About OMICS Group

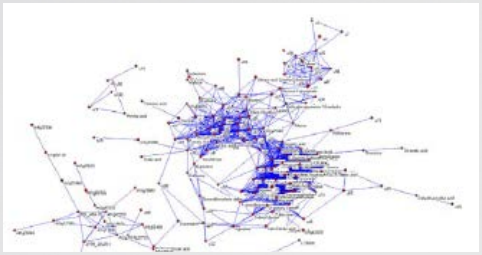
OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

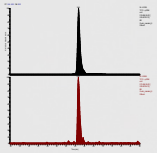
About OMICS Group Conferences

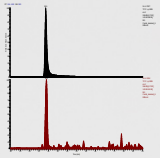
OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

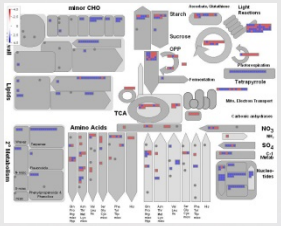
OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

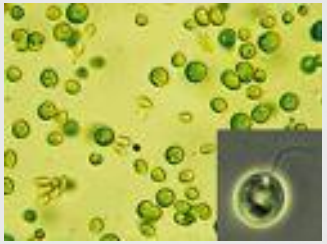
Comprehensive Protein turnover analysis of a partial ^{15}N stable isotope metabolic labelling experiment *In Planta*

