Mosys
Department
Molecular Systems Biology
universität wien
University of Vienna | Faculty of Life Sciences | Althanstrasse 14 | 1090 Vienna | Austria

Project Practical Course 300094
Proteomics in Systems Biology

Trainer: Stefanie Wienkoop

Tutor: Sebastian Schneider

Additional lectures:

safety instructions – Lena Fragner
statistics – Gert Bachmann
systems biology – Wolfram Weckwerth

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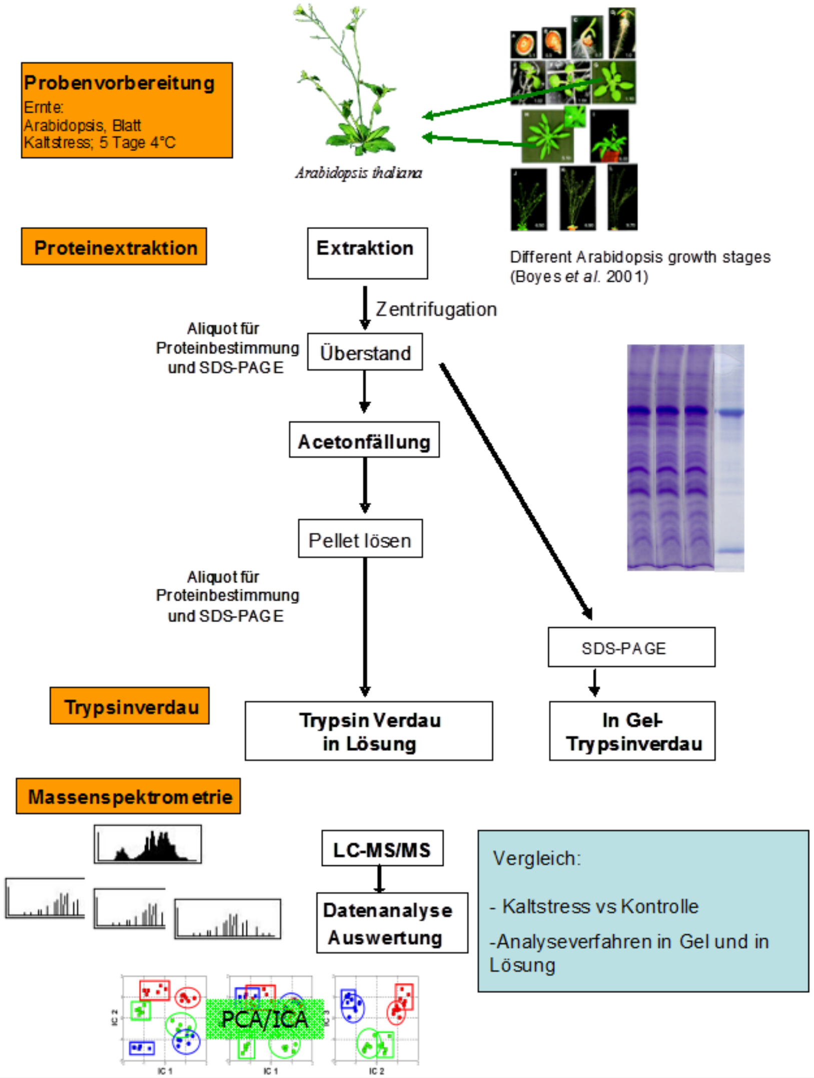
Full Scientific Experiment !

- Harvest
- Extraction
- Analytics
- Statistics



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Schedule

Tu	protein extraction / SDS-gel
We	in-solution digestion / introduction MS instruments
Th	in-gel digestion / data analysis training
Fr	desalting / data analysis training
Sat/Sun	MS analyses – *free time for the students!*
Mo	data mining
Tu	data mining
	further data mining possible until Friday!

