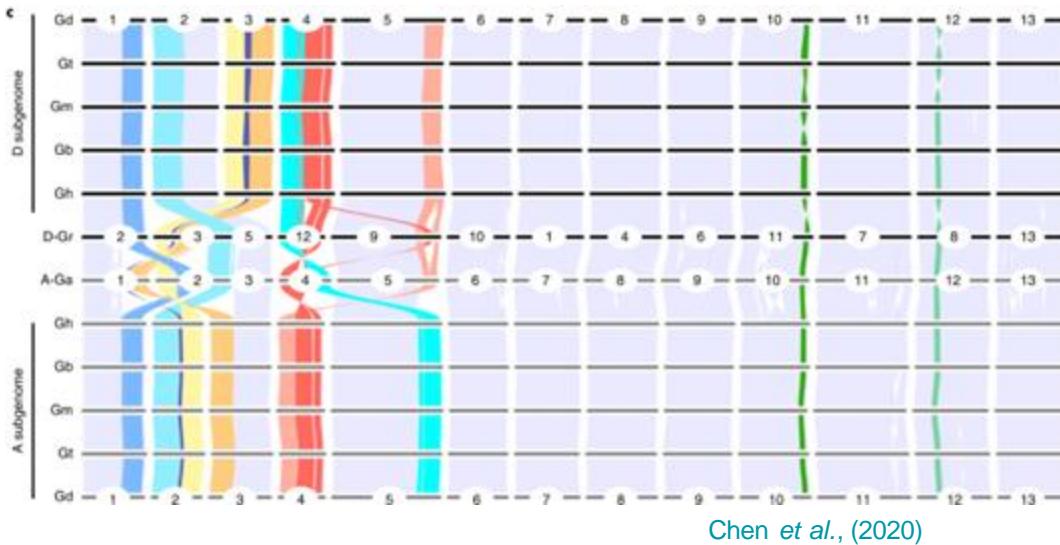
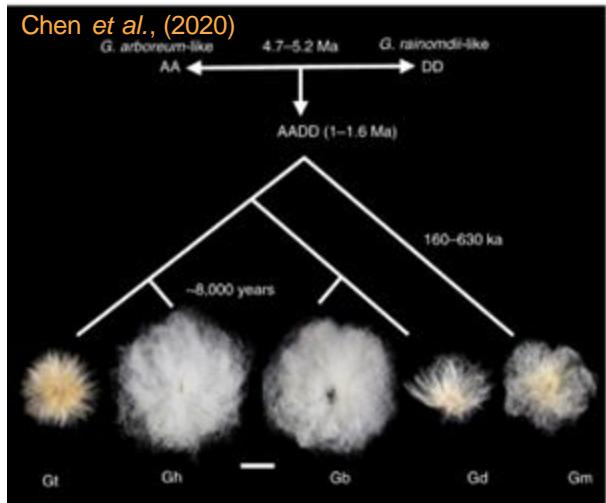


Up Enriched pathways in D130 vs WT roots

- 9e-02 Transferase activity-transferring acyl groups
- 4e-02 ATP hydrolysis coupled proton transport
- 1e-01 Transporter activity
- 1e-01 Vesicle-mediated transport



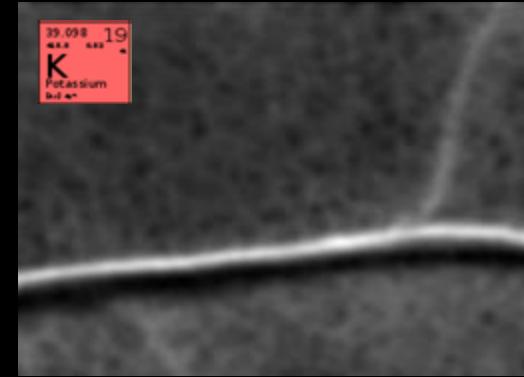
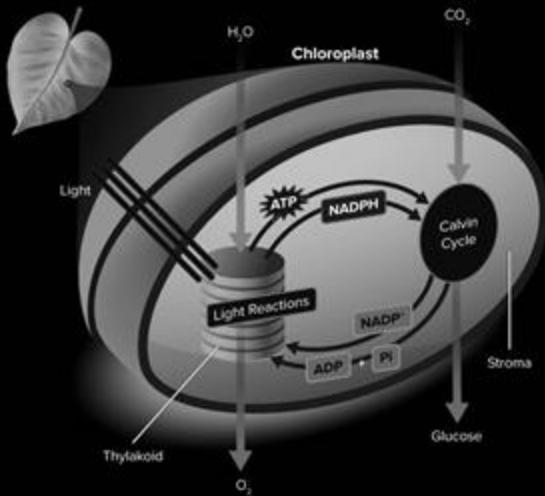
Cotton is allotetraploid, this hybrid has 4 times the number of chromosomes of a haploid organism.
 Allotetraploids are created as a result of both chromosome sets of each parents being present in gametes.



13 haploid
 26 diploid
 52 allo-tetraploid → polyplloid vigour → suppression of recessive alleles.
 Divergent mutation accumulation → D more easily squires substitution events.

Can polyploidy provide resistance to radiation? “Genomic redundancy”

Shoot adaptation to flight also involves ion transport



Photosynthesis \rightarrow Glycolysis \rightarrow ATP \rightarrow Ion transport

$CO_2 + H_2O$

Oxygen

Catalysis of
Amino acid / Sugar / lipids

Mineral uptake

the D homoeologs generally acquire substitution mutations more quickly than the A homoeologs in most lineages.

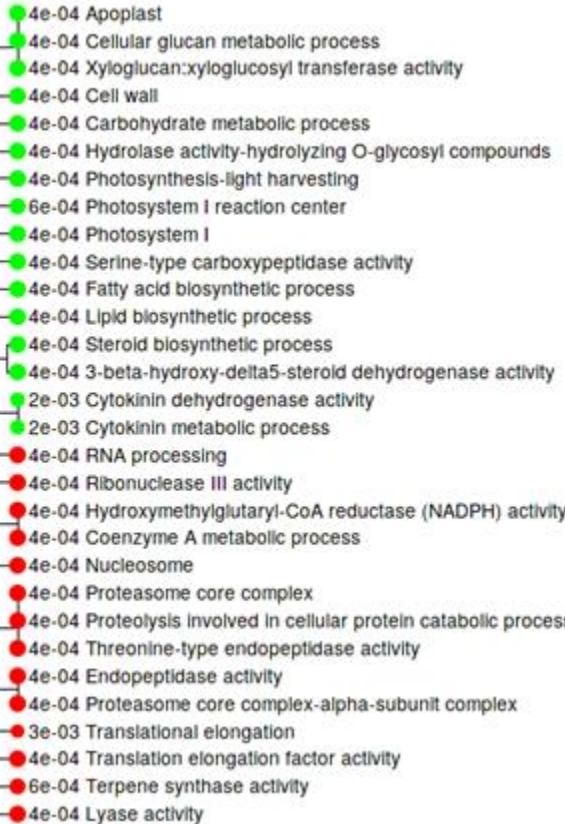
This relative acceleration of A-homoeolog divergence is mirrored in lineage-specific rate tests in Upland cotton has the fastest evolving A homoeologs and the slowest evolving D homoeologs among five polyploids.

lineage-specific rate heterogeneities between subgenomes and among different polyploid cottons.

GO Biological process enrichment

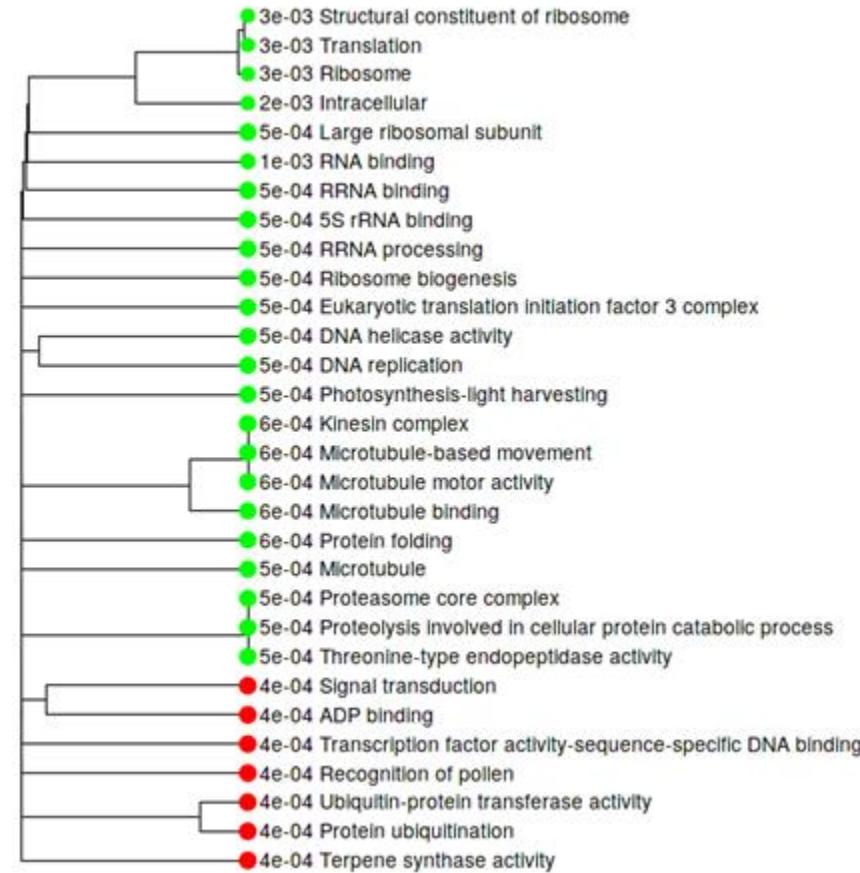
TICTOC RNAseq just WT Shoot

● Down ● Up



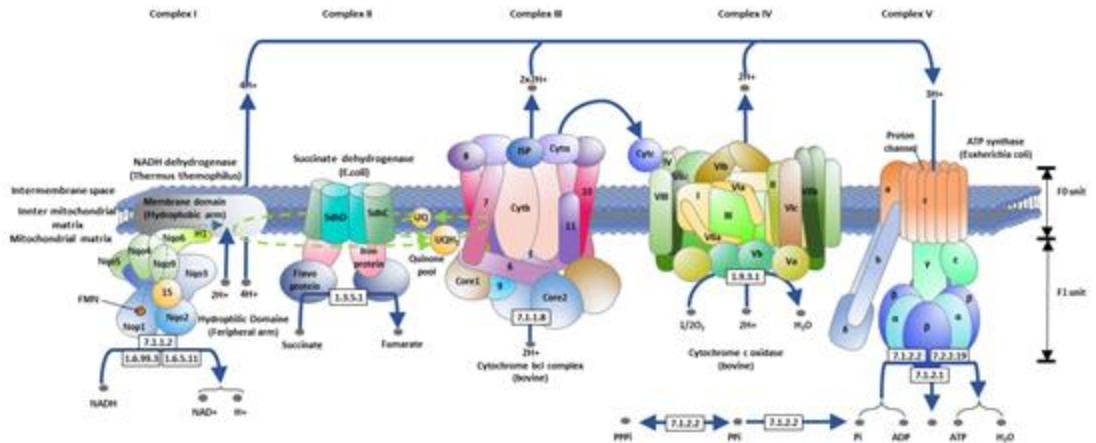
TICTOC RNAseq just WT Root

● Down ● Up

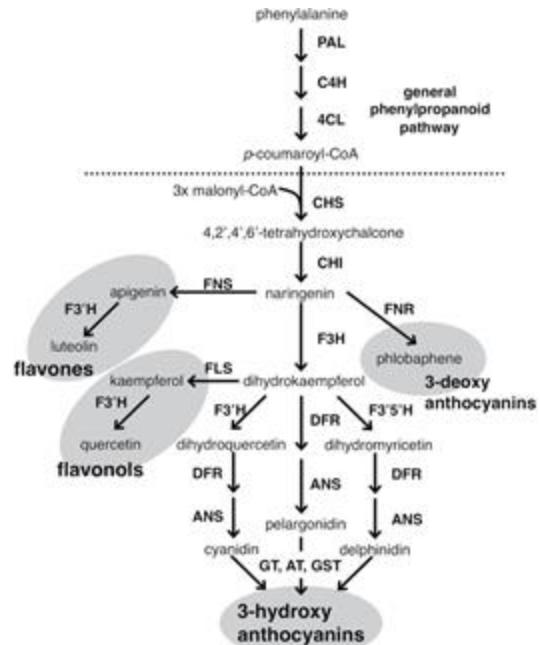
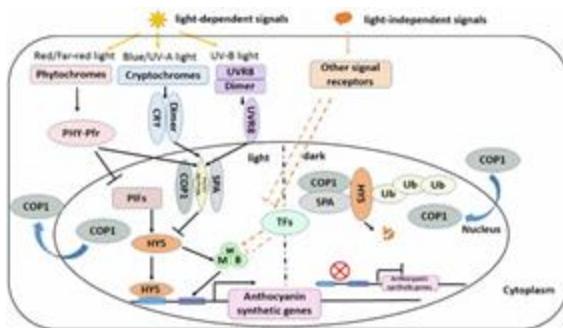


KEGG pathway

“How is OXPHOS both up and down in WT Shoots?”