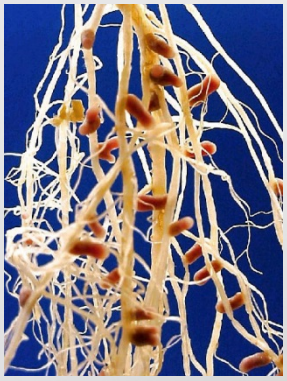










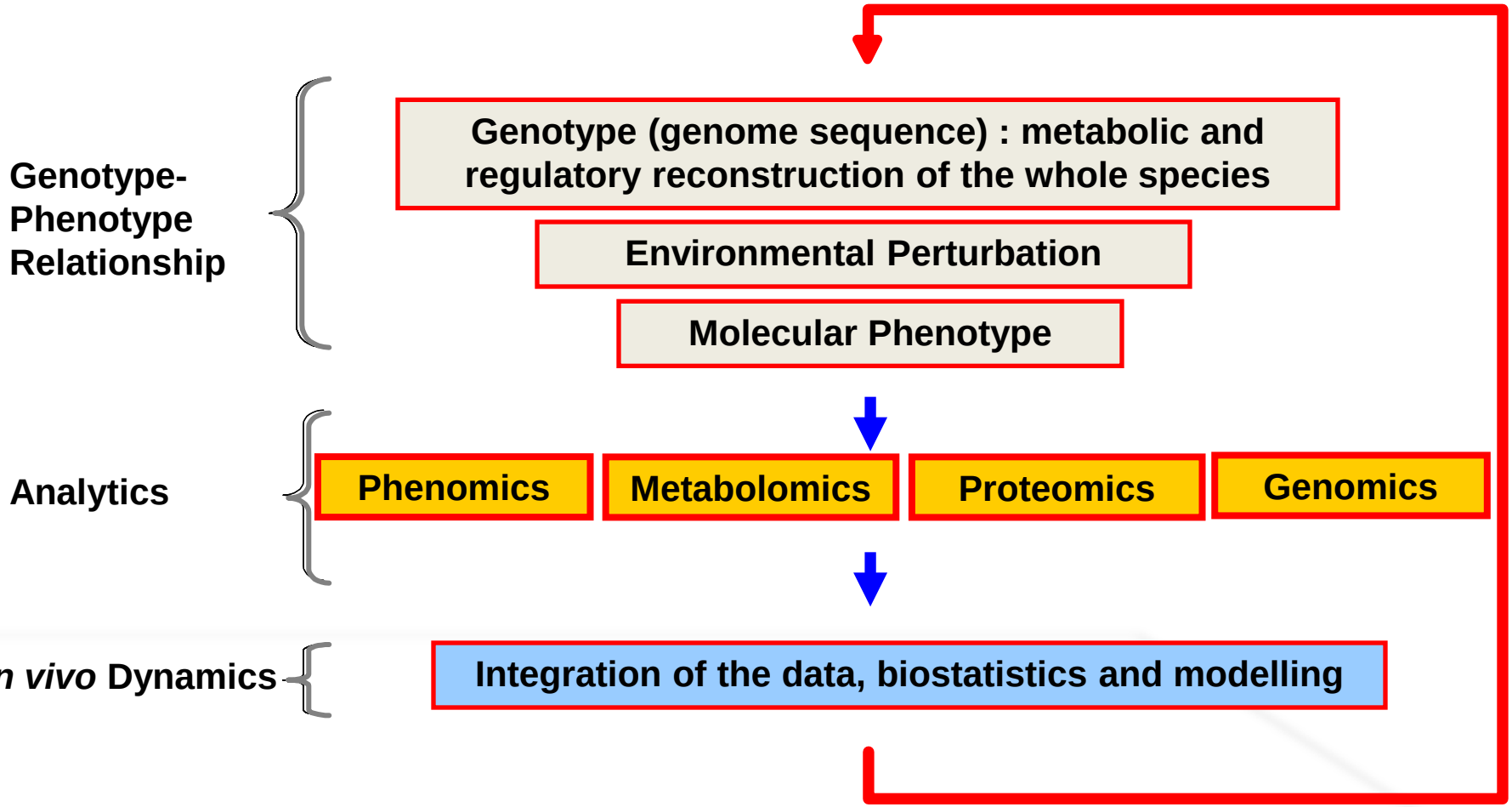




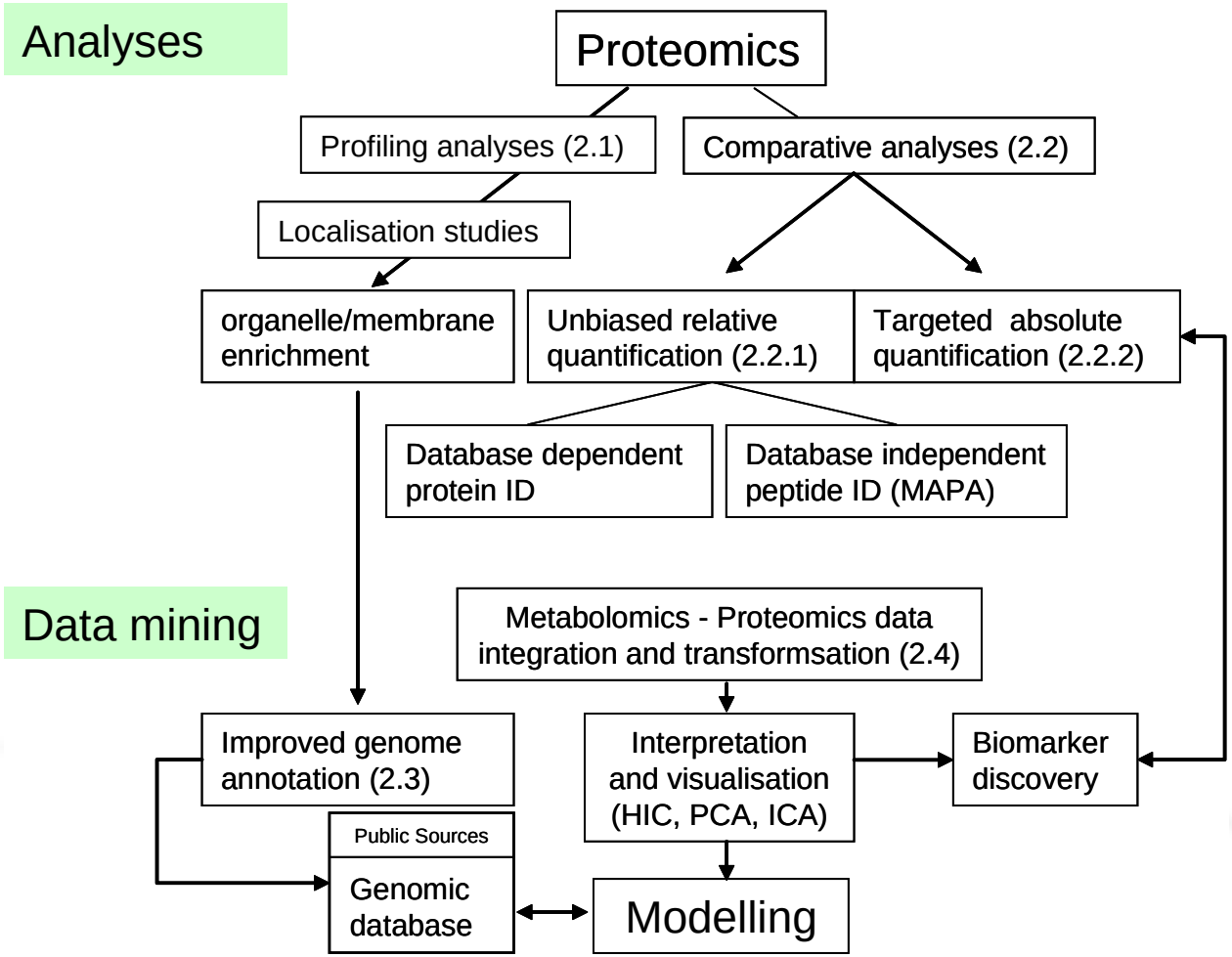
Stefanie Wienkoop

http://homepage.univie.ac.at/stefanie.wienkoop//

Molecular Systems Biology and The Molecular Plant Phenotype

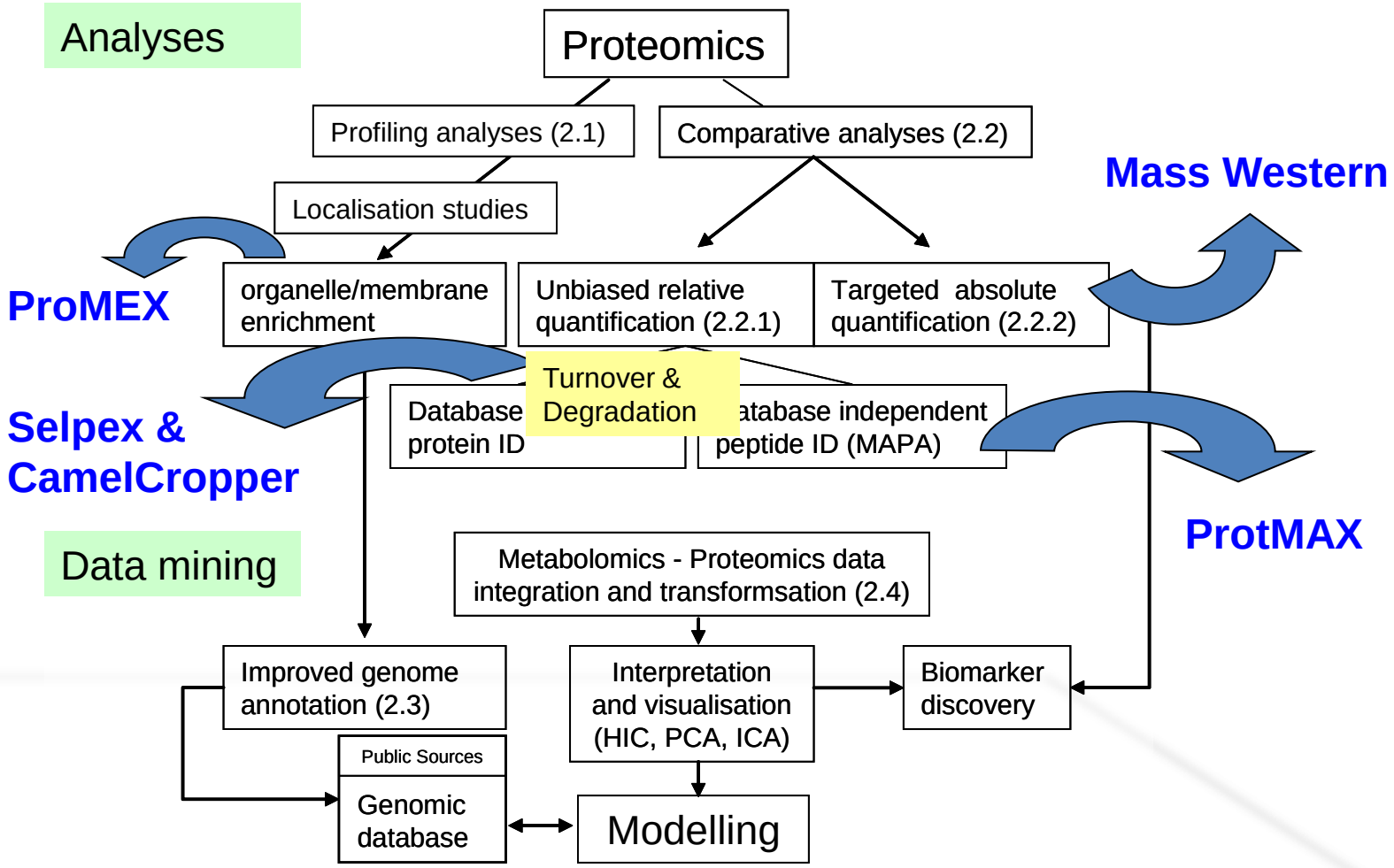


Proteomics Toolbox



Wienkoop et al. JProt (2010)

Proteomics Toolbox



Wienkoop et al. JProt (2010)

FWF Project
P23441-B20

SILIP- Drought Recovery Experiment

Medicago truncatula



In Planta

Gel-free Shotgun-LC/MS/MS

7 week old plants

10 days of drought stress

non-symbiotic (nodule free)

Experimental Setup

Drought Recovery (96 h) Experiment

Re-Watering ^{15}N (2.5 mM NH_4 NO_3)

5 TP	6 rep.	C vs D
2 h		
24 h		
48 h		
72 h		
96 h		

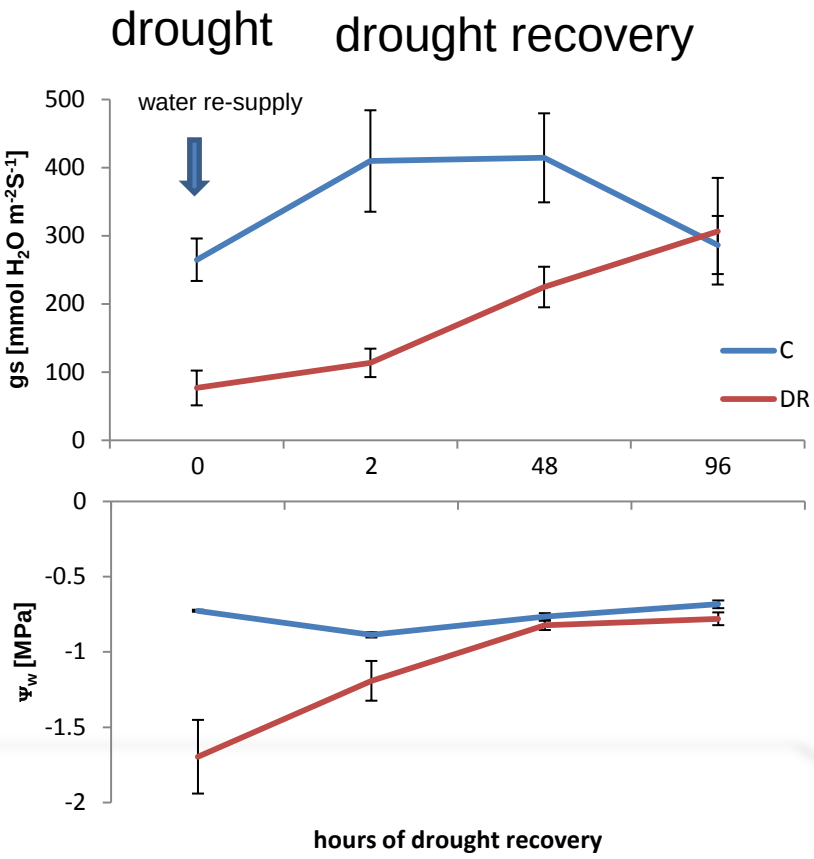
Same set:

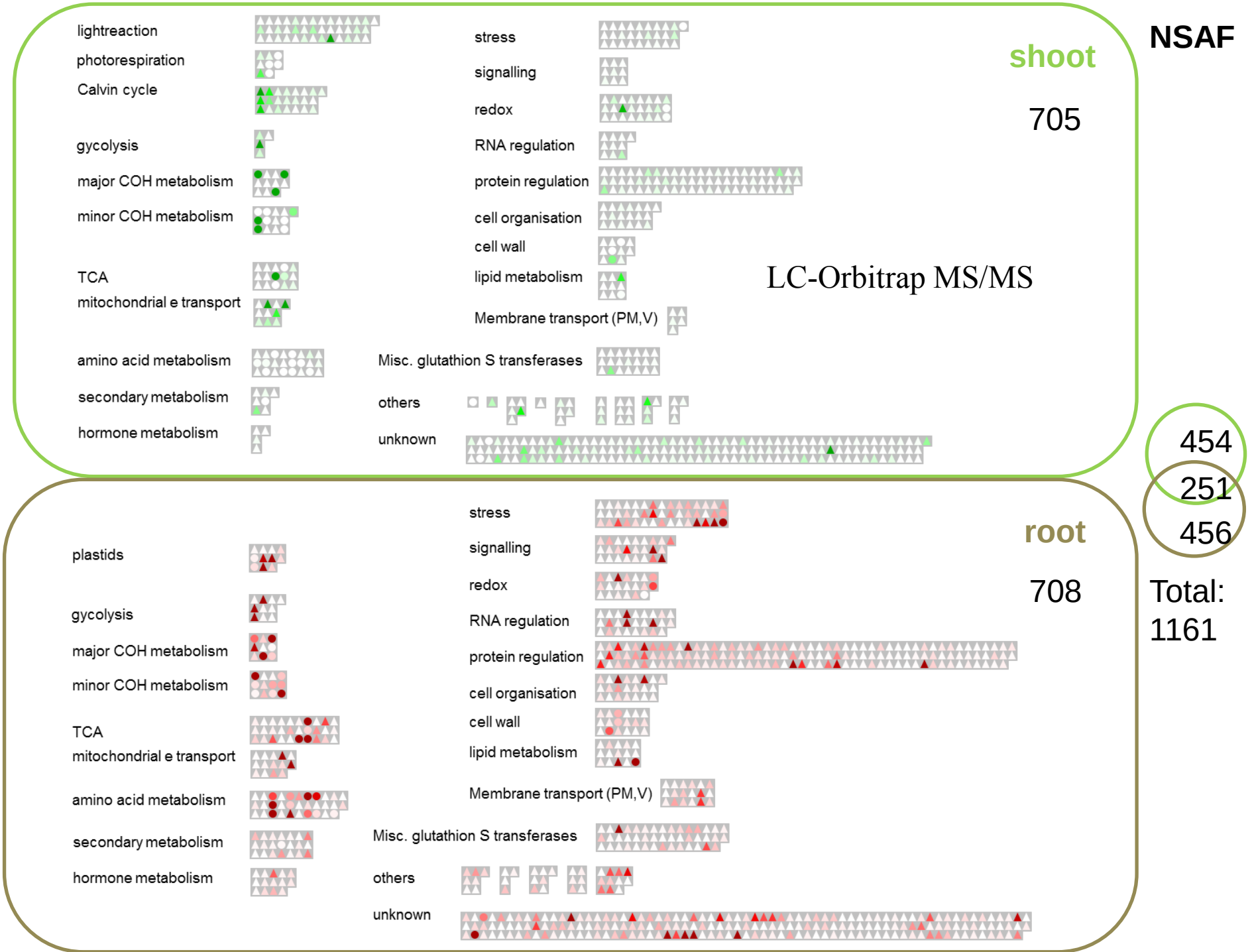
^{14}N (2.5 mM NH_4 NO_3)

Drought -> Drought Recovery

stomatal conductance

water potential (predawn)




NSAF
shoot
705
lightreaction
photorespiration
Calvin cycle
gycolysis
major COH metabolism
minor COH metabolism
TCA
mitochondrial e transport
amino acid metabolism
secondary metabolism
hormone metabolism
stress
signalling
redox
RNA regulation
protein regulation
cell organisation
cell wall
lipid metabolism
Membrane transport (PM,V)
Misc. glutathion S transferases
others
unknown
LC-Orbitrap MS/MS
454
251
456
Total:
1161
root
708
plastids
gycolysis
major COH metabolism
minor COH metabolism
TCA
mitochondrial e transport
amino acid metabolism
secondary metabolism
hormone metabolism
stress
signalling
redox
RNA regulation
protein regulation
cell organisation
cell wall
lipid metabolism
Membrane transport (PM,V)
Misc. glutathion S transferases
others
unknown

HIC: Protein and Metabolite Cluster Analysis

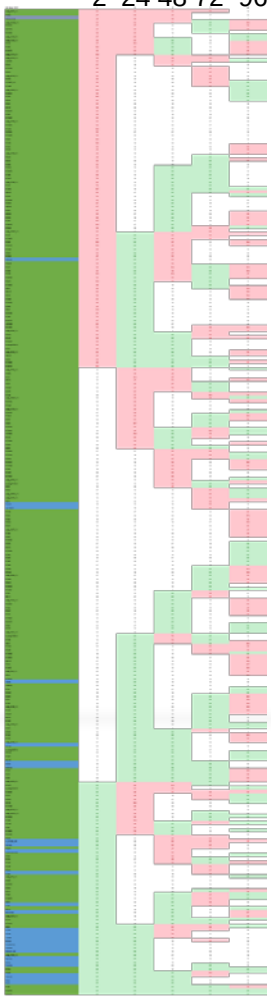
Significant changed only (DR/C)!