12 Students total

Material – Extraction – LC-MS:

4 groups á 3 persons

Experiment - Data mining:

4 groups á 3 persons

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4 Groups a 3 persons)

Material – Extraction – GC-MS – Data mining:

Medicago truncatula (Rhizobium inoculated) : **leaves**

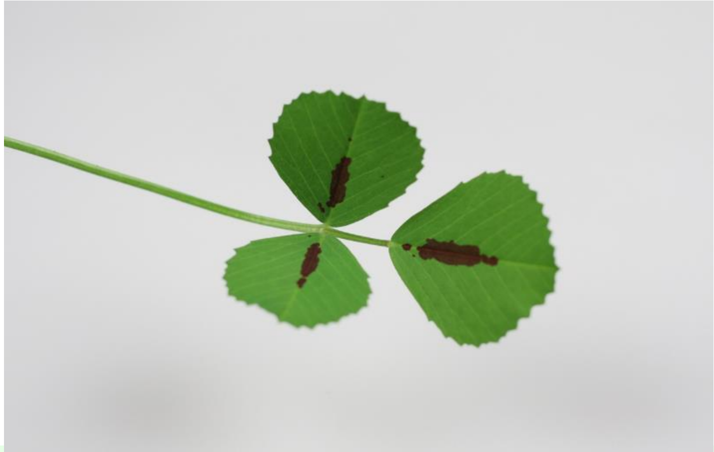
Group 1) wt

Group 2) dnf5-2 (N-fixation deficient)

Group 3) NF11301 (ferritin F3)

Group 4) NF9644 (ferritin F2)

each: control and drought stress (7 days)



What do we expect from you?

1) **Excellent collaboration** **20%**

2) **Final presentation of your results preferable in english:**

   a) Protocol (in groups, written in english, article formate) 40%
      please label your paragraphs with Author names!

   b) Oral presentation (date according to prior agreement) 40%

Part I
Technical Strategies

Complementary and Integrative, quantitative Proteomics and Metabolomics MS Analysis techniques in Systems Biology

GC-Triple-Quadrupole-MS

TARGETED

LC-Triple-Quadrupole-MS

GCxGC-Tof MS

UNBIASED

LC-Orbitrap-MS

MS-Techniques:

GEL-based (1D or 2D) and/or GEL-free

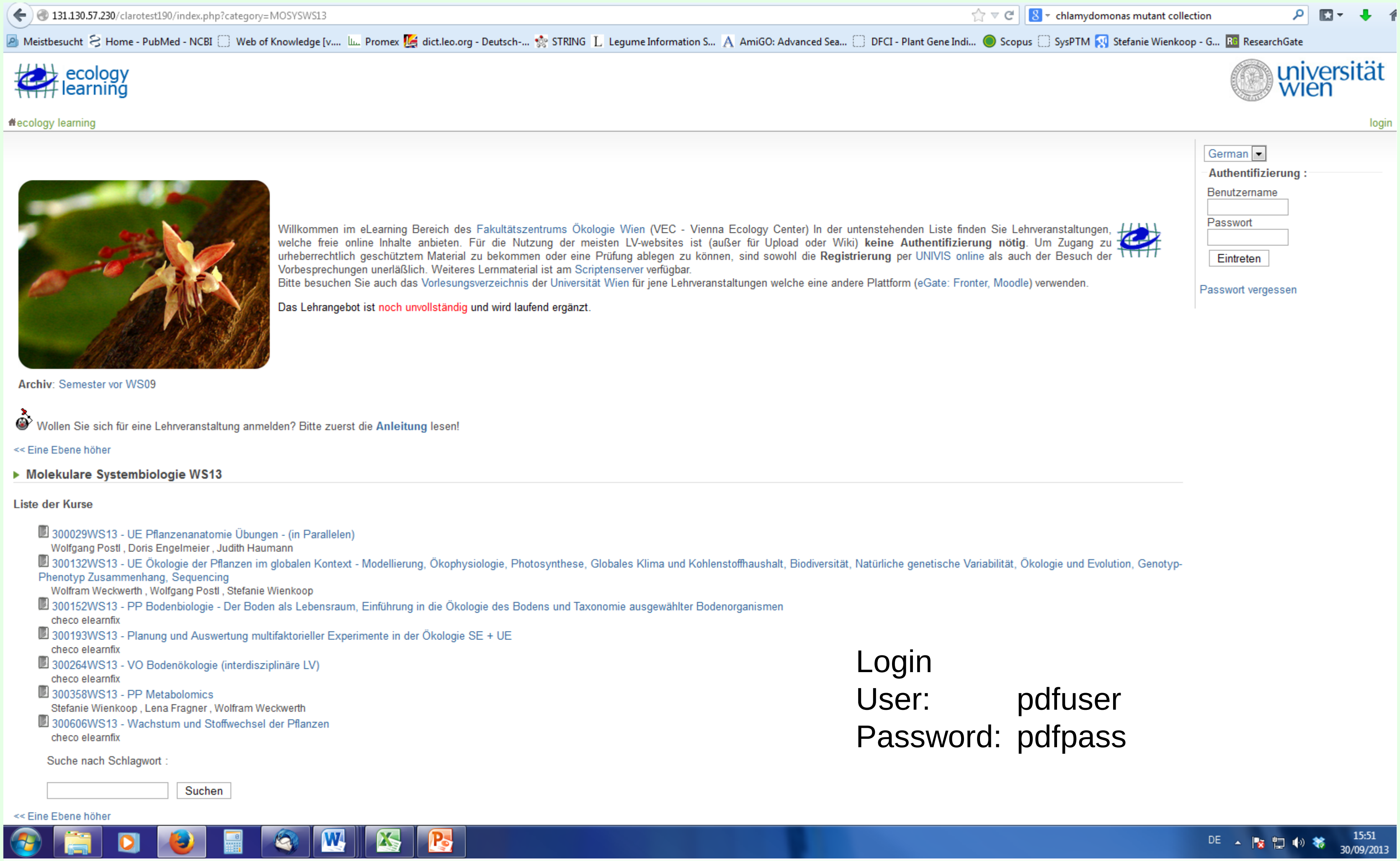
1) LC-Ion (Orb)trap MS (Protein Profiling and semi-quantitativ analysis)