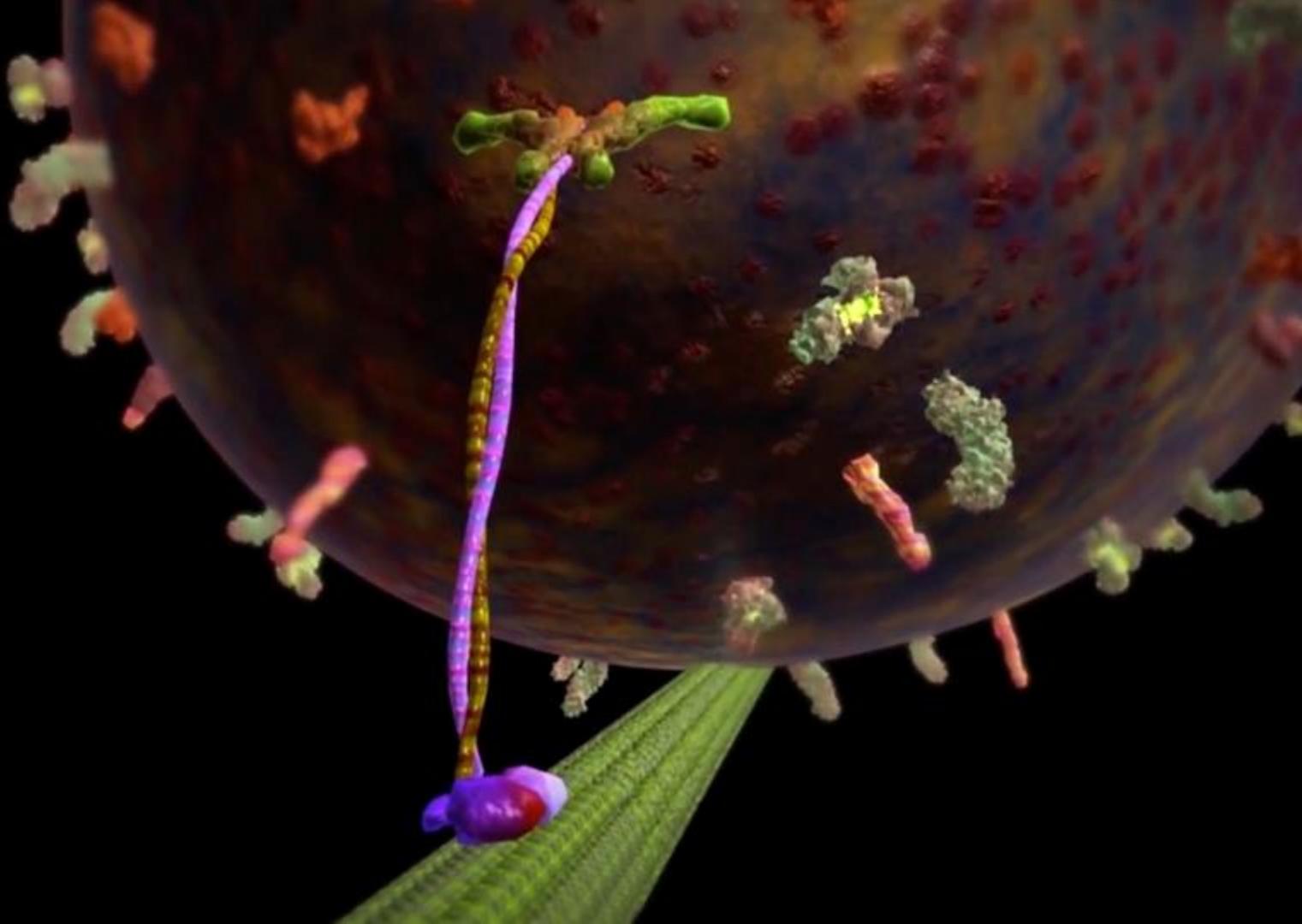


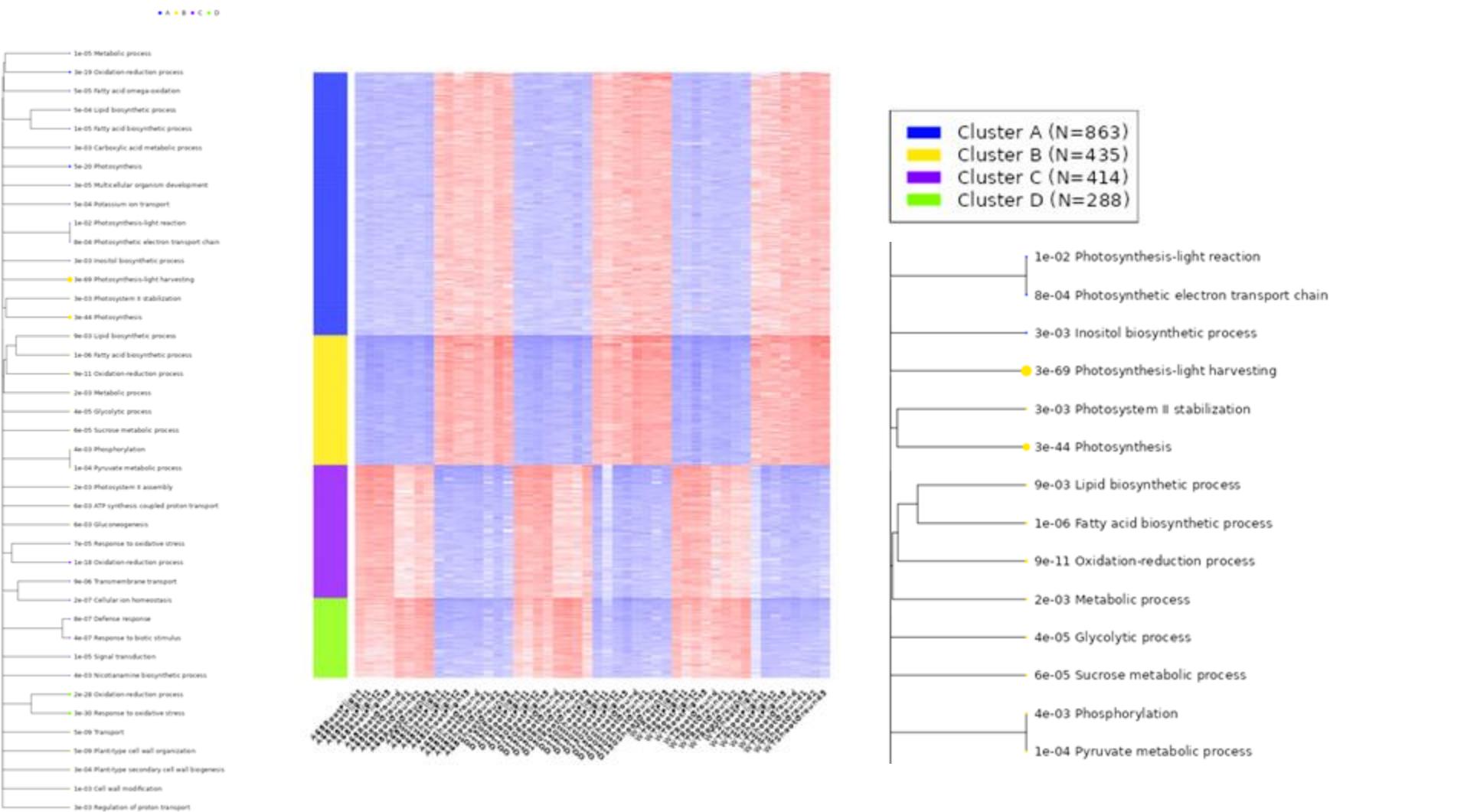
“How does this influence Anthocyanin metabolism?”



WT Shoot biochemistry showing increase in anthocyanin

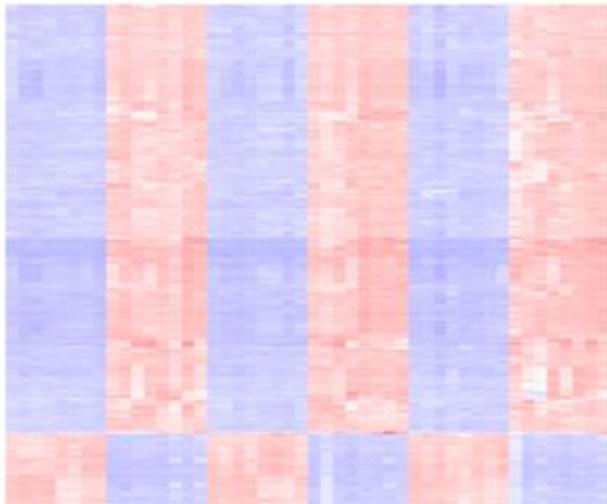
Insert WT anthocyanin data from Arko?





BCC cluster identification base on most variable 1000 loci

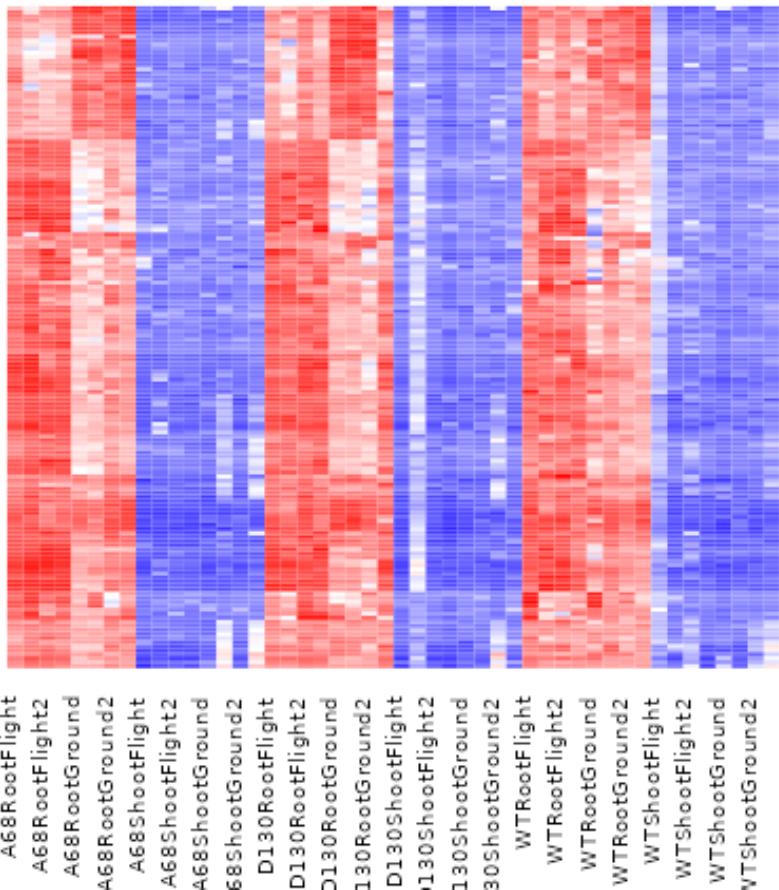
Cluster 1



A68RootFlight
 A68RootFlight2
 A68RootGround
 A68RootGround2
 A68ShootFlight
 A68ShootFlight2
 A68ShootGround
 A68ShootGround2
 D130RootFlight
 D130RootFlight2
 D130RootGround
 D130RootGround2
 130RootFlight
 130RootFlight2
 130RootGround
 130RootGround2
 WTRootFlight
 WTRootFlight2
 WTRootGround
 WTRootGround2
 WTSshootFlight
 WTSshootFlight2
 WTSshootGround
 WTSshootGround2

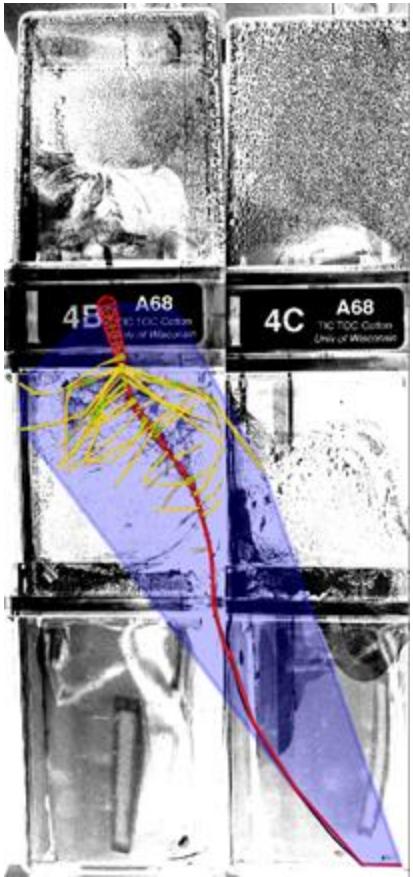
adj.Pval	Genes	Pathways
1.6e-62	39	Photosynthesis-light harvesting
4.6e-41	48	Photosynthesis
3.7e-26	114	Oxidation-reduction process
2.7e-25	16	Photosystem I reaction center
2.7e-25	92	Membrane
4.7e-23	27	Photosystem II
1.8e-21	21	Photosystem I
4.6e-19	16	Photosystem II oxygen evolving complex
8.6e-17	14	Extrinsic component of membrane
2.7e-14	56	Oxidoreductase activity
1.3e-13	45	Heme binding
1.4e-12	39	Iron ion binding
1.6e-09	32	Oxidoreductase activity-acting on paired donors-with incorporation
1.8e-09	7	Fructose-bisphosphate aldolase activity
6.3e-09	14	Fatty acid biosynthetic process

Cluster 2



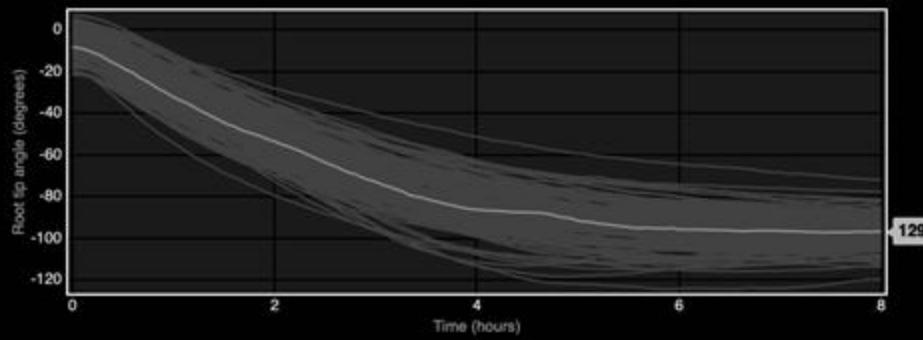
adj.Pval	Genes	Pathways
1.2e-20	18	Peroxidase activity
1.0e-15	37	Oxidation-reduction process
1.2e-15	23	Heme binding
2.2e-13	15	Transporter activity
1.8e-10	25	Membrane
2.6e-05	3	Voltage-gated anion channel activity
3.2e-05	17	Integral component of membrane
5.6e-05	6	Transmembrane transporter activity
8.0e-05	13	Oxidoreductase activity
2.5e-03	2	Carbonate dehydratase activity
3.1e-03	3	O-methyltransferase activity
3.1e-03	3	Response to biotic stimulus
4.2e-03	9	Transmembrane transport
4.6e-03	2	Growth factor activity
4.6e-03	3	Terpene synthase activity