≥ 2 fold change

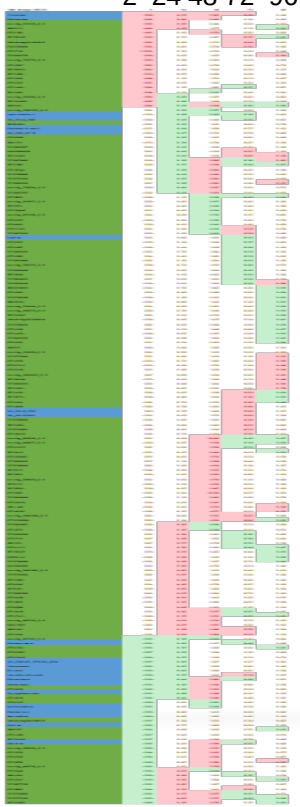
p ≤ 0.05

n = 6

Roots (278)



Shoots (174)



Proteins

Metabolites

MoSys universität wien

Protein and Metabolote Response Overview

fold change (DR/C)	2	24	48	72	96



Roots

Proteins	140	132	126	115	142
Metabolites	20	20	17	13	3





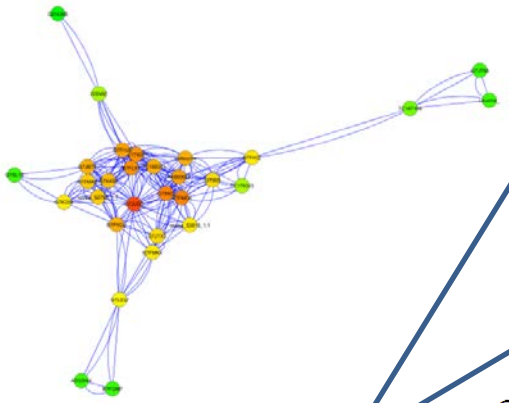
Shoots

Proteins	43	50	60	47	51
Metabolites	21	13	7	10	0

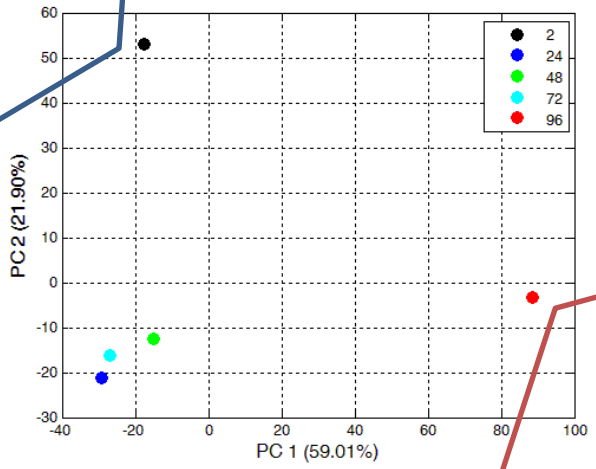


Metabolites fully recover!

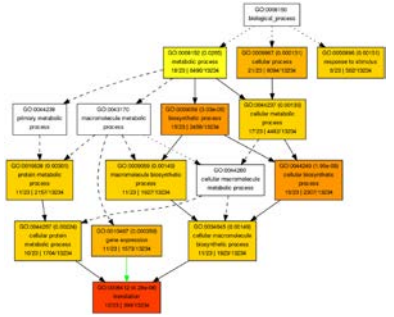
Correlation Network & Functional GO Analysis

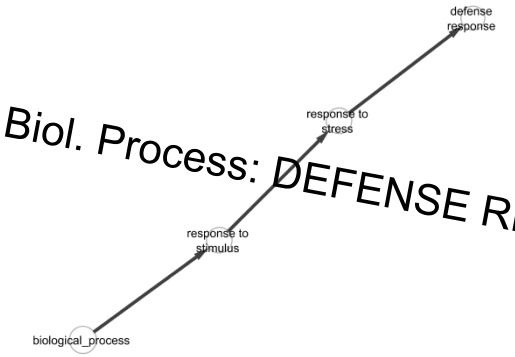


2 h



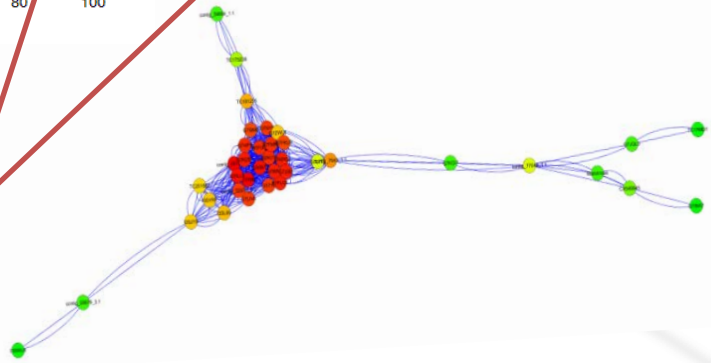
Biol. Process: TRANSLATION





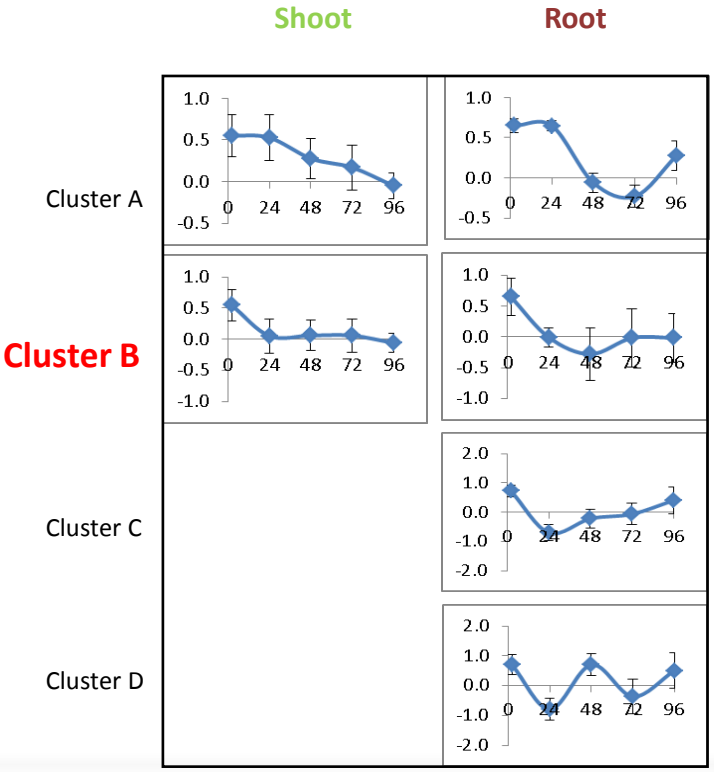
Biol. Process: DEFENSE RESPONS

96 h



Regulatory Relevant Protein Correlation-Cluster

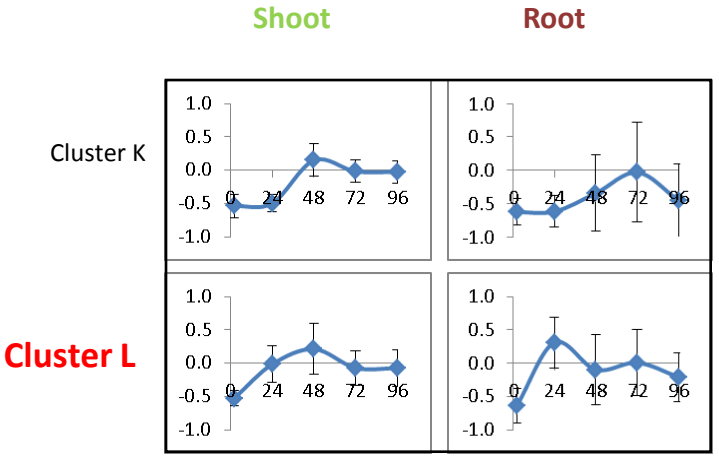
2h after Re-Watering



Group 1:

Time point 1

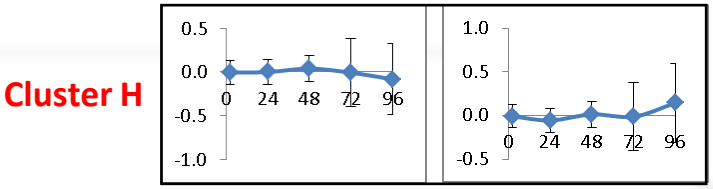
up-regulation



Group 3:

Time point 1

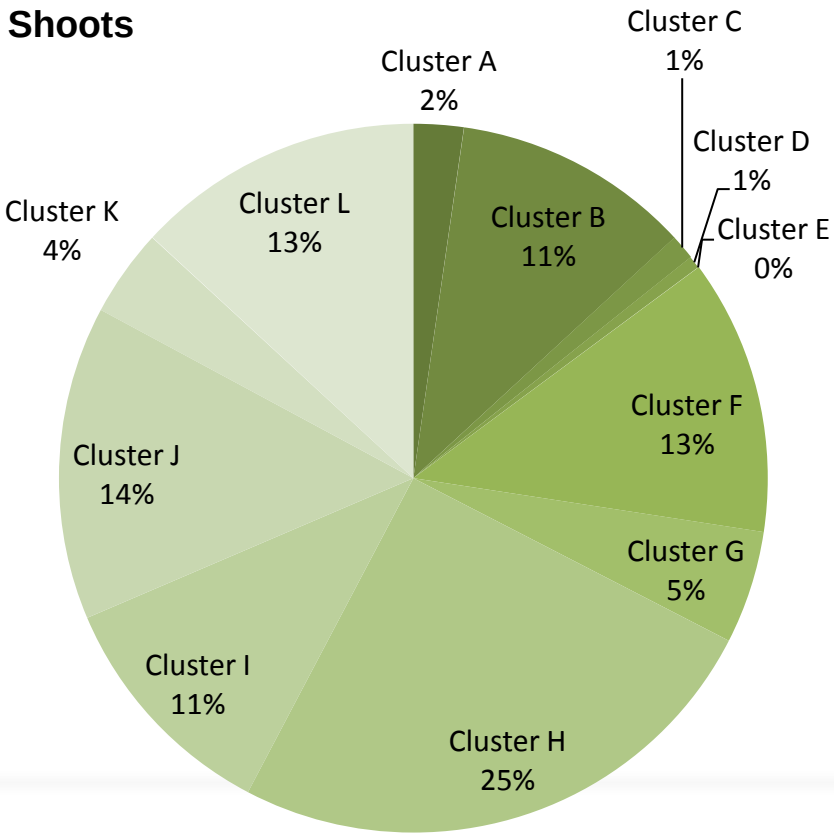
down-regulation

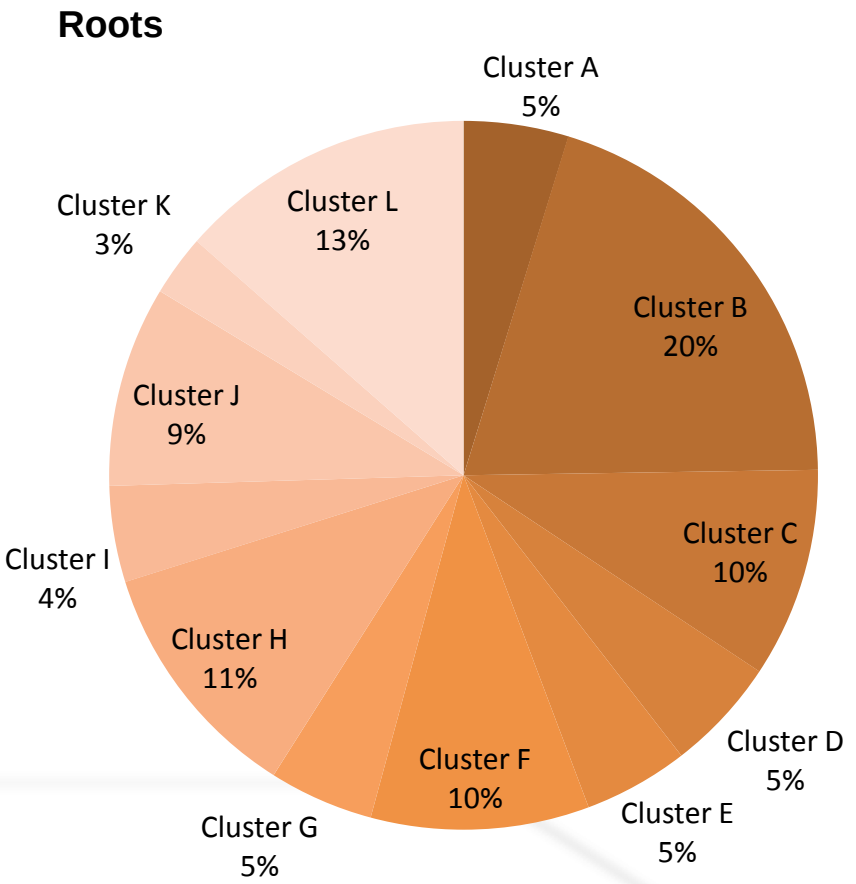


Group 5: Time point 5

up- or down-regulation

Relative cluster proportion of stress recovery response proteins





First Conclusions

- Translational Regulation within first 2 hours of rewatering.
- While physiological and metabolic levels fully recovered after 96 hours, protein regulation still in process

Why studying protein turnover?

1. Transcript and protein measurements are poorly correlated in experiments of abundance and stability when exposed to environmental perturbations.

2. Understanding the mechanisms controlling whole plant nitrogen-to-protein incorporation and protein-to-nitrogen breakdown will advance our knowledge of plant nitrogen acquisition and allocation.

How studying protein turnover?

1. 15N stable isotope metabolic labeling is one of the major approaches for Mass Spectrometry based proteomics.

2. Additionally to protein turnover, AA turnover can be monitored.

3. Protein turnover analysis involves the partial-labeling strategy.

Challenge 1

1. Partial metabolic labelling => high complexity!
2. Identification of all proteinogenic AAs => possible?
3. Absolute levels from all proteinogenic AAs => very difficult!
4. Absolute levels of all identified proteins => possible?
5. Proteotypic peptides (>2) for all proteins => possible?