- Non-targeted approach using spectral count (SC)

2) LC-Triple quadrupole MS (Absolute quantitativ protein analysis)

- Targeted approach

PPMetabolomics



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For private use only!

Drought Experiment *Medicago truncatula*

3 students per group!

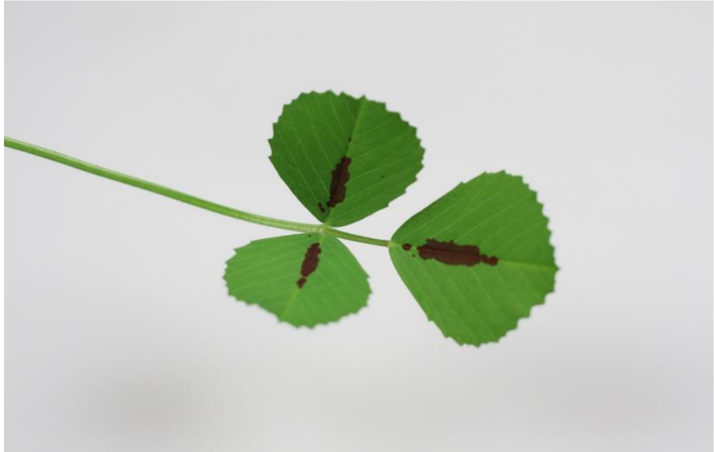
Medicago truncatula (Rhizobium inoculated) : **leaves**

Group 1) wt

Group 2) dnf5-2 (N-fixation deficient)

Group 3) NF11301 (ferritin F3)