Extra art page



Gravity influence auxin and other cation distribution

Natural variation in hormone movement influence gravitropic reorientation

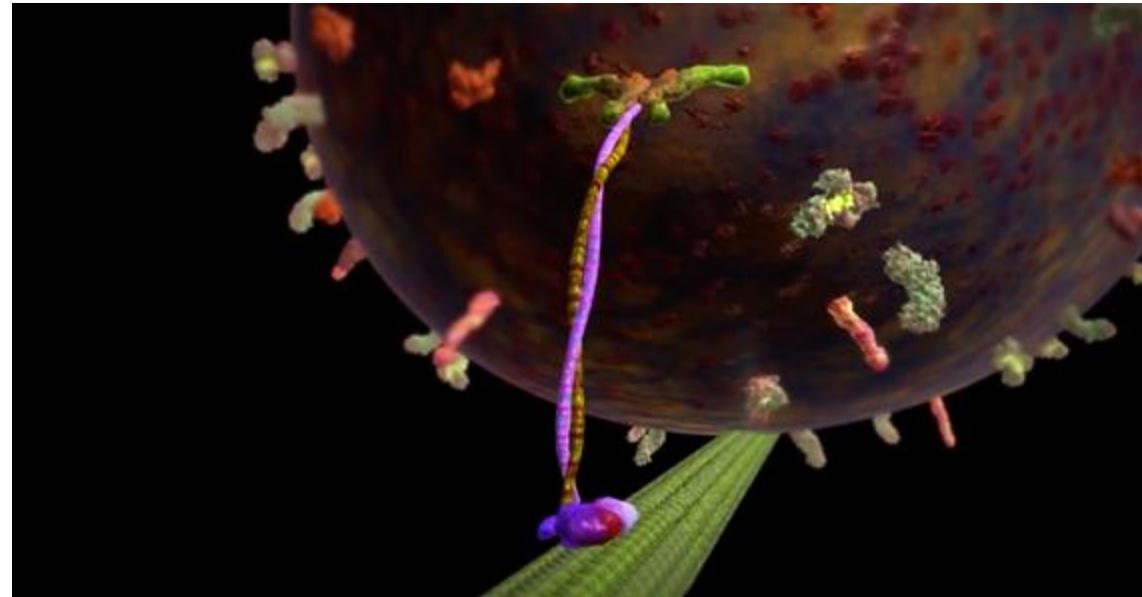


One historical example of how Cotton irrigation systems have influenced the Ariel sea

1kg of cotton is **10,000 litres**. glbau



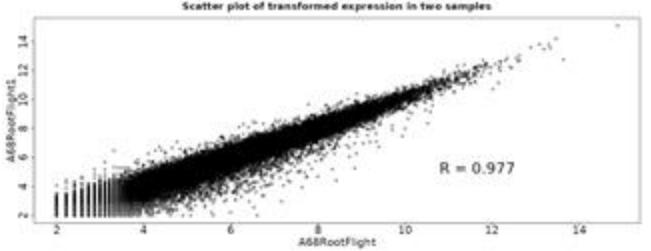
DNA repair slide



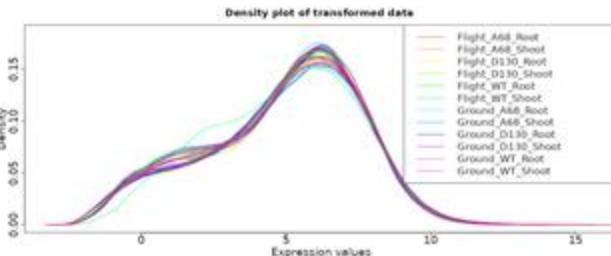
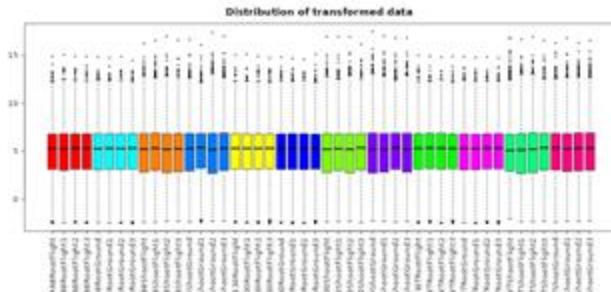
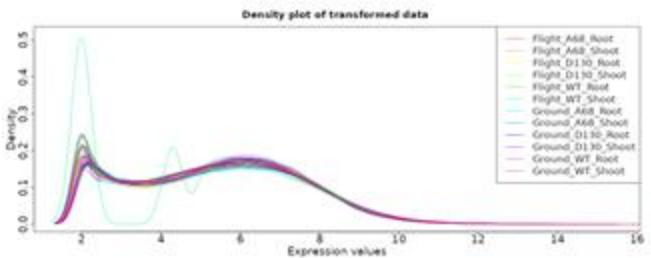
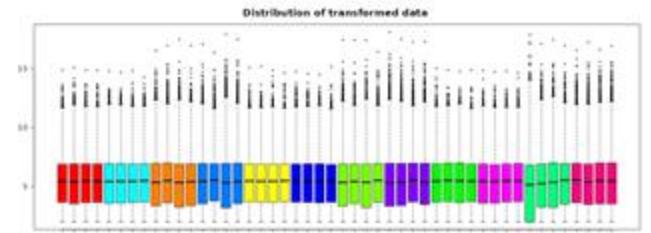
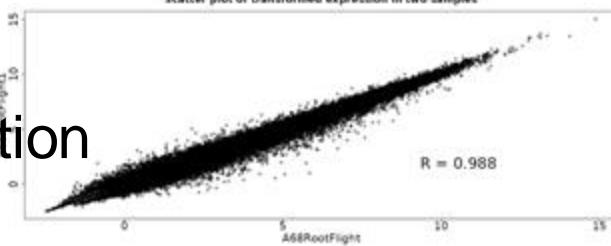
DNA repair slide

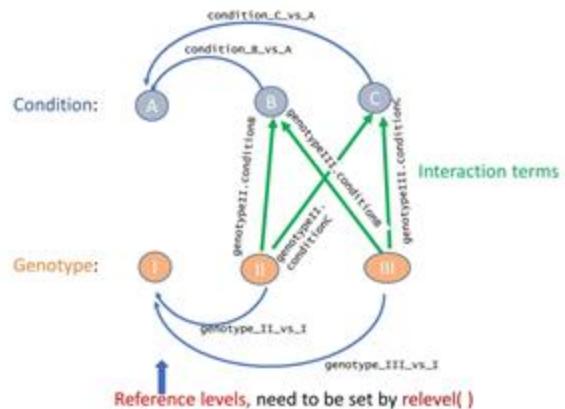


EdgeR

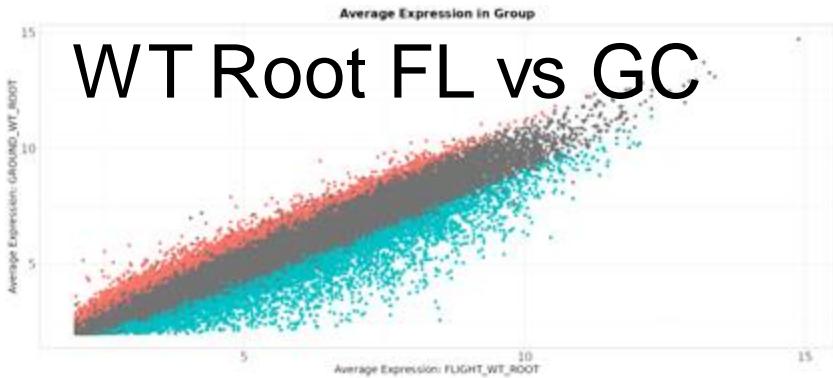


Log regularisation





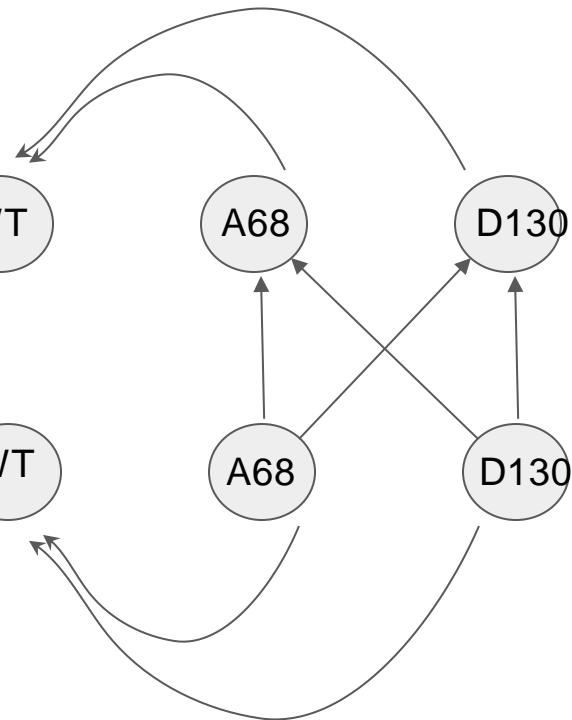
- The specific effect of condition B in genotype III: `genotypeIII.conditionB` (interaction)
- For genotype III, what is the difference between conditions B and A: $B - A + II.B = \text{condition}_B_vs_A + \text{genotypeIII.conditionB}$
- For condition B, what is the difference between genotypes II and I: $II.B + II.B = \text{genotype}_II_vs_I + \text{genotypeIII.conditionB}$
- For condition C, what is the difference between III and I: $(III.I - III.I) + III.C - II.C$
= $\text{genotype}_III_vs_I - \text{genotype}_II_vs_I + \text{genotypeIII.conditionC} - \text{genotypeII.conditionC}$



Reference level

Condition: uG

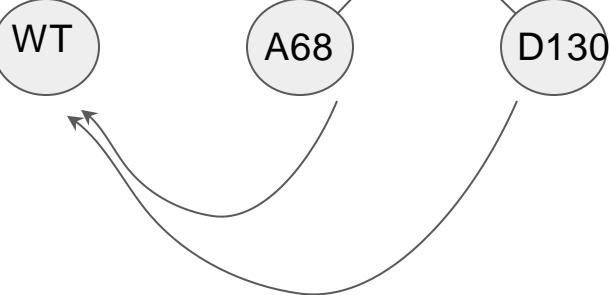
Genotype: WT



Reference level

Condition: 1G

Genotype: WT



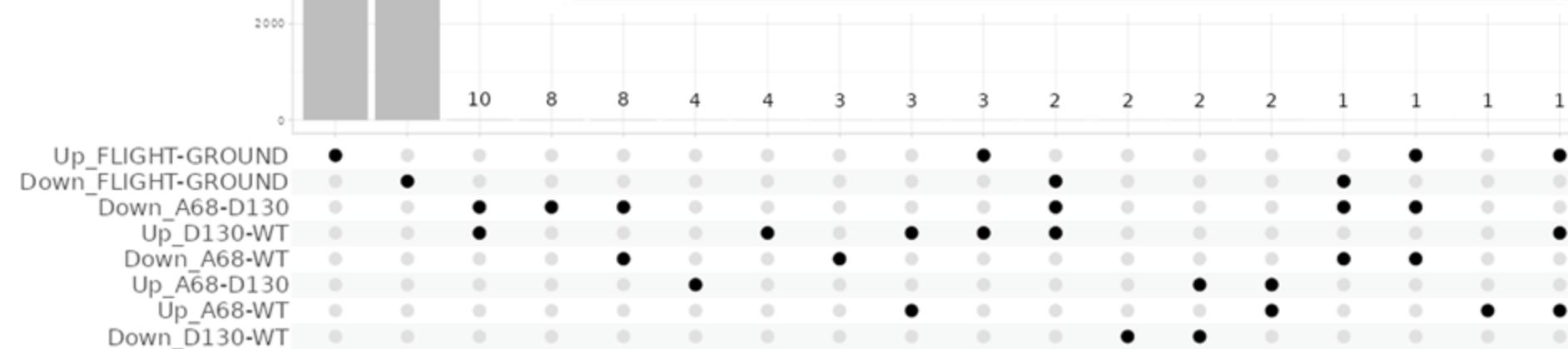
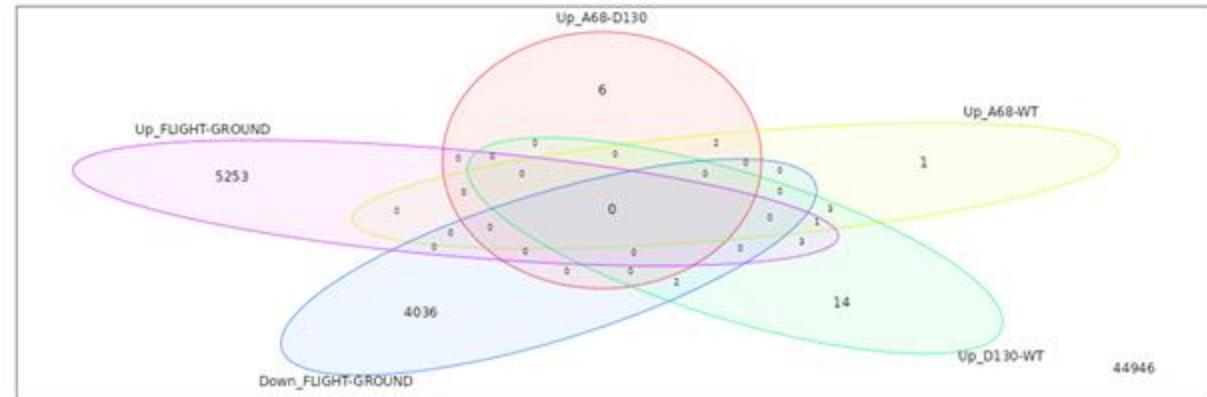
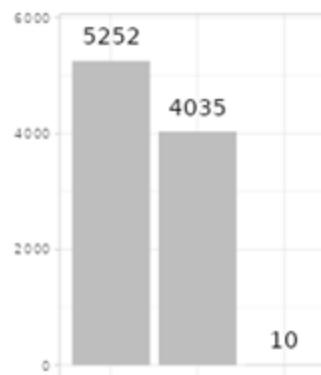
Baseline Genotype Effect
A68 vs WT
D130 vs WT