root

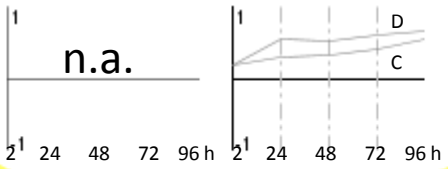
Glucose ← *Photosynthesis*

Fructose 1,6-bisphosphate

Phosphoglycolate

Cysteine

Glycine* ← Serine*



Erytrose 4-phosphate

Shikimate → Chorismate

Prephenate Anthranilate

Tryptophan

Tyrosine Phenylalanine

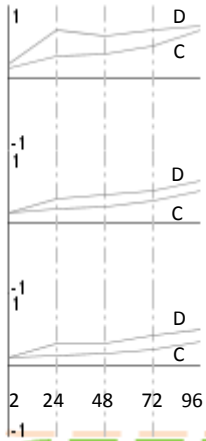
Phosphoenolpyruvate

Pyruvate

D = drought-recovery

C = control

* = display for marked AA



Acetyl-CoA

Oxaloacetate

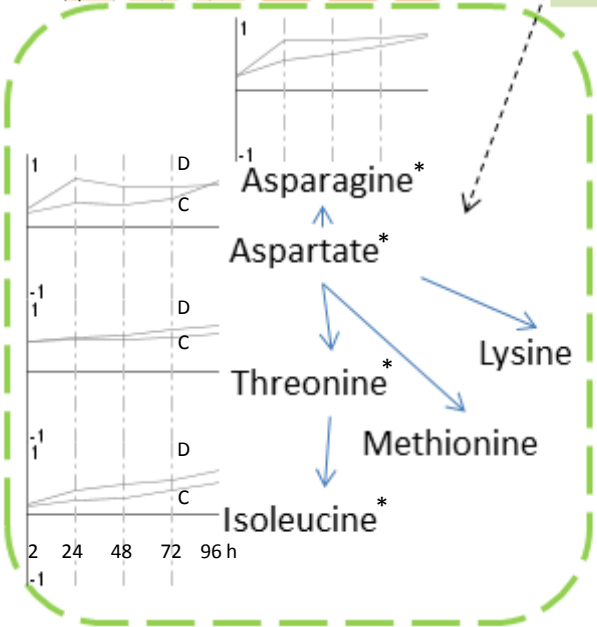
Citrate

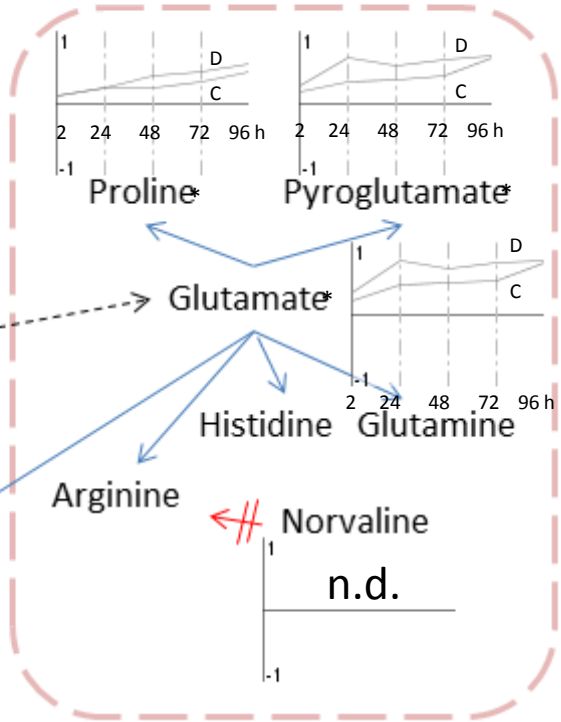
Isocitrate

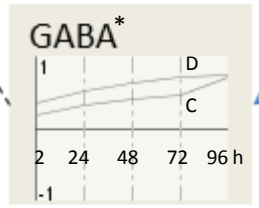
Malate *TCA* Fumarate

Succinate

α-Ketoglutarate

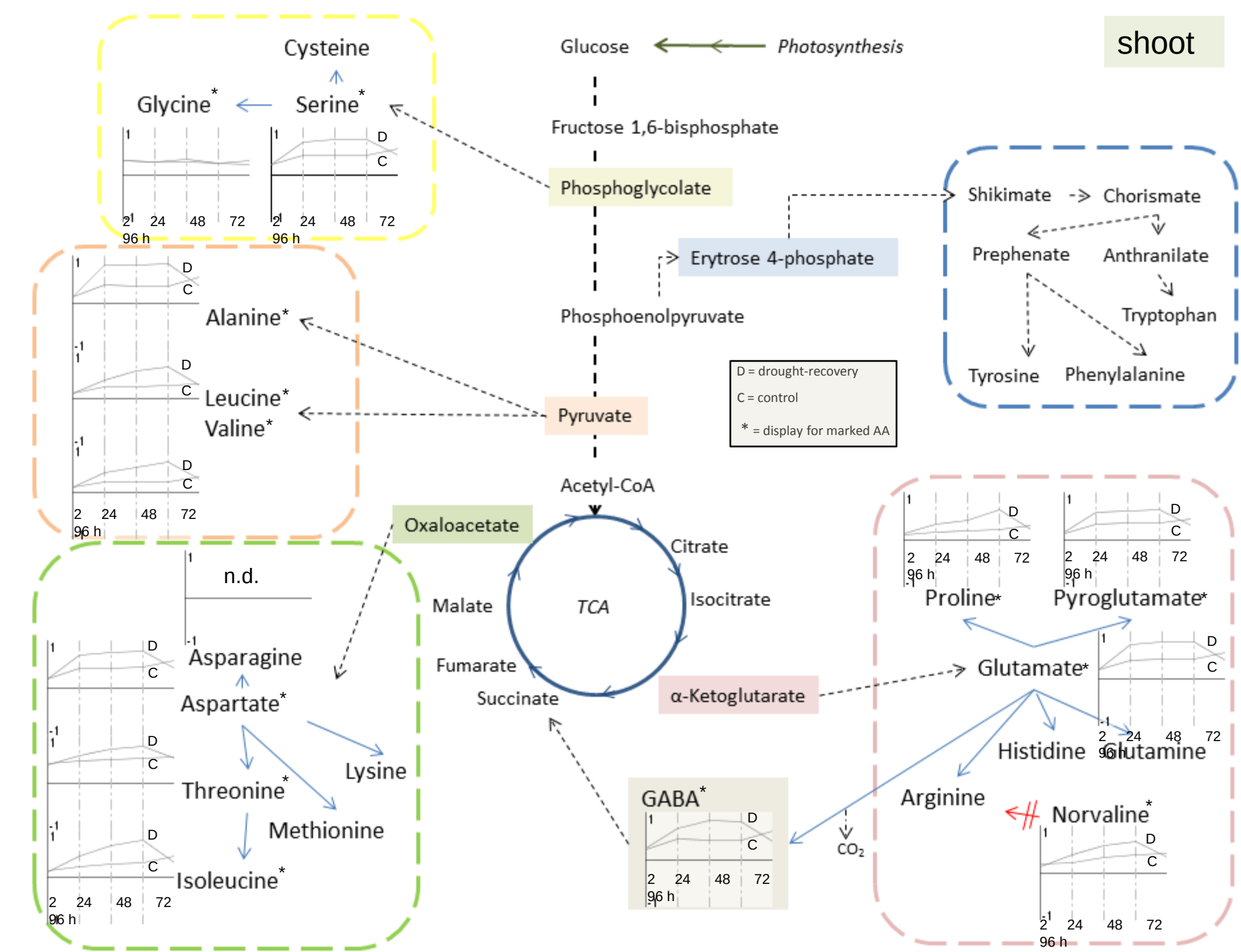






CO_2

shoot


Cysteine
Glycine*
Serine*
Glucose
Photosynthesis
Fructose 1,6-bisphosphate
Phosphoglycolate
Erytrose 4-phosphate
Shikimate
Chorismate
Prephenate
Anthranilate
Tryptophan
Tyrosine
Phenylalanine
Alanine*
Leucine*
Valine*
Phosphoenolpyruvate
Pyruvate
D = drought-recovery
C = control
* = display for marked AA
Acetyl-CoA
Oxaloacetate
Citrate
Isocitrate
Malate
TCA
Fumarate
Succinate
α-Ketoglutarate
n.d.
Asparagine
Aspartate*
Lysine
Threonine*
Methionine
Isoleucine*
Proline*
Pyroglutamate*
Glutamate*
Histidine
Glutamine
Arginine
Norvaline*
GABA*
CO2
96 h

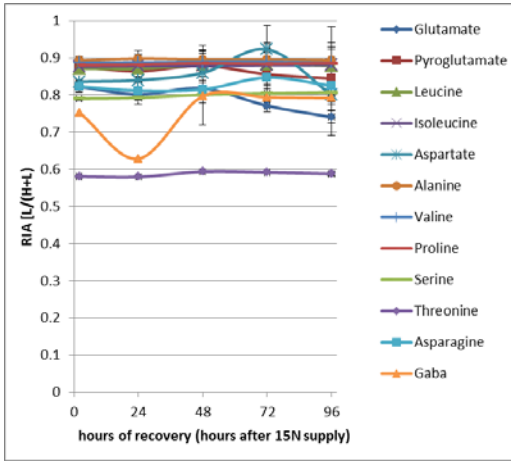
GC-MS analysis of 15N incorporation into Amino Acids

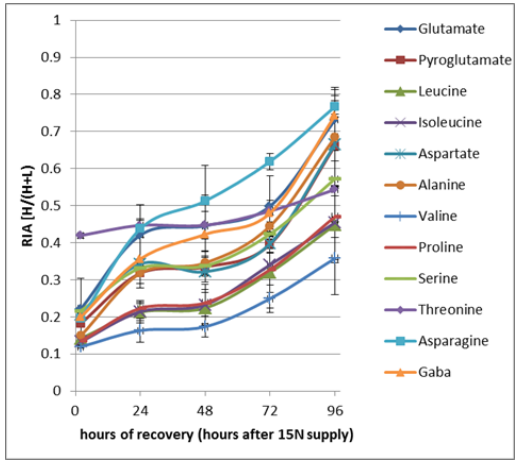
roots

No labelling
RIA_0 $14N/15N_{NA}$

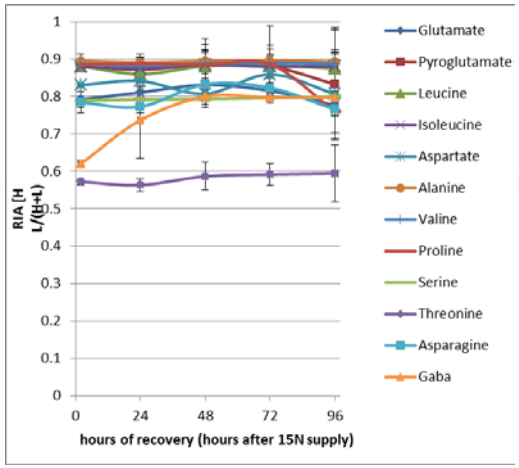
15N labelling
RIA of 15N enrichment

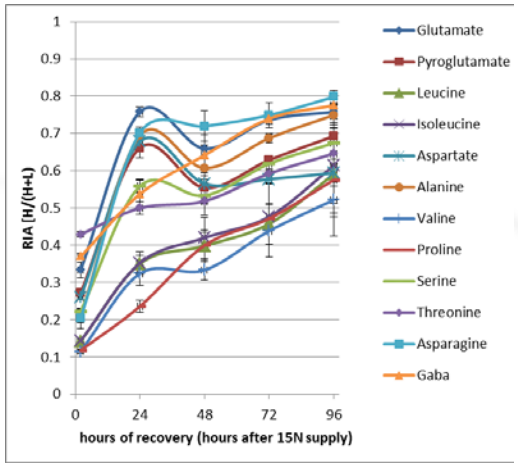
C





D

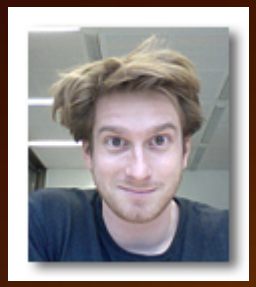




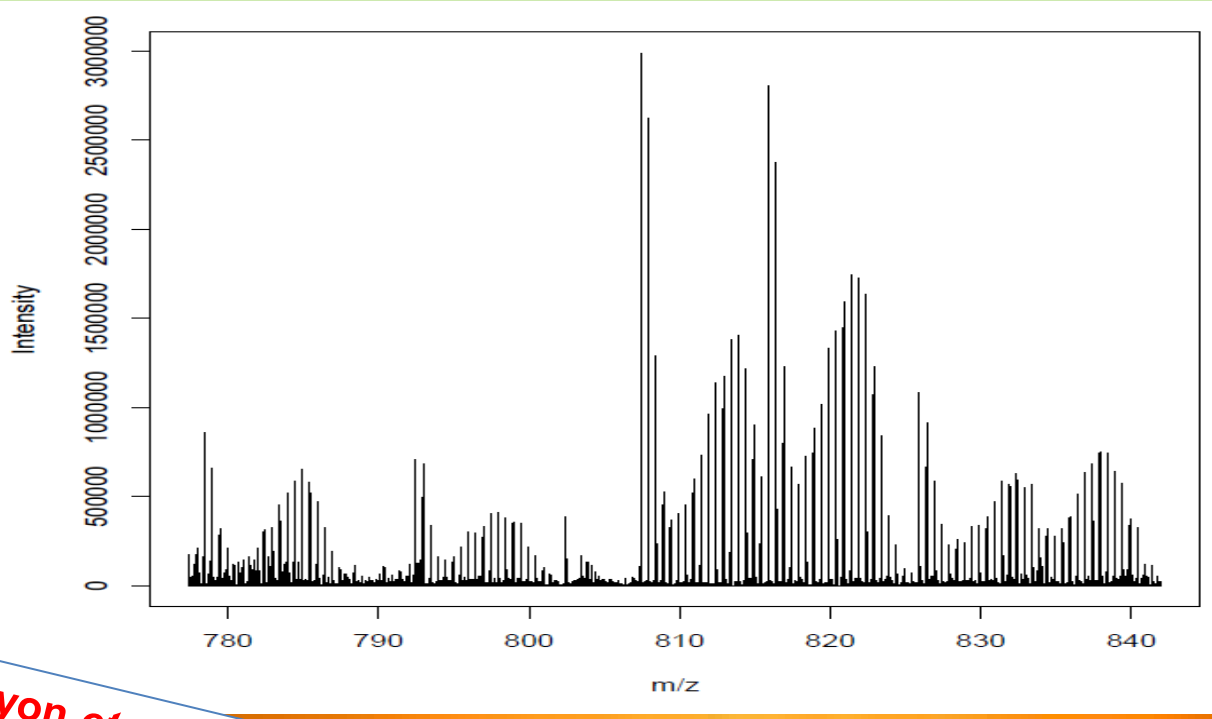
Callenge 2

How to retrieve the relative isotope abundance (RIA) of partial metaboloc labelling from MS data?