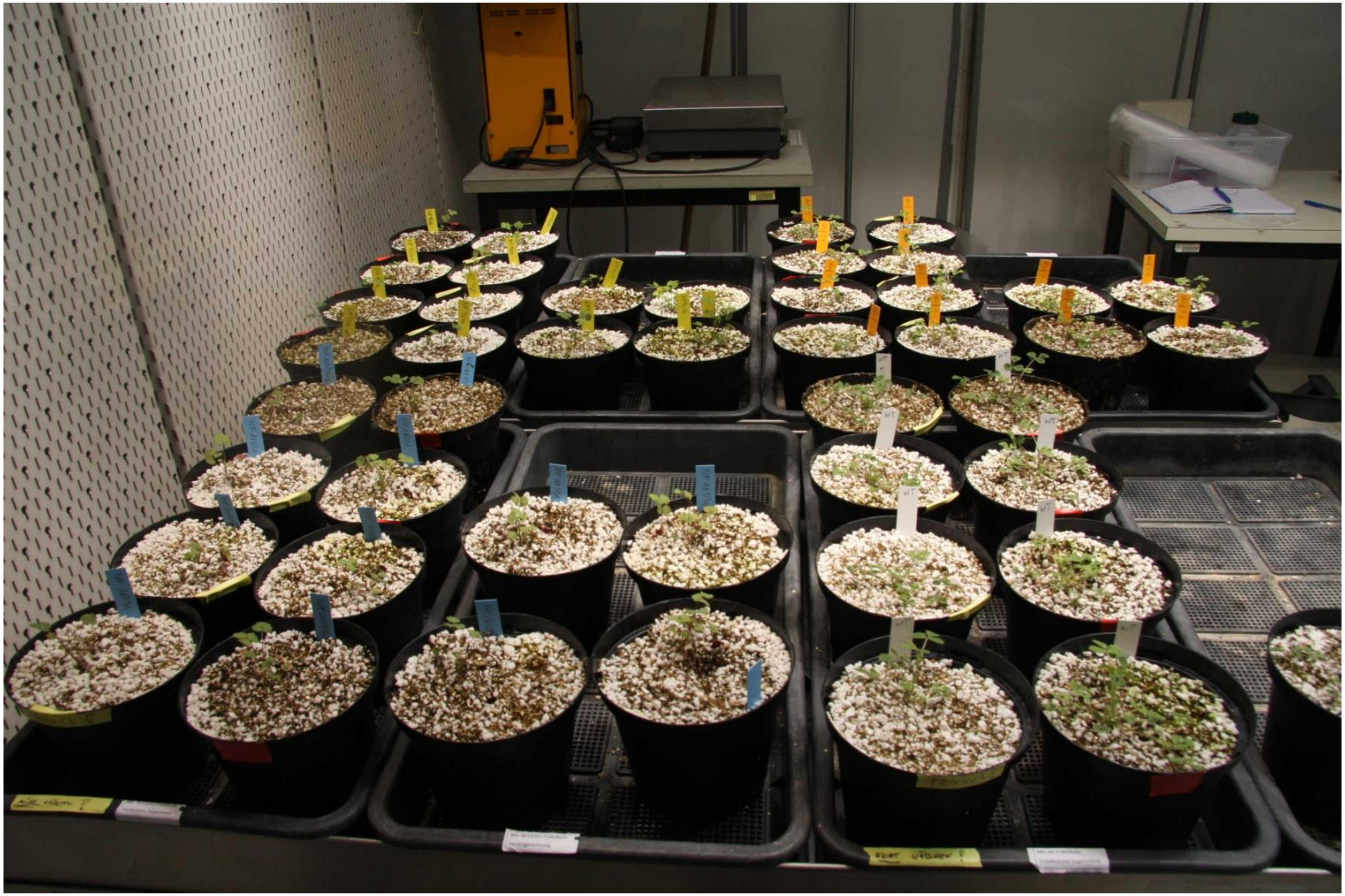
Group 4) NF9644 (ferritin F2)



each: control and drought stress (8 days water withold)