3 factor model

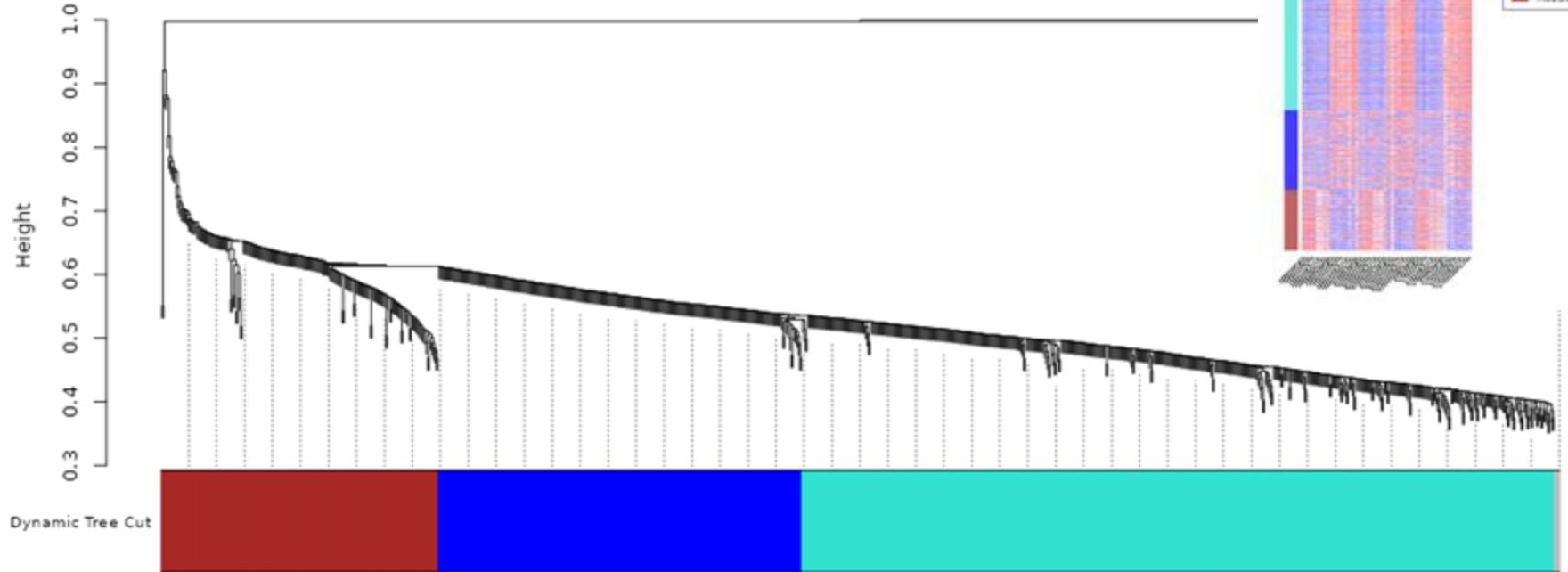
Tissues: Root

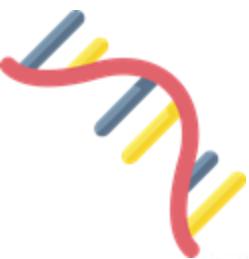
Environment:GC

Genotype

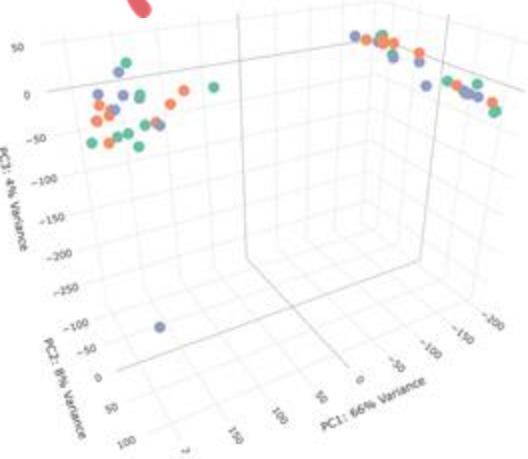


Gene dendrogram and module colors





Principal component analysis



Genotype Tissue

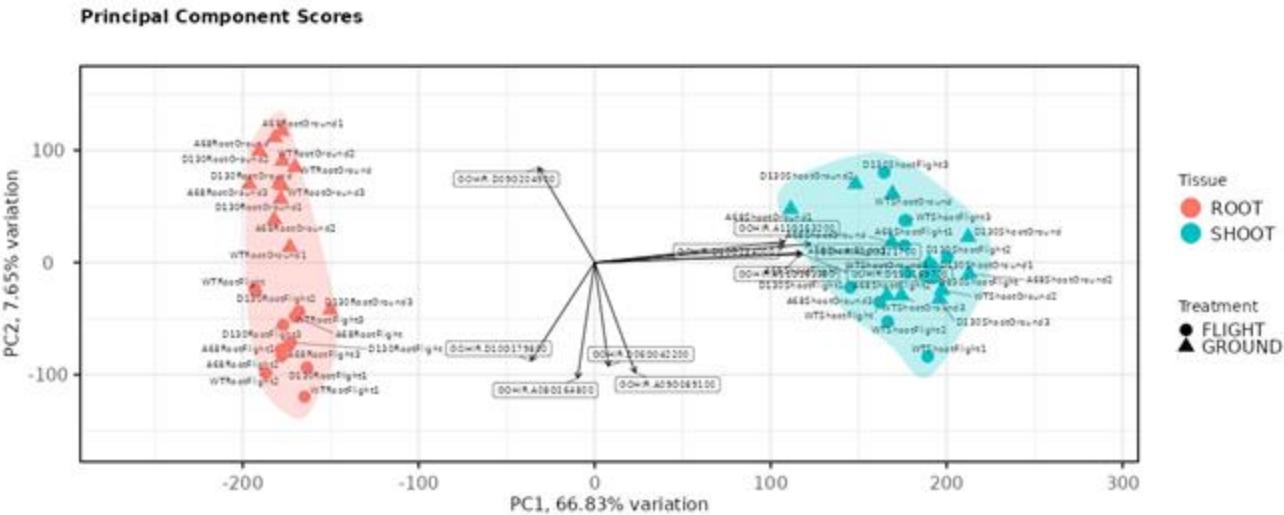
- A6B ROOT
- A6B SHOOT
- D13D ROOT
- D13D SHOOT
- WT ROOT
- WT SHOOT

PC1 is correlated with Tissue ($p=3.14e-47$).

PC2 is correlated with Treatment ($p=9.39e-05$).

PC3 is genotype

PC4 is correlated with Treatment ($p=4.01e-04$).



Tissue

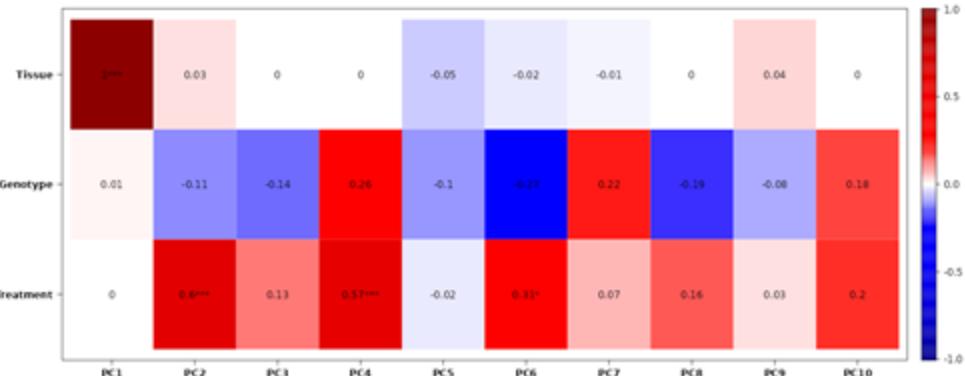
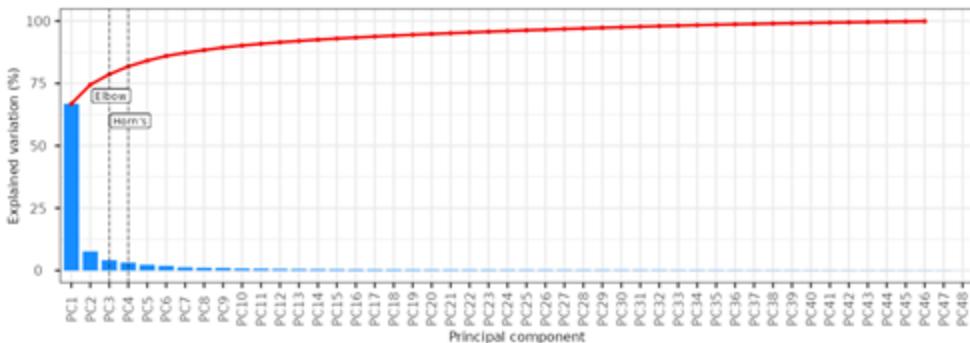
- ROOT
- SHOOT

Treatment

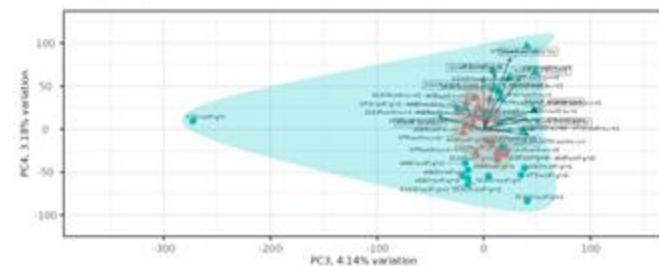
- FLIGHT
- GROUND

All samples

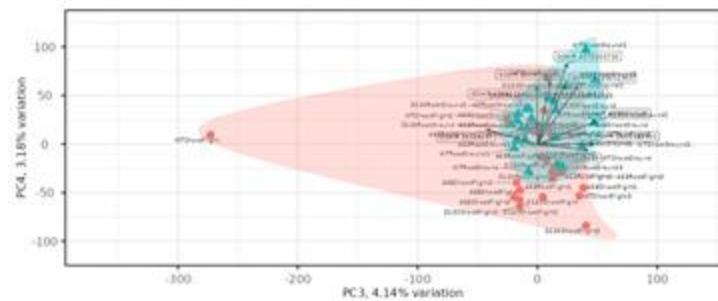
SCREE plot



Principal Component Scores



Principal Component Scores



Treatment
FLIGHT
GROUND

- Treatment
- Genotype
- Tissue

Reference/baseline level for Treatment

Reference/baseline level for Genotype

Reference/baseline level for Tissue

GROUND

WT

ROOT

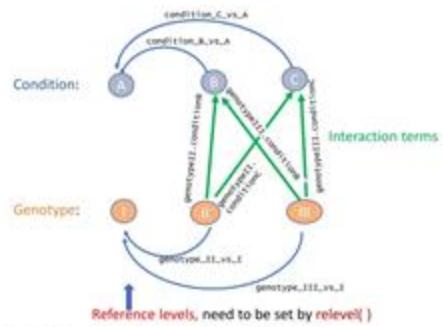
Interaction terms between factors (e.g., genotypes respond differently to treatment):

- Treatment:Genotype
- Treatment:Tissue
- Genotype:Tissue

Model: expression = Treatment + Genotype + Tissue + Treatment:Genotype + Treatment:Tissue + Genotype:Tissue

2. Select one or more comparisons:

- Treatment: FLIGHT vs. GROUND
- Treatment: GROUND vs. FLIGHT
- Genotype: A68 vs. D130
- Genotype: A68 vs. WT
- Genotype: D130 vs. A68
- Genotype: D130 vs. WT
- Genotype: WT vs. A68
- Genotype: WT vs. D130
- Tissue: ROOT vs. SHOOT
- Tissue: SHOOT vs. ROOT



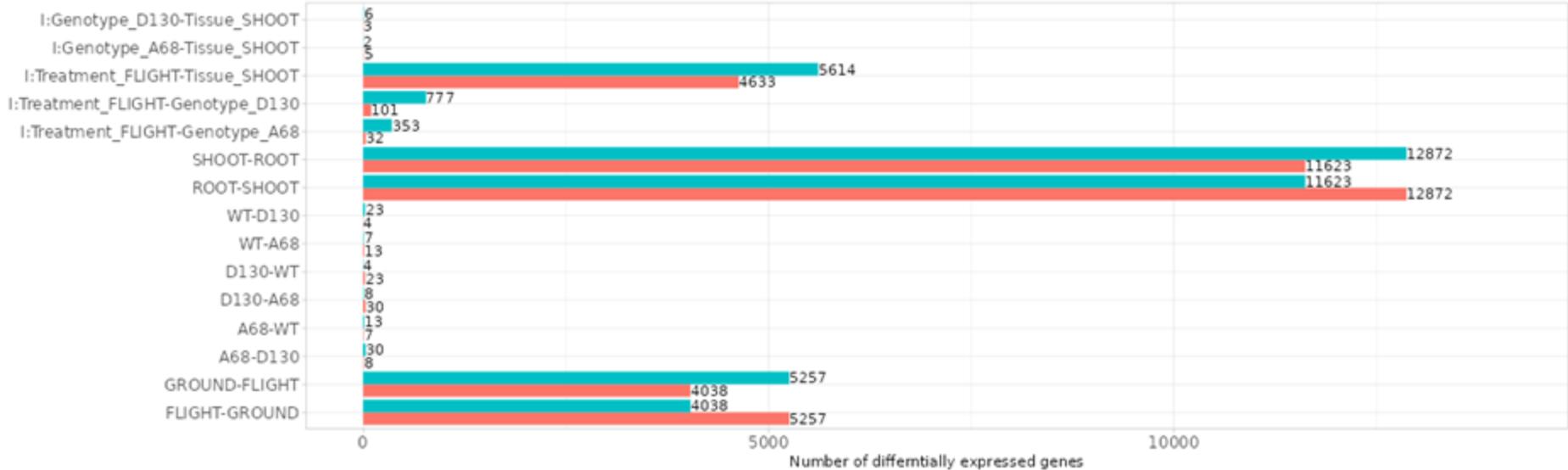
Condition:

Genotype:

Reference levels, need to be set by relevant()

- The specific effect of condition B in genotype III: genotypeIII*conditionB [interaction]
- For genotype III, what is the difference between conditions B and A: -B + H.B + condition_B_vs_A + genotypeIII*conditionB
- For condition B, what is the difference between genotypes I and II: H.I - H.II + genotype_I_vs_II + genotypeII*conditionB
- For condition C, what is the difference between II and III: (H.II) - (H.III) + H.C - H.C
= genotype_II_vs_III - genotype_I_vs_III + genotypeIII*conditionC - genotypeII*conditionC

Regulation Up Down



- Treatment
 Genotype
 Tissue

Reference/baseline level for Treatment

Ground

Reference/baseline level for Genotype

WT

Reference/baseline level for Tissue

Root

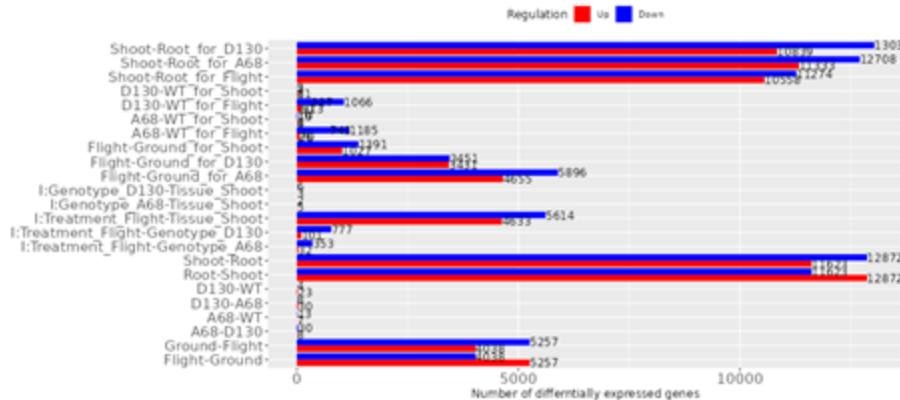
Interaction terms between factors(e.g.
genotypes respond differently to treatment?):

- Treatment:Genotype
 Treatment:Tissue
 Genotype:Tissue

Model: expression ~ Treatment + Genotype + Tissue + Treatment:Genotype + Treatment:Tissue + Genotype:Tissue

2. Select one or more comparisons:

- Treatment: Flight vs. Ground
 Treatment: Ground vs. Flight
 Genotype: A68 vs. D130
 Genotype: A68 vs. WT
 Genotype: D130 vs. A68
 Genotype: D130 vs. WT
 Genotype: WT vs. A68
 Genotype: WT vs. D130
 Tissue: Root vs. Shoot
 Tissue: Shoot vs. Root



Data

Species: *Gossypium hirsutum*(phytozomev12_v1.1)

Number of samples: 48

Number of genes converted and filtered: 54267

Model: ~ Treatment + Genotype + Tissue + Treatment:Genotype + Treatment:Tissue + Genotype:Tissue

Input file type: RNA-seq read count file

Pre-processing and exploratory data analysis settings:

Min. counts: minCounts= 0.5

Min. counts samples: NminSamples= 1

Counts data transformation method: rlog: regularized log

Method for differential expression: CountsDEGMethod= 3 (DESeq2)

number of genes in heatmap: nGenes= 100

number of genes in k-means clustering: nGenesKNN= 2000

number of clusters in k-means clustering: nClusters= 4

Promoter analysis for k-means clustering: radioPromoterKmeans= 600 bp

Differential expression settings:

FDR cutoff: limmaPval= 0.1

Fold-change cutoff: limmaFC= 2

Promoter analysis for DEGs: radio.promoter= 600 bp

Pathway analysis settings:

Pathway analysis methods: pathwayMethod= GAGE

FDR cutoff: pathwayPvalCutoff= 0.2

Min size for gene set: minSetSize= 15

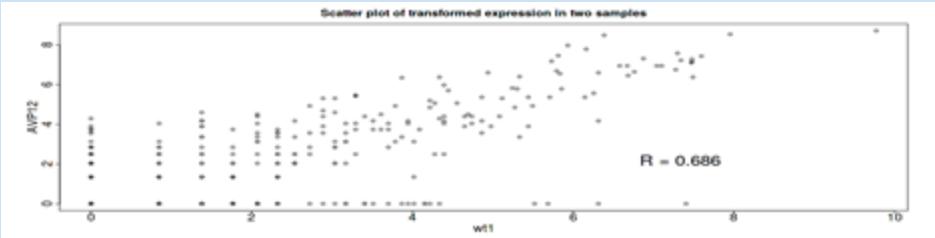
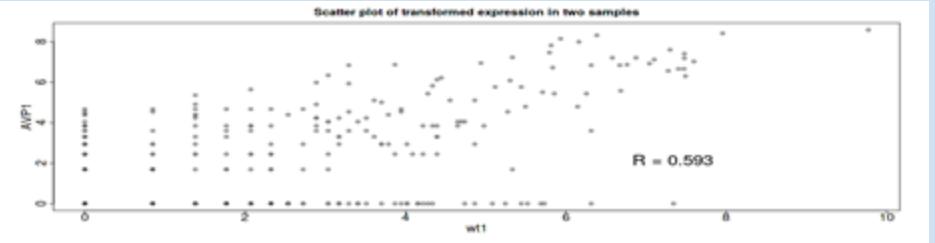
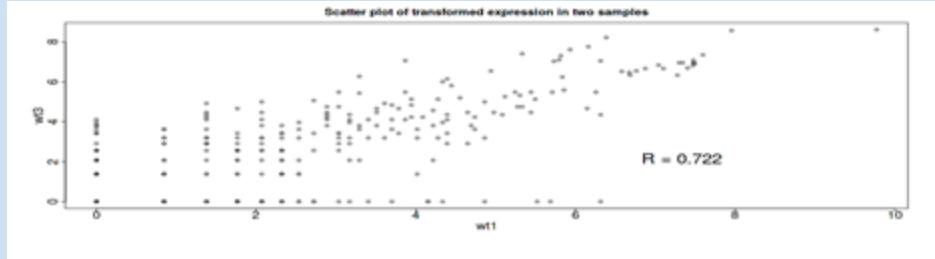
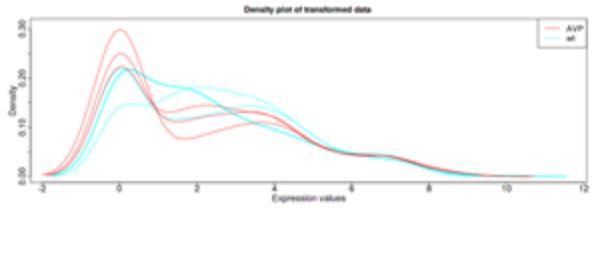
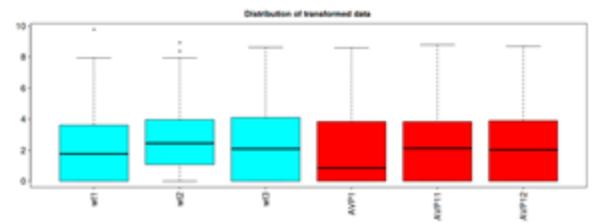
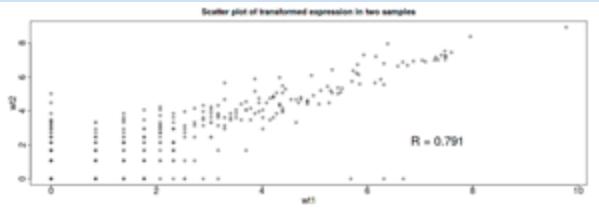
Max size for gene set: maxSetSize= 2000

PREDA settings:

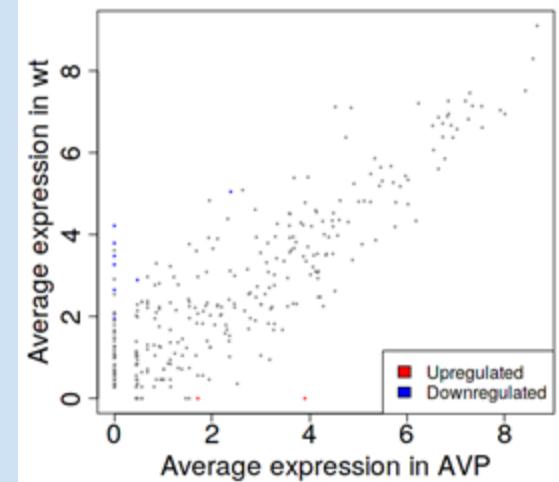
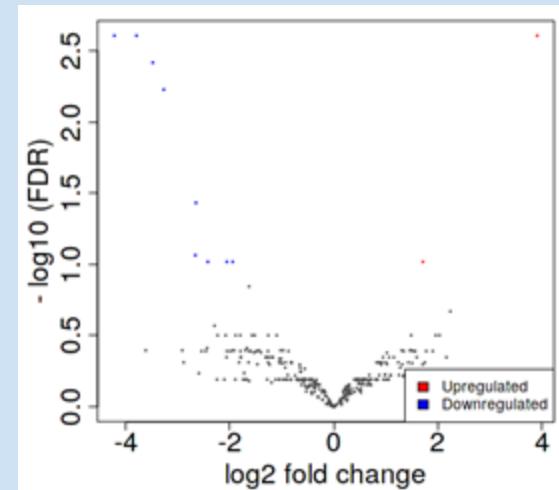
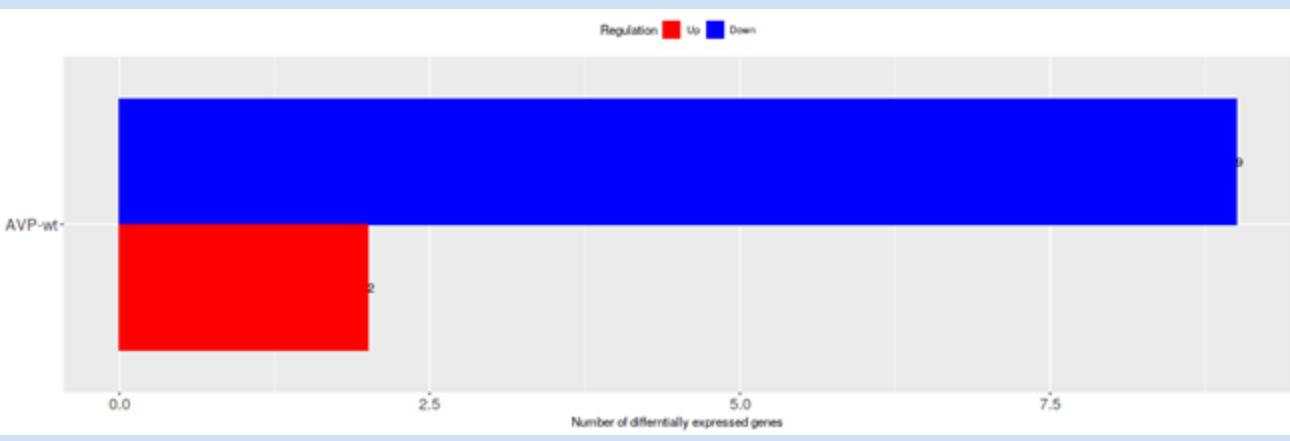
FDR cutoff: RegionsPvalCutoff= 0.01