CamelCropper



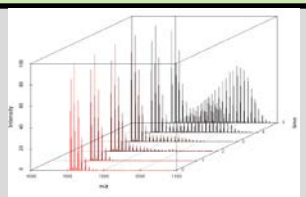
David Lyon



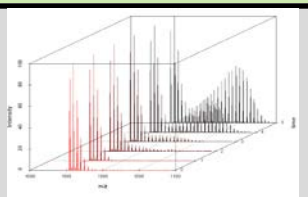
Lyon et al. 2014 Plos One Volume 9 | Issue 4 | e94692

FWF Project P23441-B20

FWF Der Wissenschaftsfonds.



CamelCropper for ^{15}N partial labelling – protein turnover detection



SelPEx- Selective Peptide Extraction

List of identified peptides with high quality MS properties:

- Robust and reproducible MS signals / peak shape

- m/z ratio, RT and ID

MS data upload into CamelCropper

CamelCropper

Castillecho *et al.* 2013

IN: Plant Proteomics Methods and Protocols. Ed. J.V. Jorrin Novo, S. Komatsu, S. Wienkoop, W. Weckwerth: Springer New York.

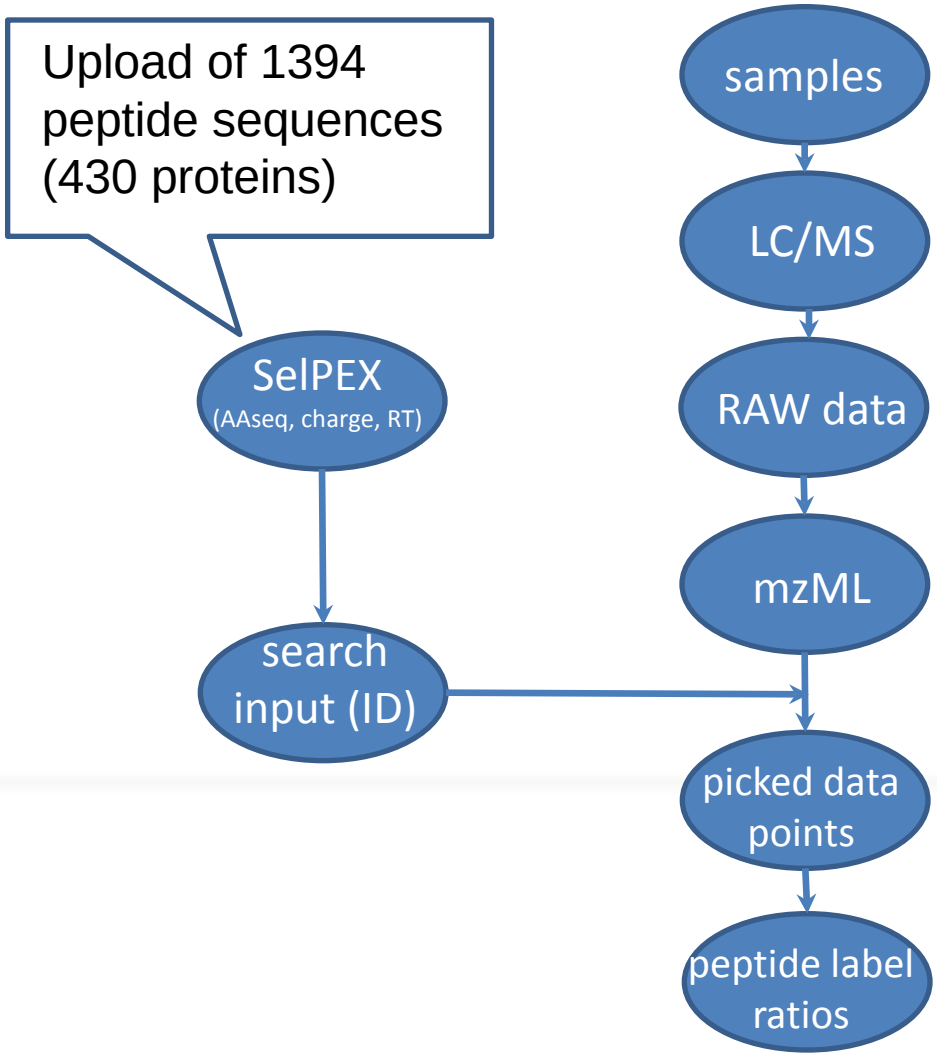
- Calculation of all possible m/z values of the ^{15}N isotope pattern for each peptide.

- Ancoring to the monoisotopic precursor (MIP0)

- ID of the „Camel neck“ and „Camel back“

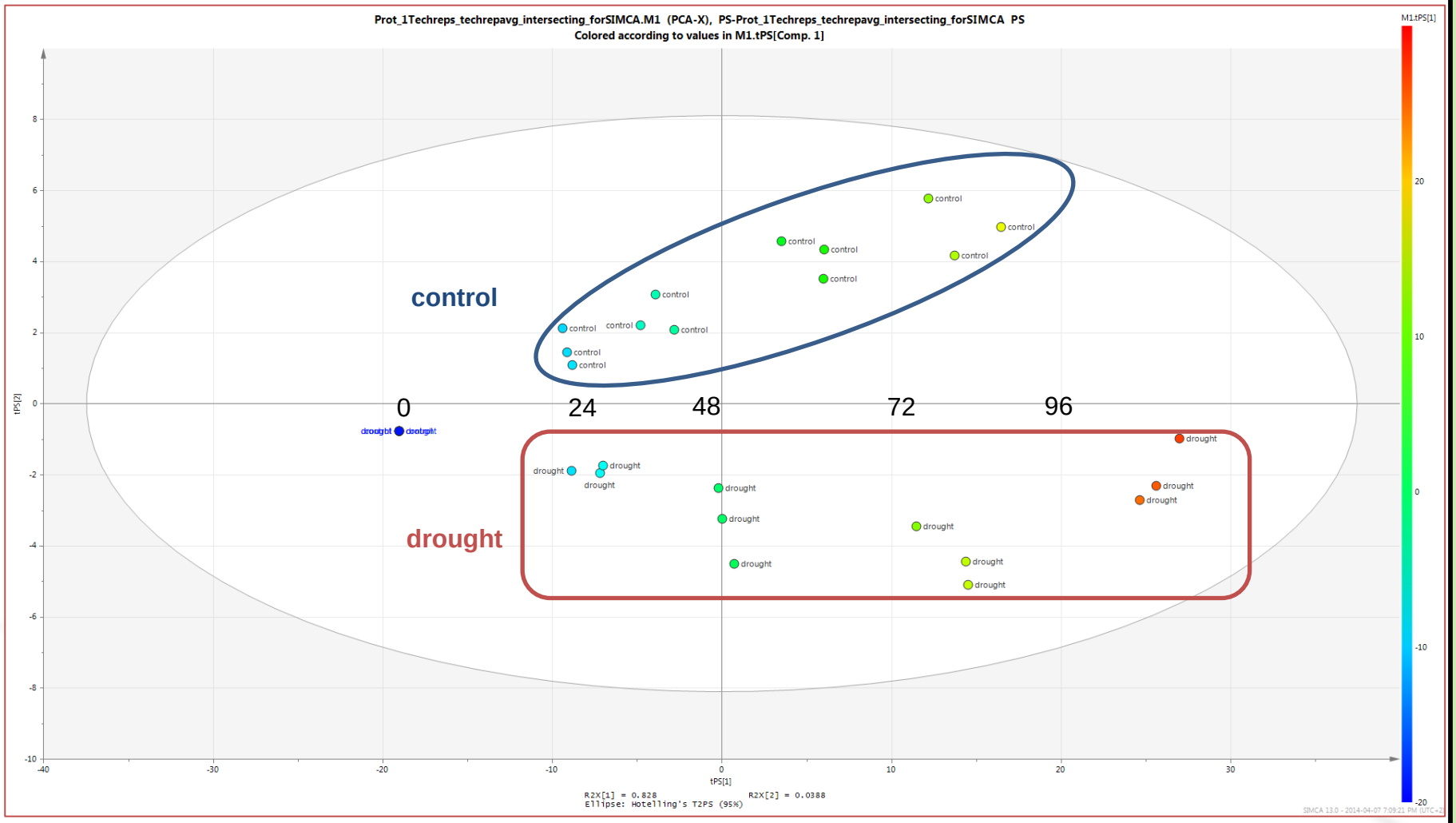
- Calculation of eg RIA [H]/ [H+L] for calculation protein synthesis and degradation