Proteomics

Drought Experiment
Medicago truncatula



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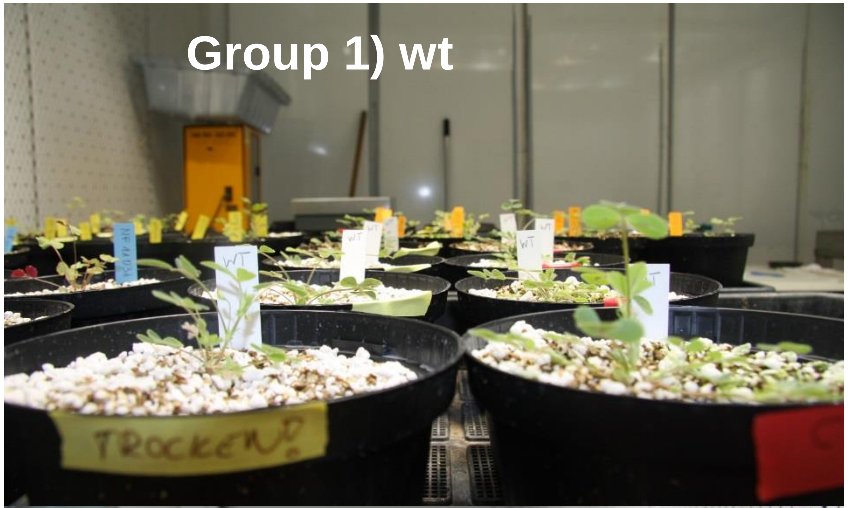
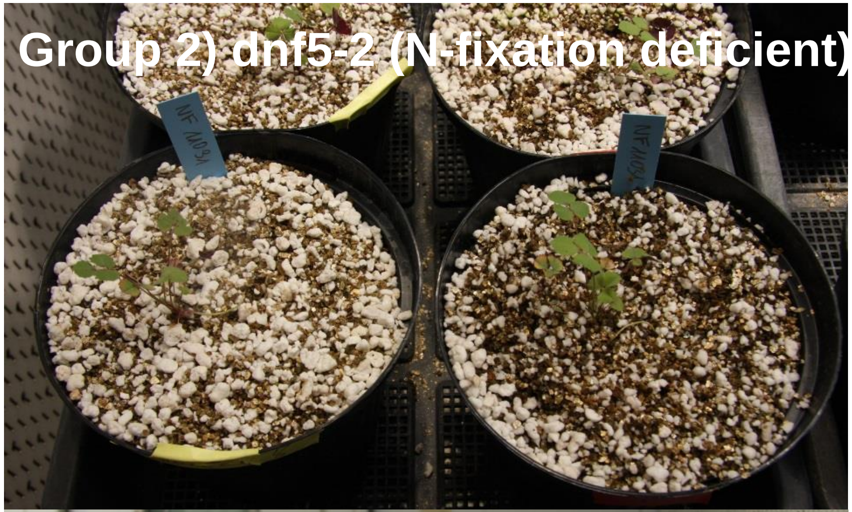
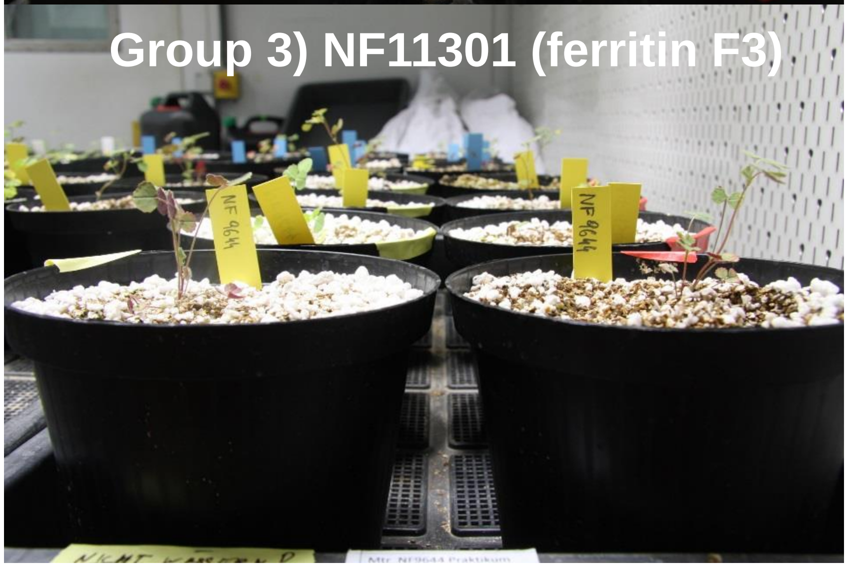
Proteomics