FDR cutoff: StatisticCutoff= 0.5

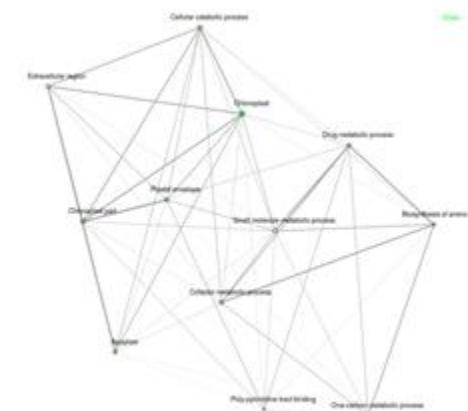
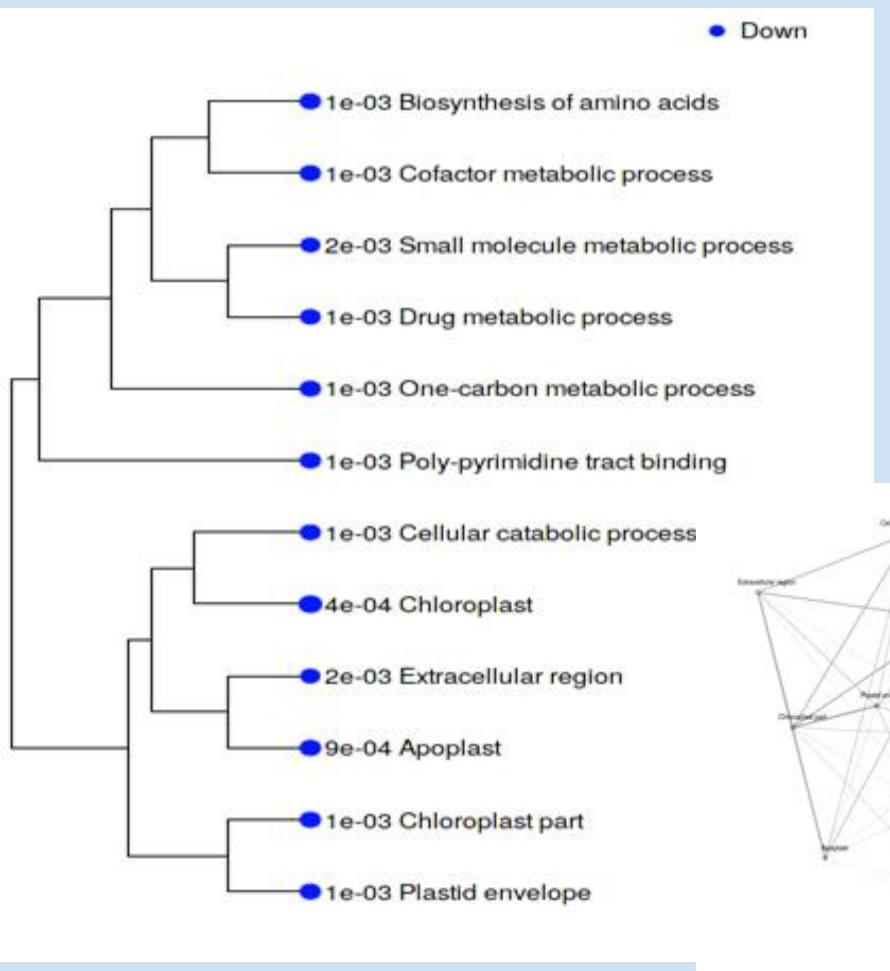
AVP - WT GC proteomics (GEO)



AVP -WT proteomics



AVP -WT proteomics

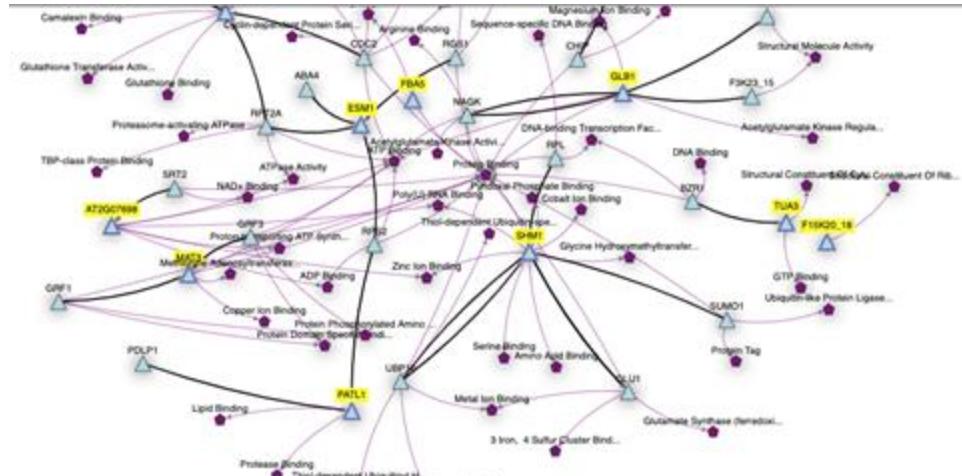


Direction	adj.Pval	nGenes	Pathways
Down regulated	4.0e-04	7	Chloroplast
	9.3e-04	4	Apoplast
	1.0e-03	4	Cofactor metabolic process
	1.0e-03	4	Plastid envelope
	1.0e-03	5	Chloroplast part
	1.0e-03	2	One-carbon metabolic process
	1.0e-03	2	Poly-pyrimidine tract binding
	1.0e-03	3	Biosynthesis of amino acids
	1.2e-03	5	Cellular catabolic process
	1.4e-03	4	Drug metabolic process
	2.0e-03	5	Small molecule metabolic process
	2.0e-03	5	Extracellular region

AVP -WT proteomics

Regulation	EnsemblID	log2 FoldChange	Adj.Pval	wt1	wt2	wt3	AVP1	AVP11	AVP12
Up	AT2G27720	3.90591444595305	2.47E-03	0	0	0	3.84026179641587	4.13709360897494	3.74038793246835
Up	AT4G01900	1.71030951025522	9.59E-02	0	0	0	1.68728469191784	1.41115503695776	2.03248880189007
Down	AT5G19770	-4.21883033587692	2.47E-03	3.94485844580754	4.47897180503294	4.23266075679027	0	0	0
Down	AT2G36880	-3.79336434630723	2.47E-03	3.51096191927738	4.06177619758669	3.8073549220576	0	0	0
Down	AT3G14210	-3.47995193718071	3.82E-03	3.40599235967584	3.84799690655495	3.18586654531133	0	0	0
Down	AT4G26530	-3.27005664588822	5.92E-03	3.16992500144231	3.73335434061383	2.90689059560852	0	0	0
Down	AT4G37930	-2.66318032302784	8.62E-02	5.3327079336406	5.06608919045777	4.74953426766926	1.68728469191784	2.11066391270741	3.36084181805888
Down	AT4G02520	-2.65101079241775	3.69E-02	2.0703893278914	3.32192809488736	2.56071495447448	0	0	0
Down	AT1G72150	-2.4232556789072	9.59E-02	3.03562390973072	2.4594316186373	3.18586654531133	0	1.41115503695776	0
Down	AT2G07698	-2.05712232520541	9.59E-02	2.0703893278914	2.72246602447109	1.37851162325373	0	0	0
Down	AT1G02560	-1.94598108063034	9.59E-02	2.32192809488736	2.13750352374994	1.37851162325373	0	0	0

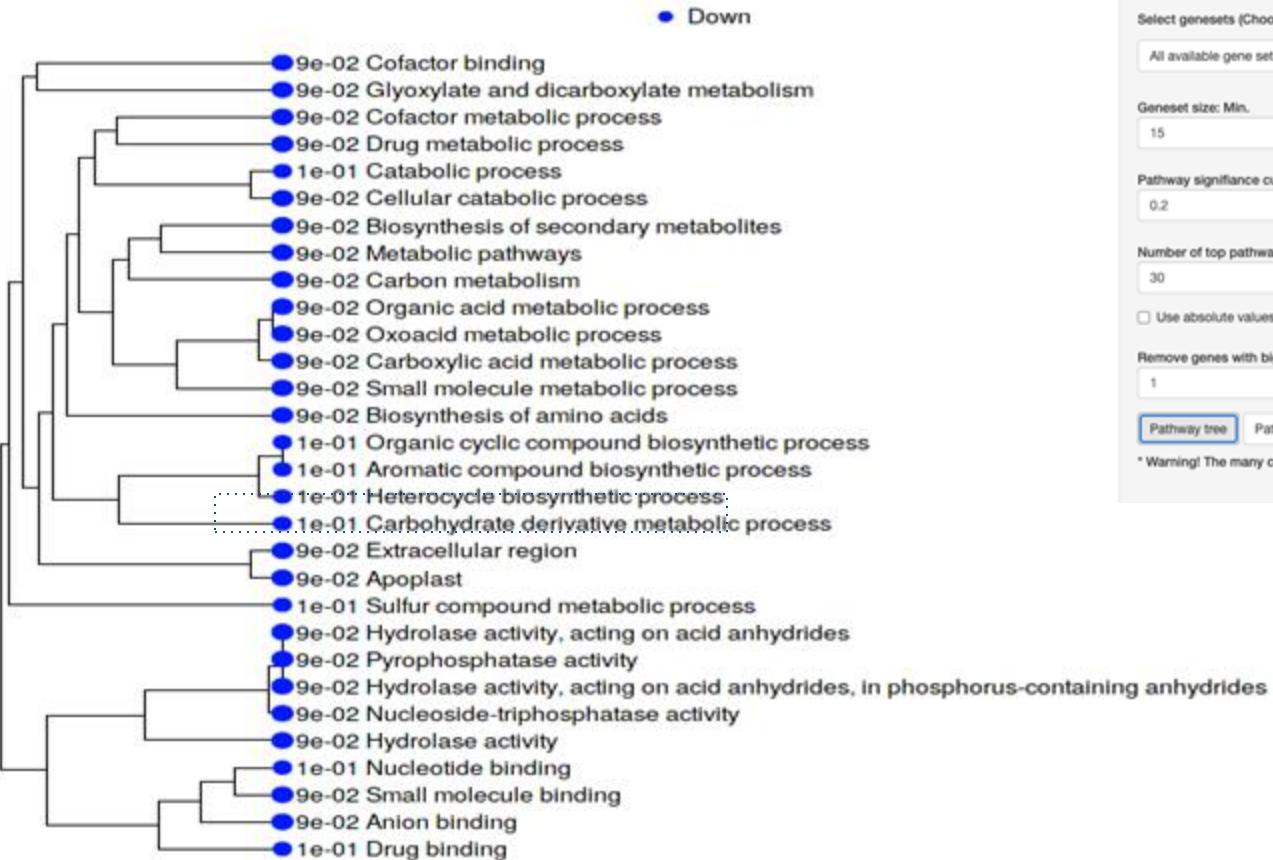
Knetminer analysis



TYPE #	DESCRIPTION	LUCINE SCORE	P-VALUE	TOTAL GENES	USER GENES
Ubiquitin-dependent Protein C...	1.00	0.00345	3062	5	
Protein Ubiquitination	1.00	0.00436	3904	5	
Response To Cold	1.00	0.00065	2270	5	
Modification-dependent Protei...	1.00	0.00099	1382	4	
Protein Transport	1.00	0.00414	2060	4	
Defense Response To Bacterium	1.00	0.00611	2305	4	
Response To UV-B	1.00	0.00499	2173	4	
Response To Cadmium Ion	1.00	0.00290	1862	4	
Root Development	1.00	0.00102	1395	4	
Sister Chromatid Cohesion	1.00	0.00009	253	3	
Defense Response	1.00	0.05258	2668	3	
Chromatin Assembly Or Disease...	1.00	0.00053	471	3	
Response To Heat	1.00	0.01268	1488	3	
Chromatin Silencing At Silent...	1.00	0.00064	505	3	
Plant-type Hypersensitive Res...	1.00	0.00654	1158	3	

GENE	SCORE	P-VALUE	TOTAL GENES	USER GENES
ATP Binding	1.00	0.00003	8666	10
Protein Tag	1.00	0.00036	6580	6
ATPase Activity	1.00	0.00021	1769	5
Zinc Ion Binding	1.00	0.00946	2622	4
Ubiquitin Protein Ligase Bind...	1.00	0.00250	1786	4
Metal Ion Binding	1.00	0.09413	5713	4
DNA-binding Transcription Fac...	1.00	0.11509	3910	3
RNA Binding	1.00	0.03229	2162	3
Poly(U) RNA Binding	1.00	0.00005	210	3
Sequence-specific DNA Binding	1.00	0.07302	3104	3
ADP Binding	1.00	0.00022	349	3
NAD+ Binding	1.00	0.00003	177	3
DNA-dependent ATPase Activity	1.00	0.01290	506	2
Structural Molecule Activity	1.00	0.01120	469	2

AVP -WT proteomics



Select a comparison to analyze:
AVP-wt

Select method:
GAGE

Select genesets (Choose KEGG to show pathway diagrams):
All available gene sets

Geneset size: Min. 15 Max. 2000

Pathway significance cutoff (FDR) 0.2

Number of top pathways to show 30

Use absolute values of fold changes for GSEA and GAGE

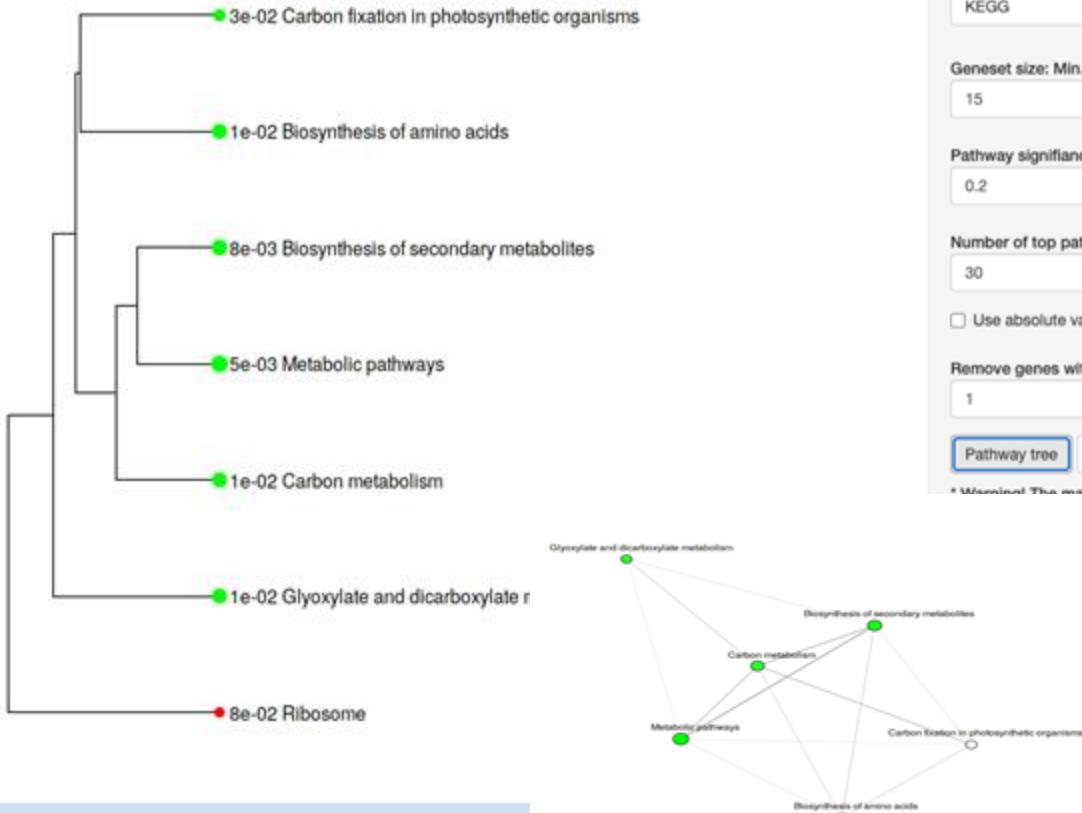
Remove genes with big FDR before pathway analysis: 1

Pathway tree Pathway network Pathway list w/ genes

* Warning! The many combinations can lead to false positives in pathway analyses.