Workflow



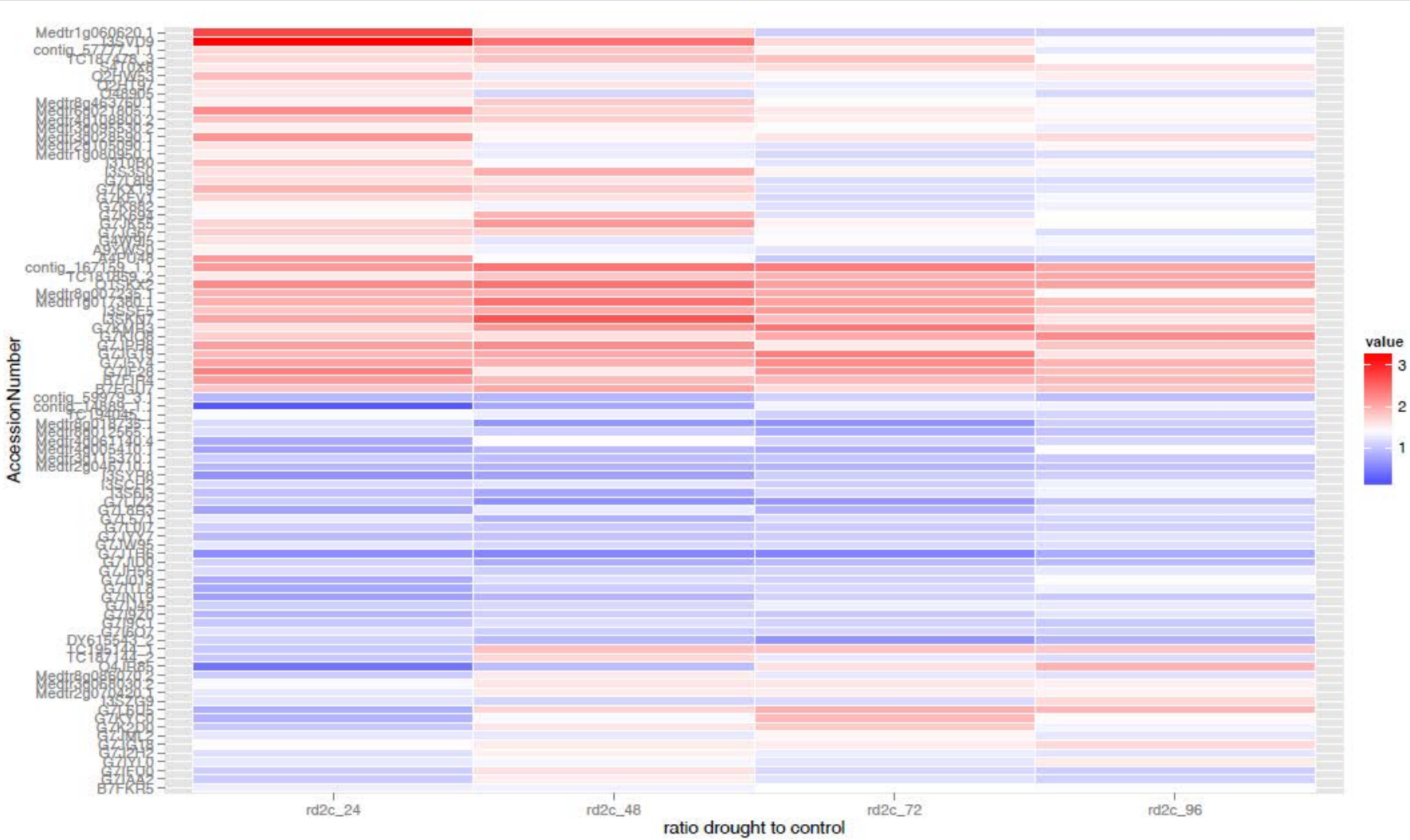
Lyon *et al.* 2014

PCA Plot: RIA Intensities of Shoot Proteins



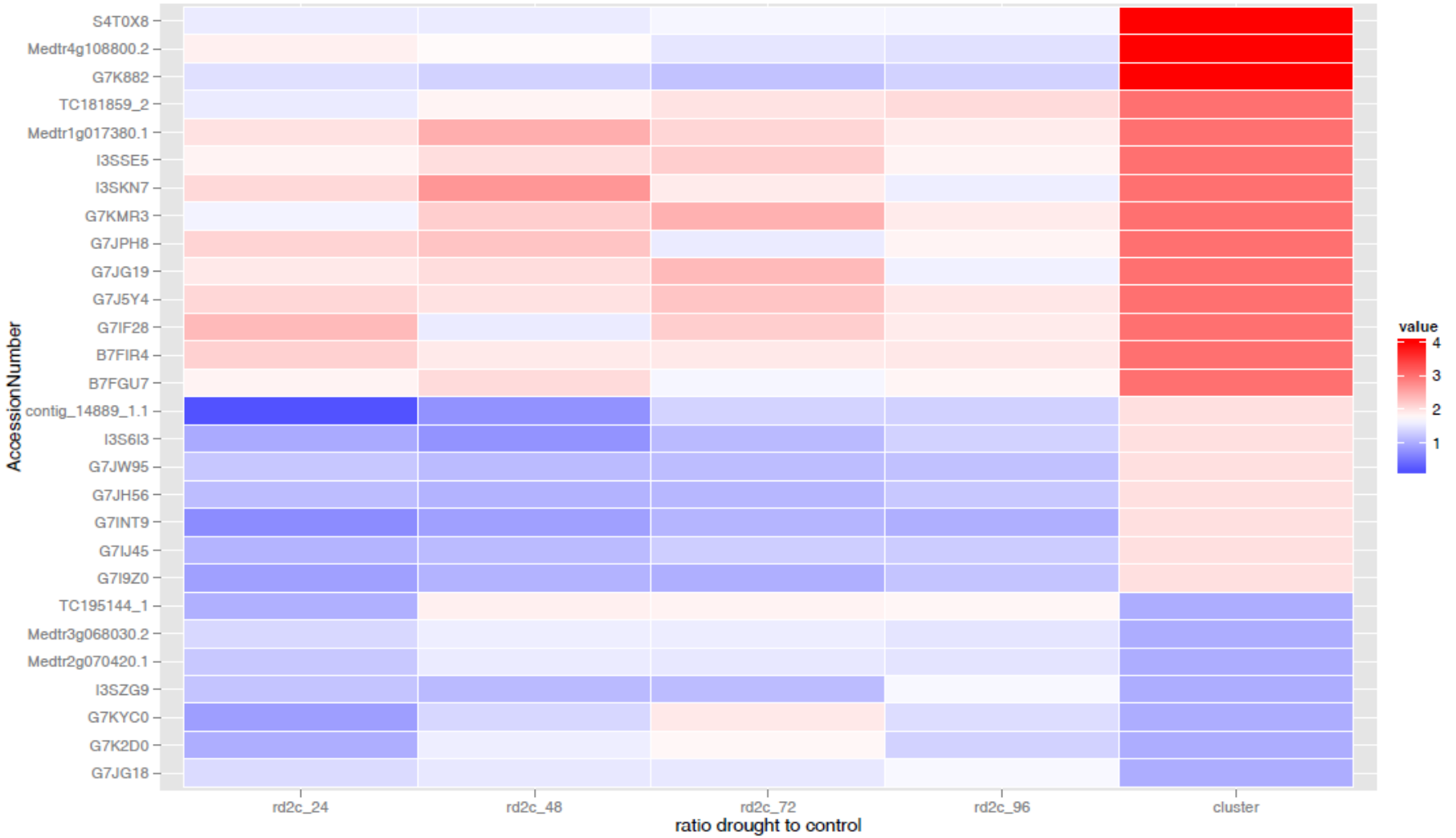
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Cluster Analysis: RIA ration DR vs. C