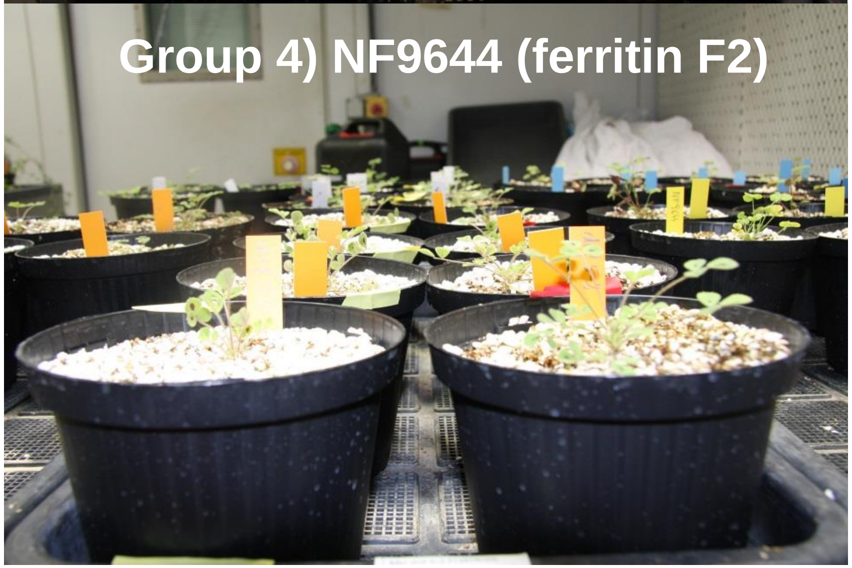
Drought Experiment *Medicago truncatula*

Plant-Systems Interaction







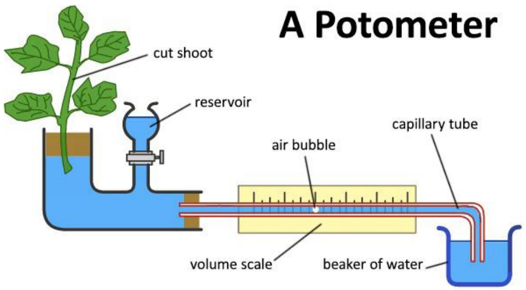


8 days of water withold!
Drought stress?

What is drought stress?

How to define drought stress?

Defining Drought Stress



Physiological definition:

1. Stomatal conductance
2. Transpiration
3. PS efficiency
4. Soil Water Content
5. ...

1 + 2. porometer

4. Time domain reflectometry (TDR)

3. PAM

Drought

Cause of water deficit

- Induced by many environmental conditions:

3. No rainfall- drought
4. High salt conc.
5. Low temp.
6. Transient loss of turgor at midday

- Rate of onset, duration, acclimatization- influence the water stress response

Drought

drought induced leaf senescense

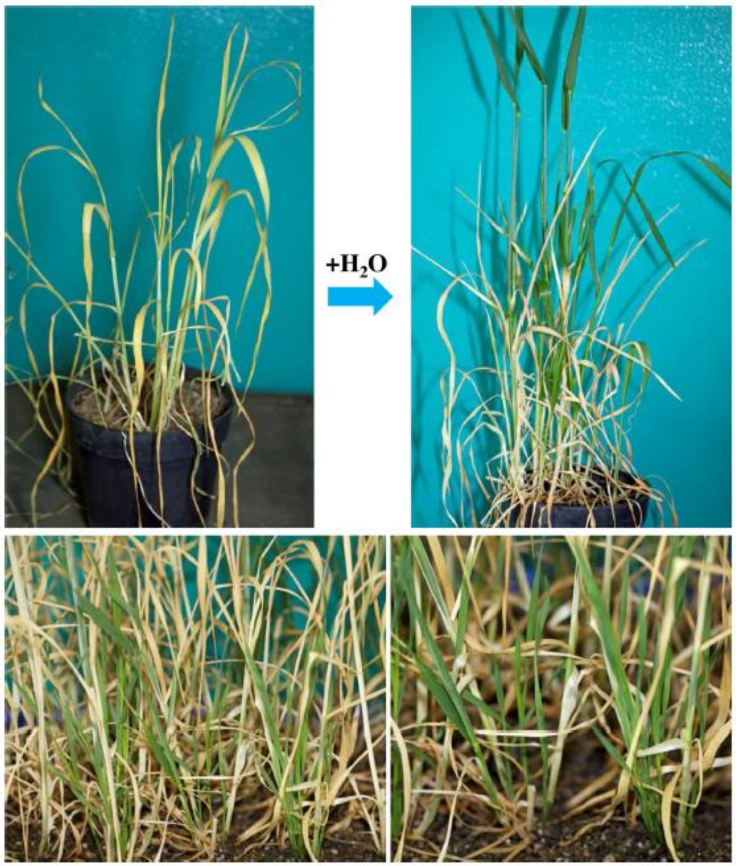


Fig. 3. Effect of drought stress at the reproductive stage in wheat. Drought stress leads to extensive leaf senescence in wheat (top left). Re-watering results in the development of new freshly green tillers that will flower and produce grains, while grain development in the older stressed tillers is either aborted or leads to spikes without grain (top right). The close-up pictures at the bottom show prolific initiation of new tillers in response to re-watering after drought treatment.

- Drought stress induces leaf senescence
- Rewatering results in development of new leaves