AVP -WT proteomics

● Down ● Up



Select a comparison to analyze:
AVP WT

Select method:
GAGE

Select genesets (Choose KEGG to show pathway diagrams):
KEGG

Geneset size: Min. 15 Max. 2000

Pathway significance cutoff (FDR) 0.2

Number of top pathways to show 30

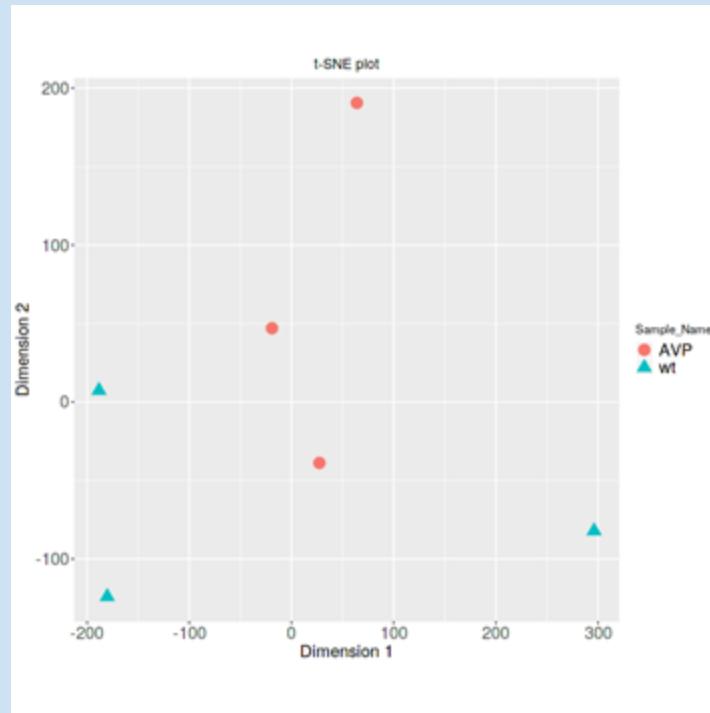
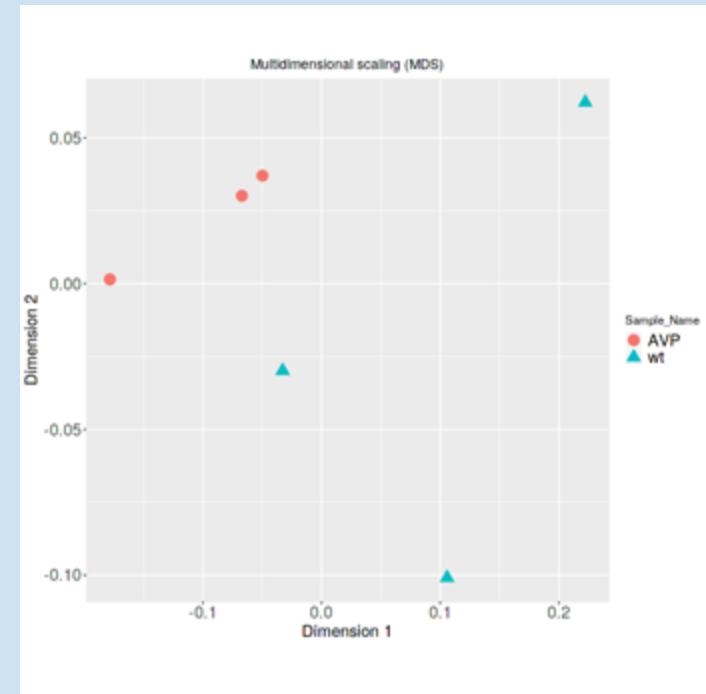
Use absolute values of fold changes for GSEA and GAGE

Remove genes with big FDR before pathway analysis:
1

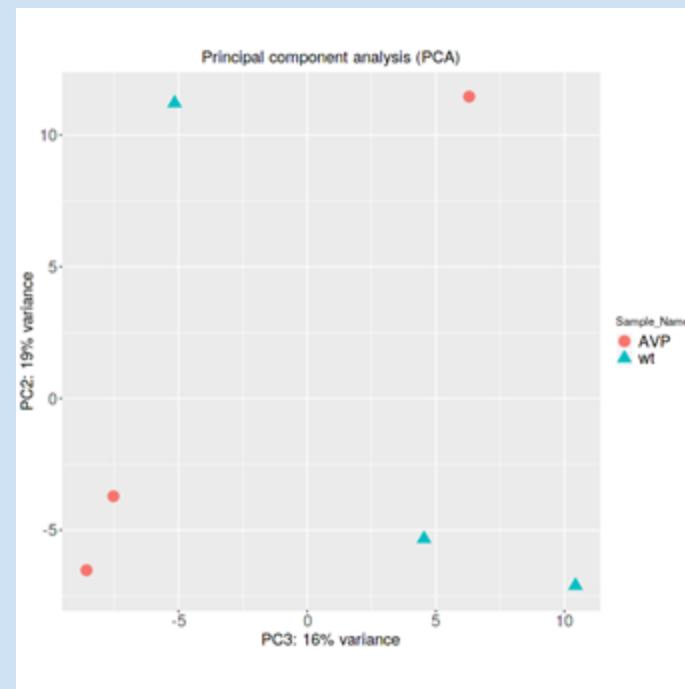
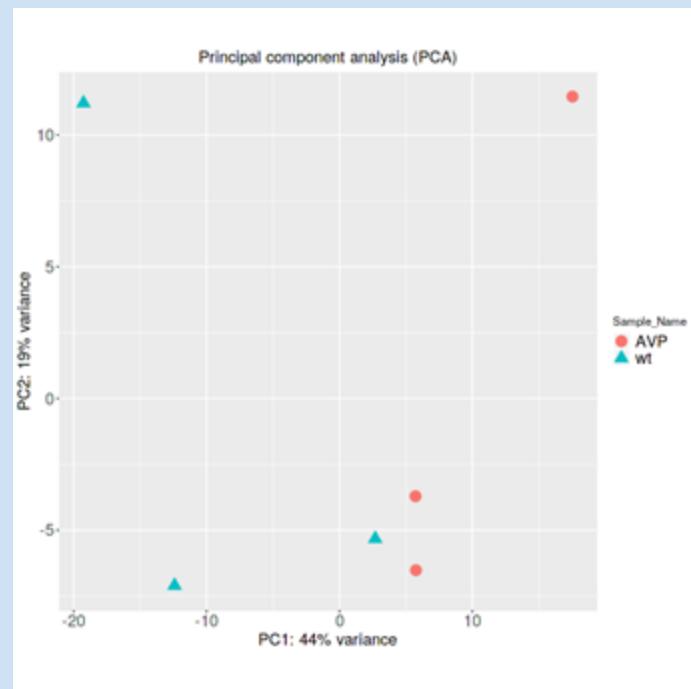
Pathway tree Pathway network Pathway list w/ genes

* Warning! The above combinations can lead to false positives in pathway analyses.