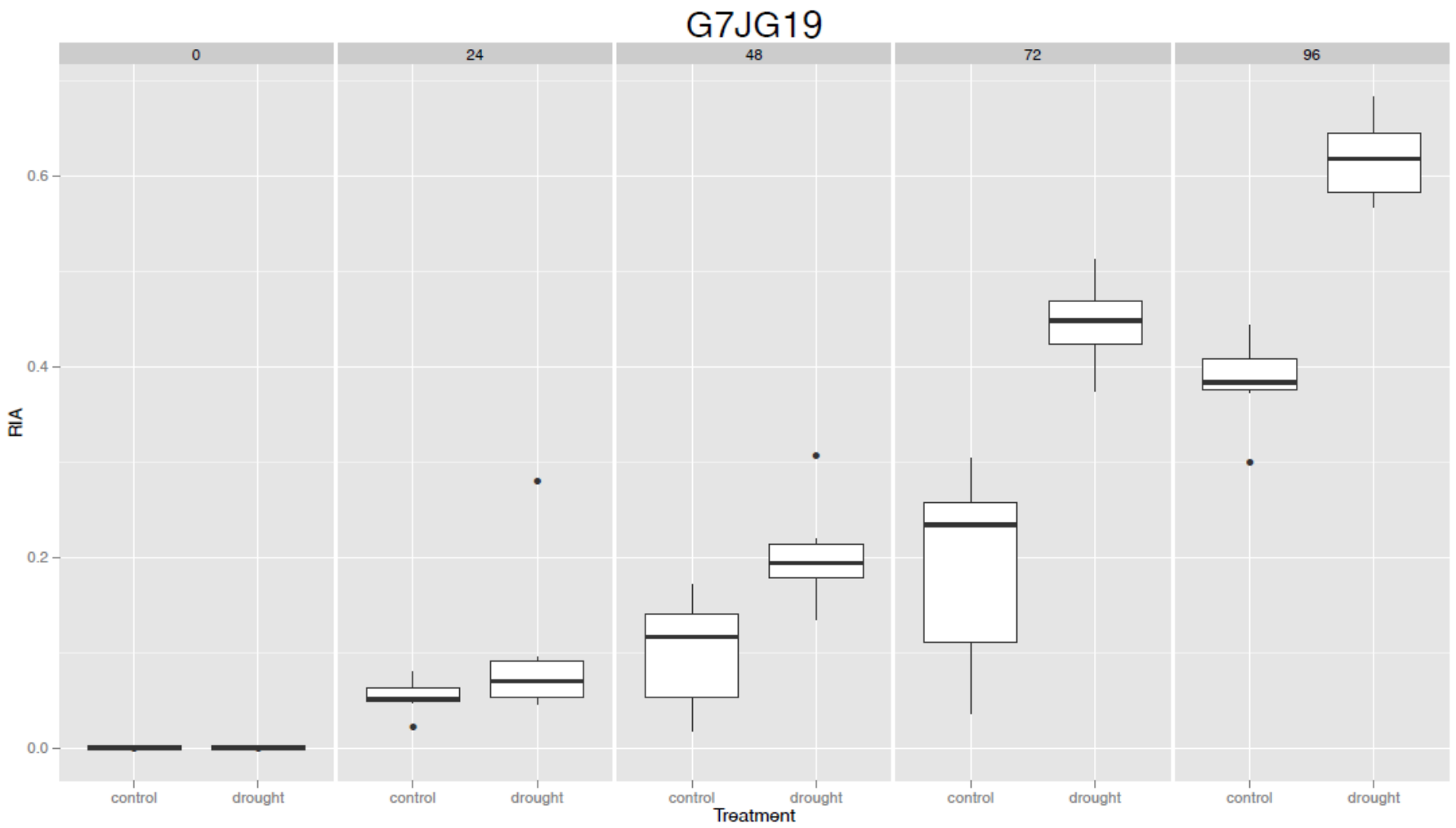


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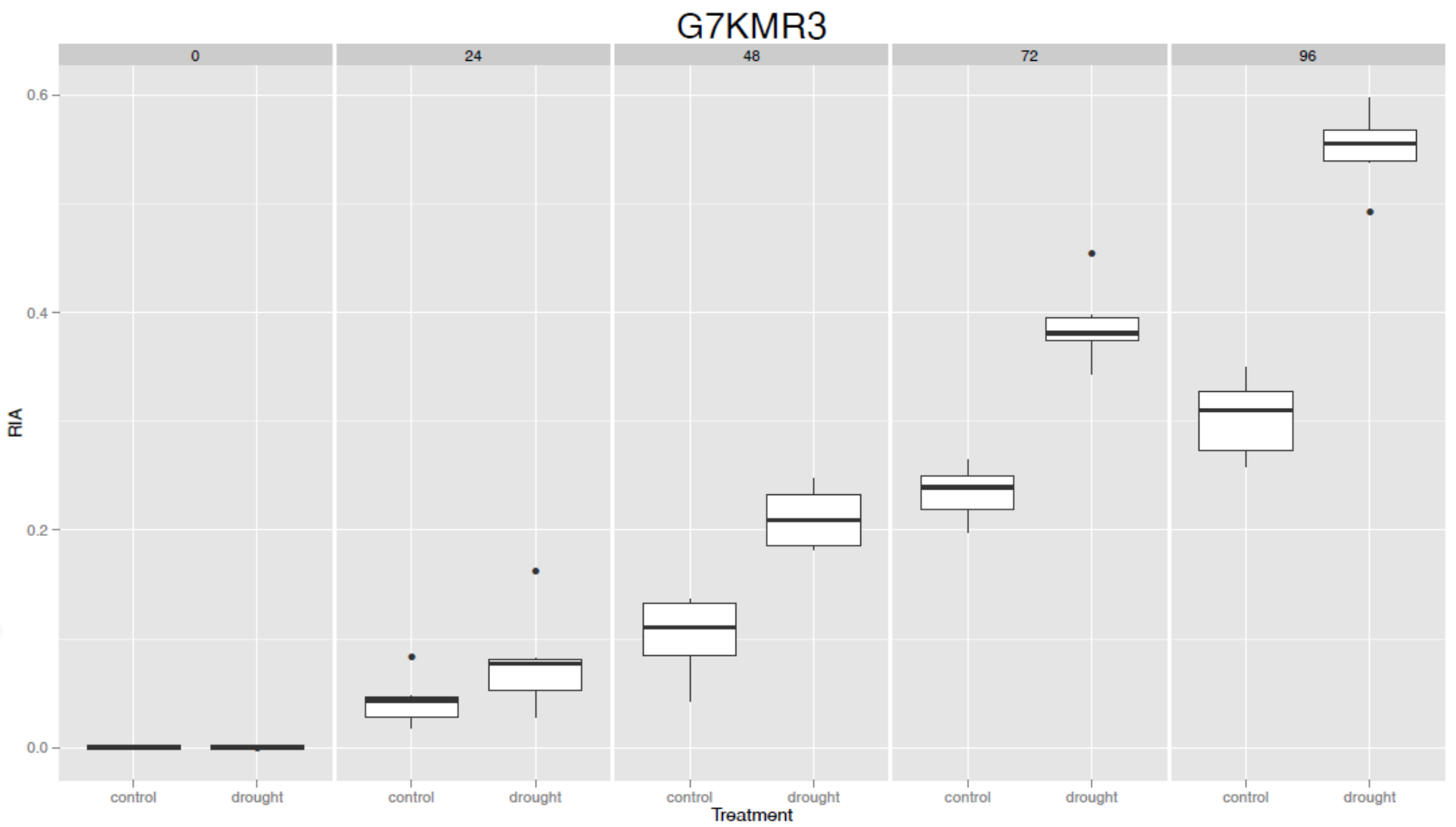
Cluster Analysis of PS: RIA ration DR vs. C



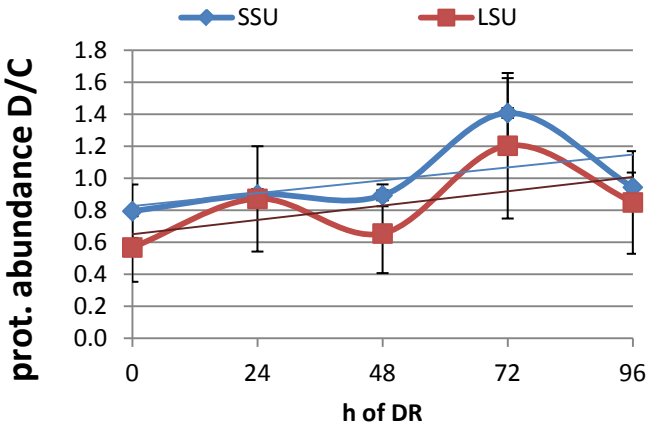
BoxPlot LSU



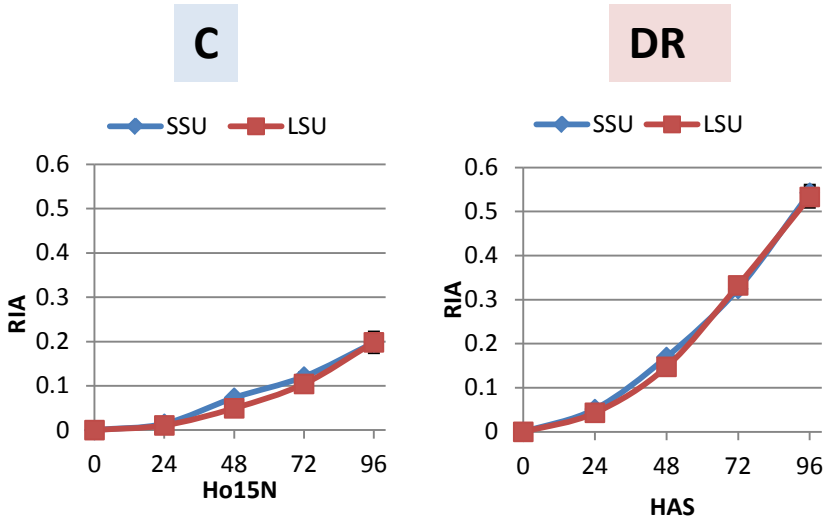
BoxPlot SSU



Protein Levels vs. Turnover



RIA



RSUs **abundance**
did not significantly change
during drought recovery!

RSUs **RIAs**
did significantly increase
during drought recovery!

=> Equal Synthesis and Degradation Rates!

Protein Levels vs. Turnover

Av. RSU synthesis rates per 24 h:

Contol = 0.05

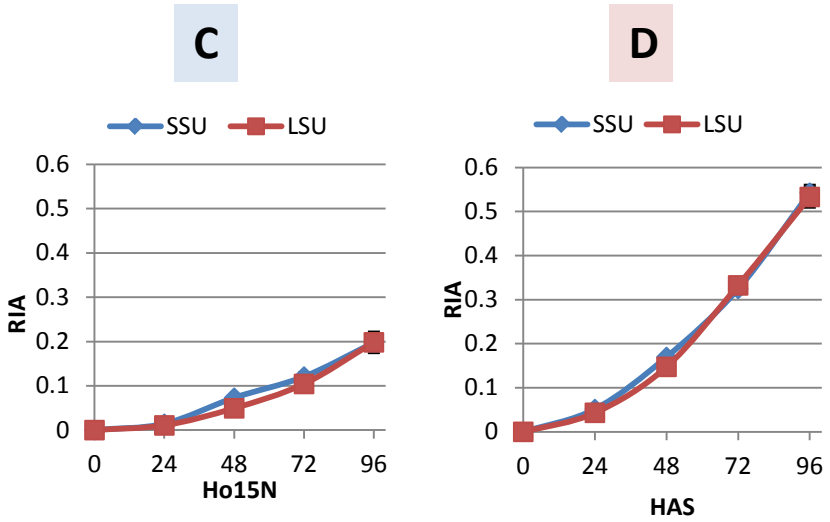
DR= 0.13

Av. RSU degradation rates per 24 h:

Control = 0.06

DR= 0.12

RIA



RSUs **RIAs**
did significantly increase
during drought recovery!

=> RuBisCO turnover ~2 fold higher during drought recovery!

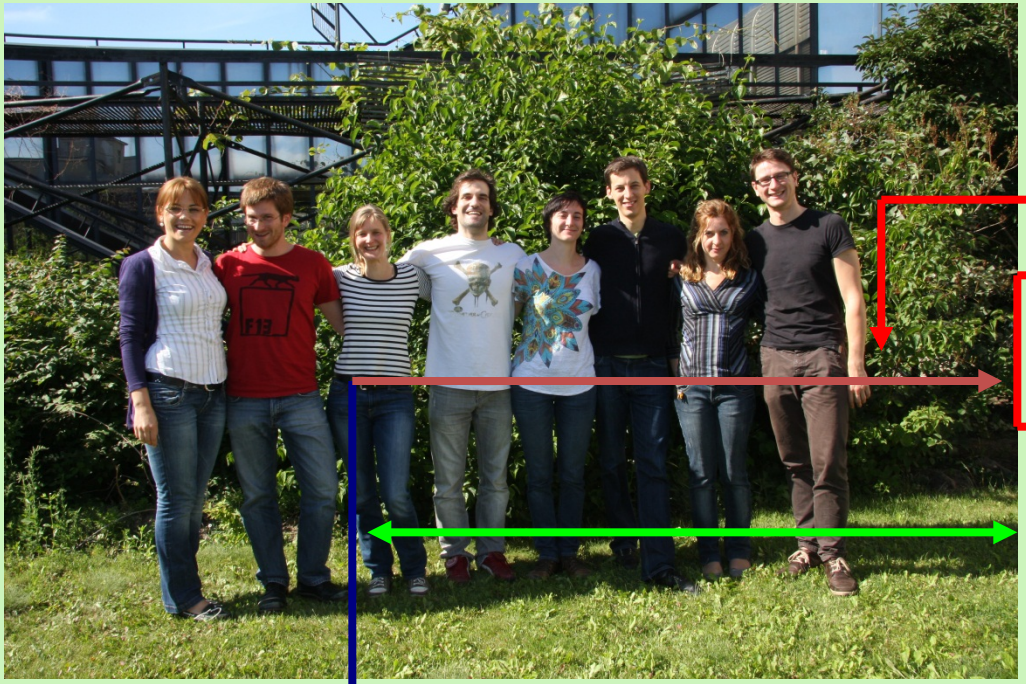
Second Conclusion

1) AA turnover increased within first 24 h of drought recovery. -> AA levels increased within 48 h -> direct incorporation into proteins
2) A strong increase in protein turnover was observed along drought recovery (synthesis and degradation rates)

❖ variation in isotope composition can be caused by multiple assimilation events, organ specific loss of nitrogen, and resorption and reallocation of nitrogen.

➢ Reduced N-uptake during drought caused decreased AA levels

Thanks to the Team!!!



+ Green team!!

[P23441-B20]
[P24870-B22]

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