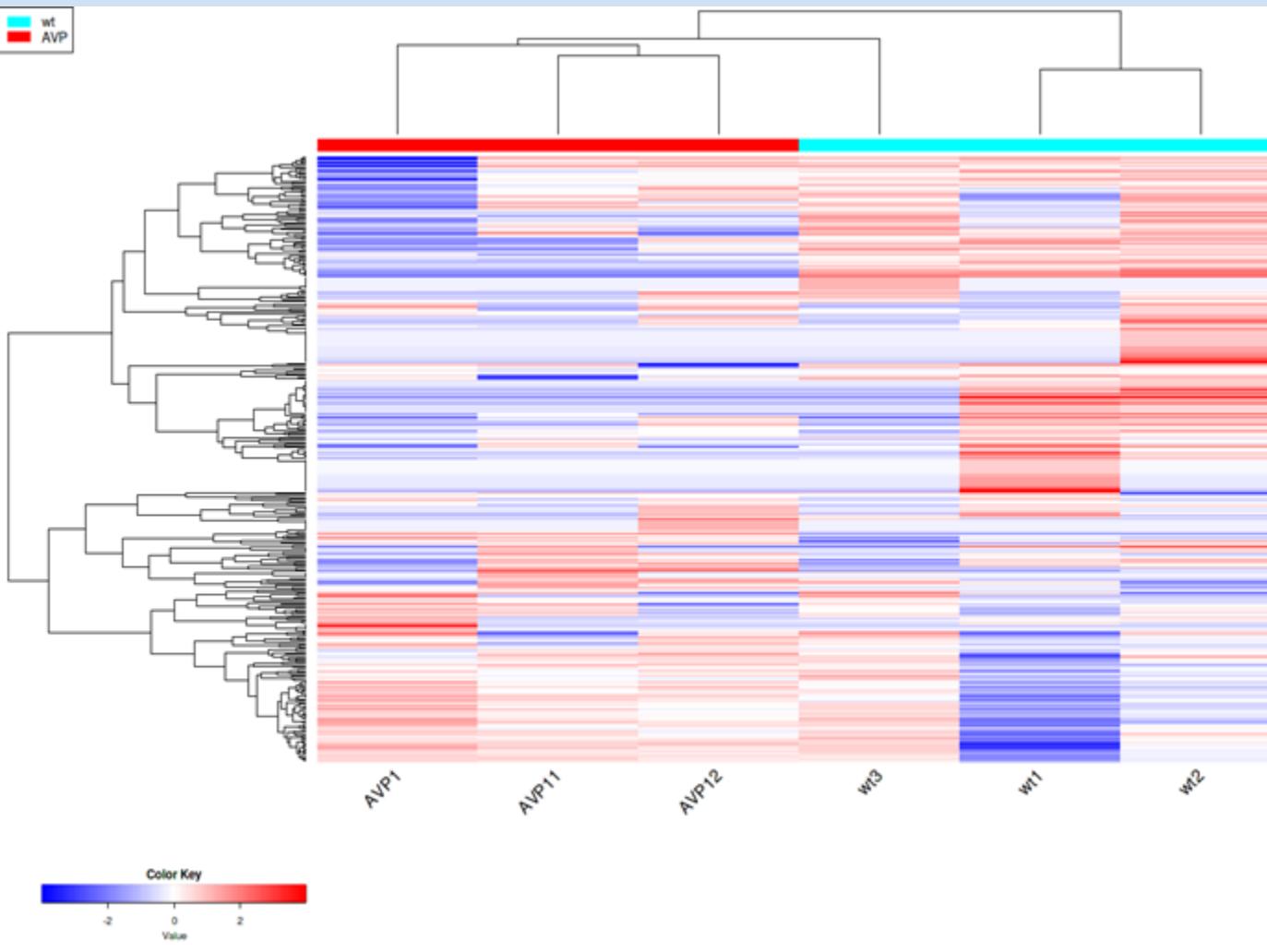
AVP -WT proteomics



AVP -WT proteomics

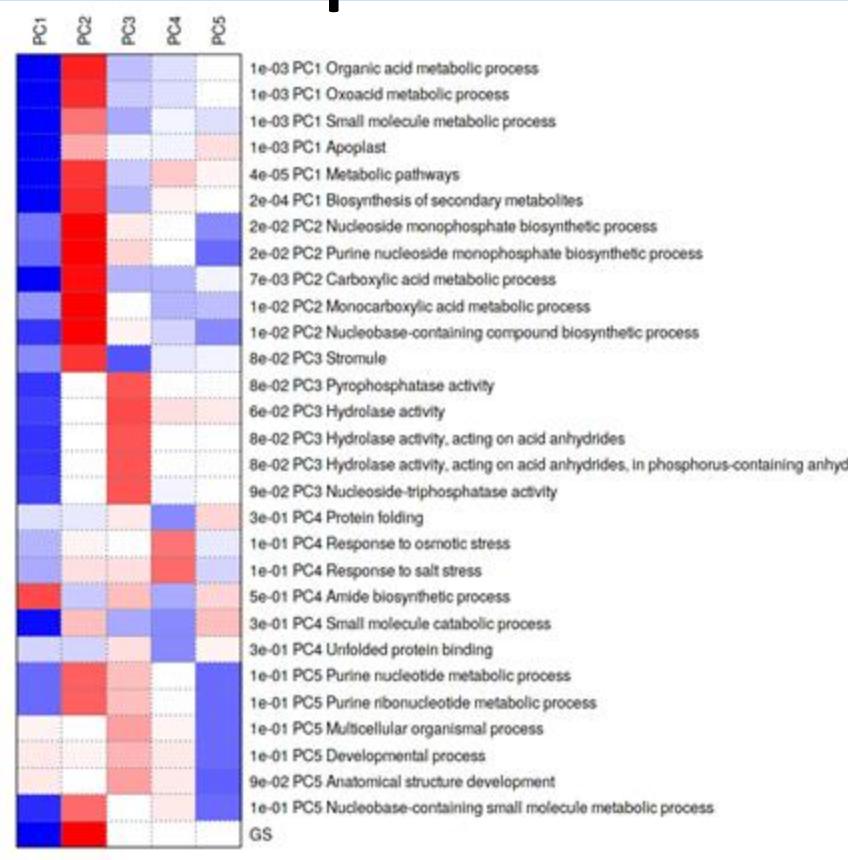


wt
AVP

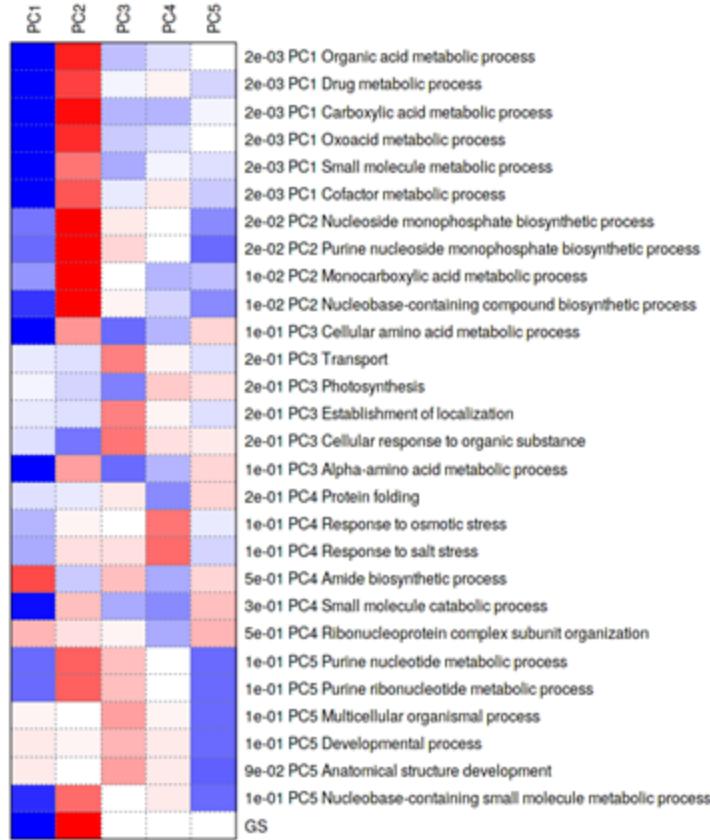


AVP -WT proteomics

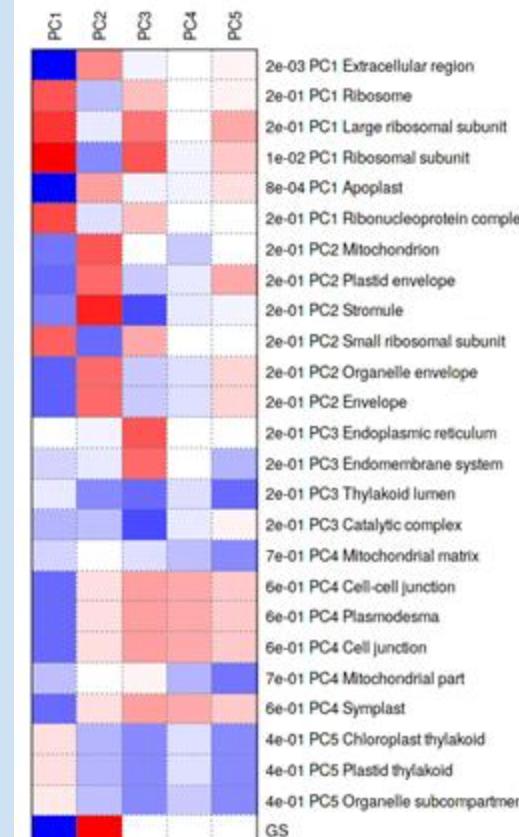
Top from GO and KEGG



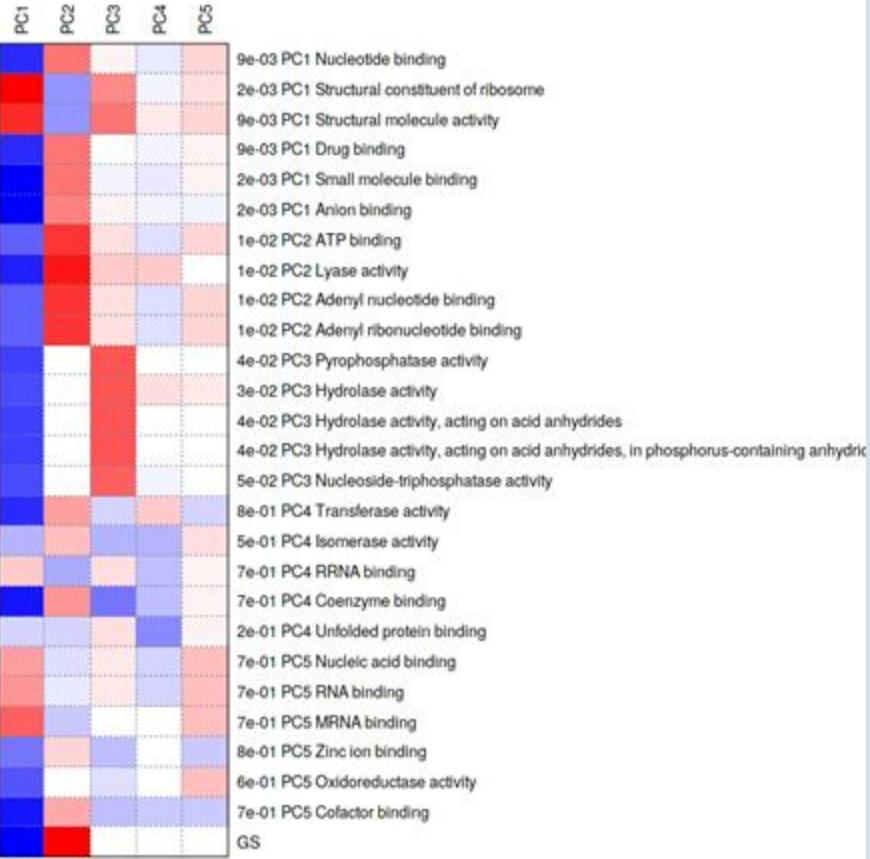
GO biological



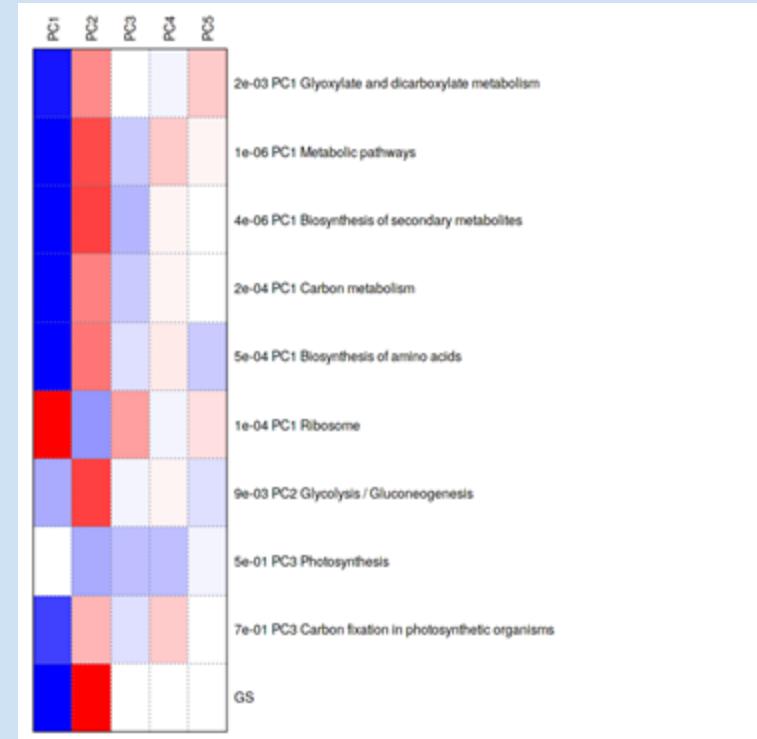
GO cellular site



GO molecular



KEGG pathway



AVP -WT proteomics

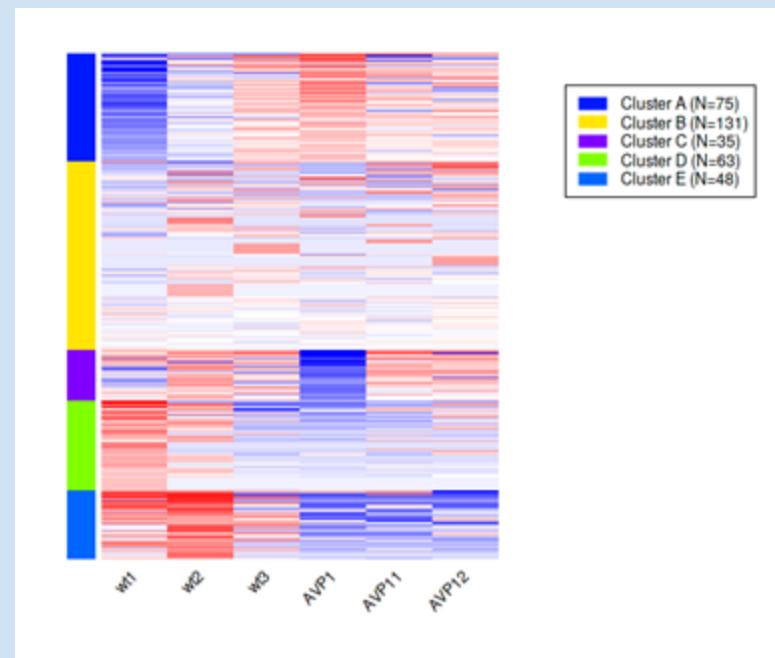
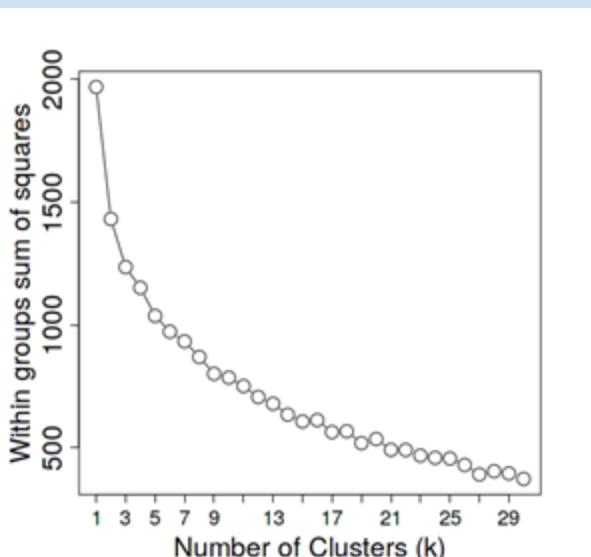
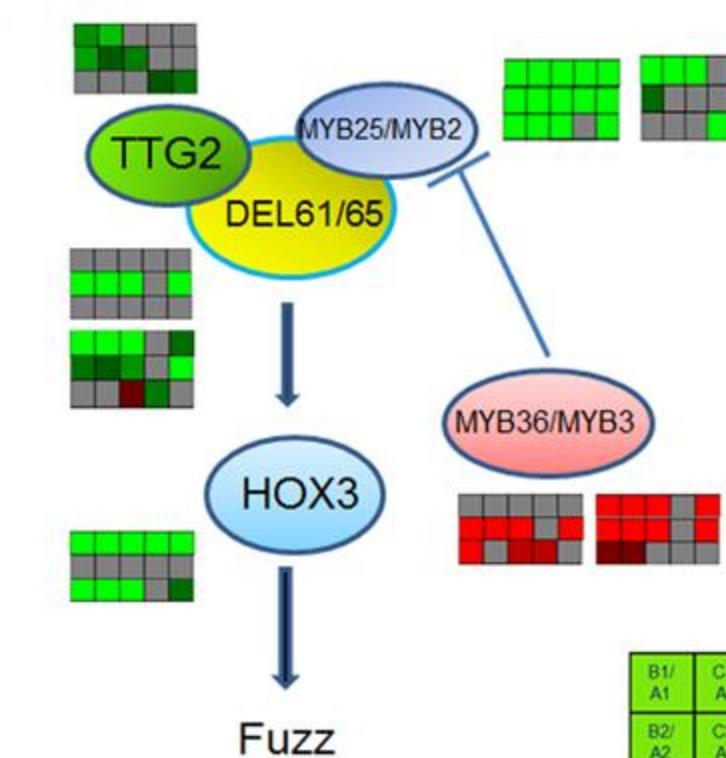
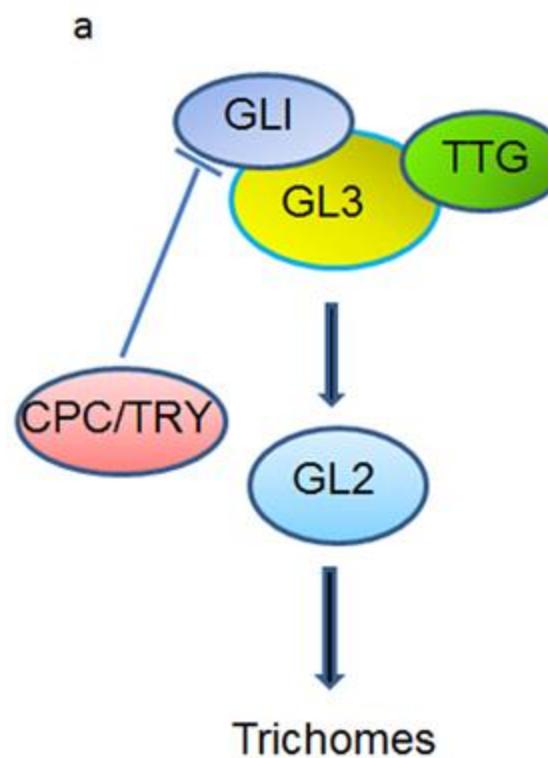


Table of functional analysis of these cluster on name slide



Arabidopsis thaliana

Gossypium hirsutum

B1/ A1	C1/ A1	D1/ A1	E1/ A1	F1/ A1
B2/ A2	C2/ A2	D2/ A2	E2/ A2	F2/ A2
B3/ A3	C3/ A3	D3/ A3	E3/ A3	F3/ A3

-3.0 0.0 3.0