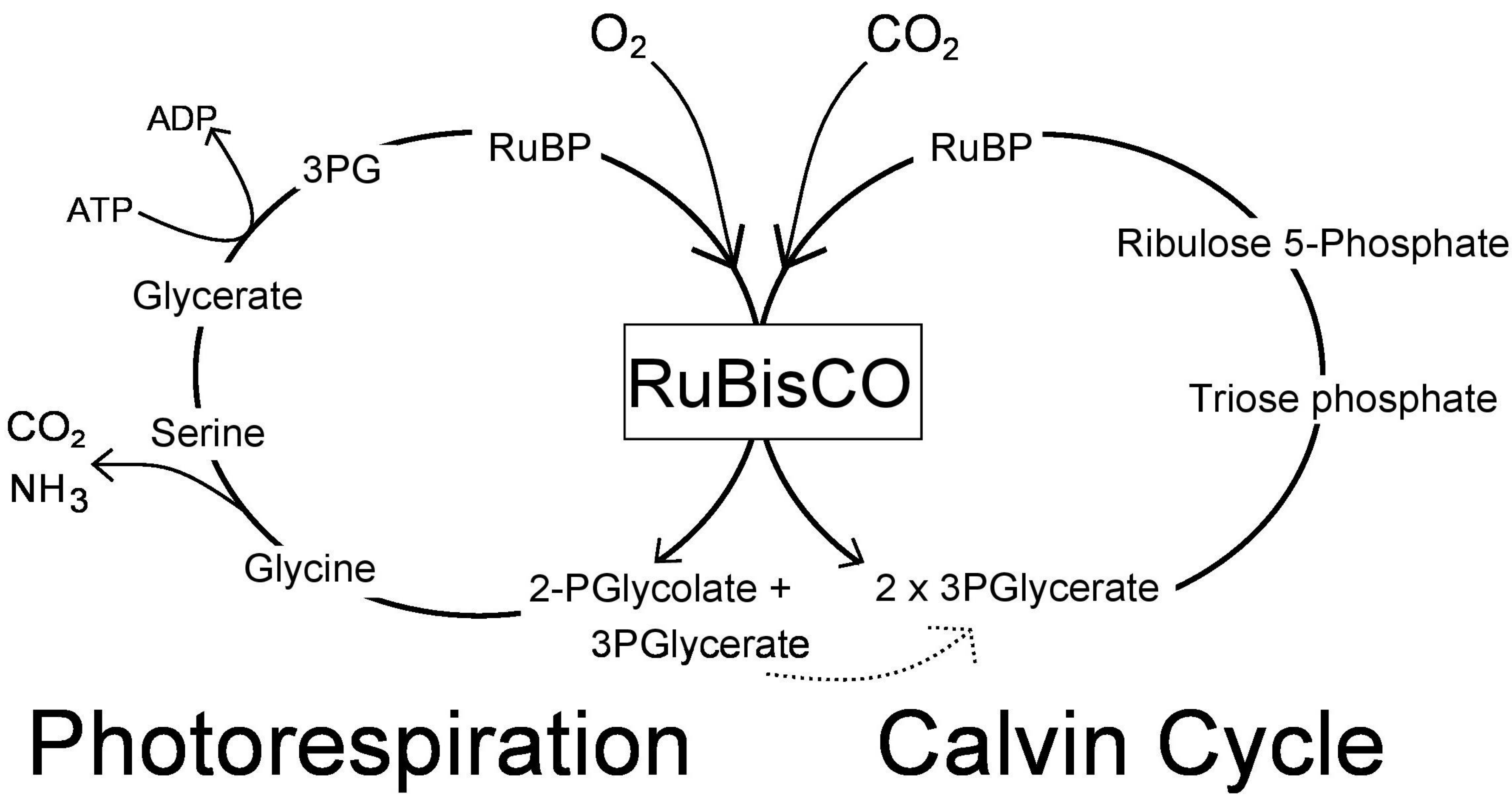
Drought

Pathways involved in drought stress adaptation

?

Drought

Photorespiration

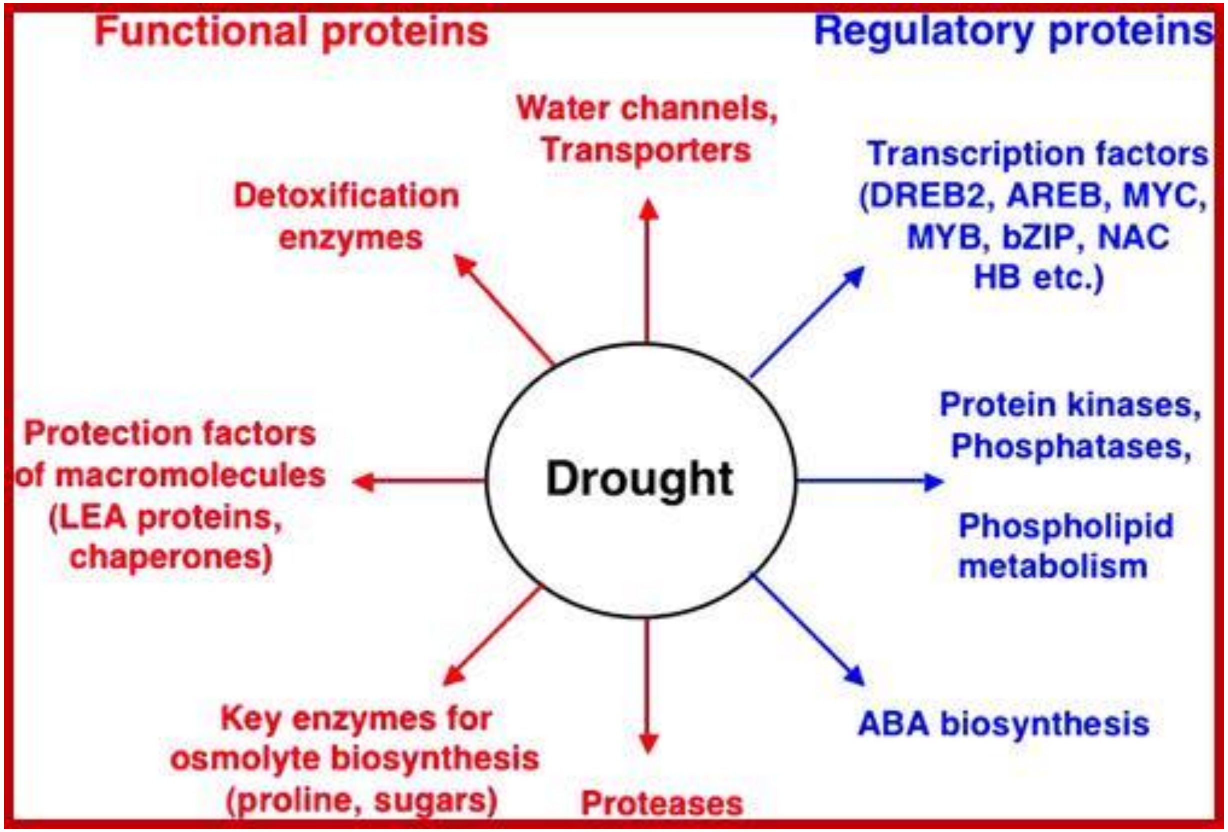


Proteins involved in drought stress adaptation

?

Drought

Proteins involved in drought stress adaptation

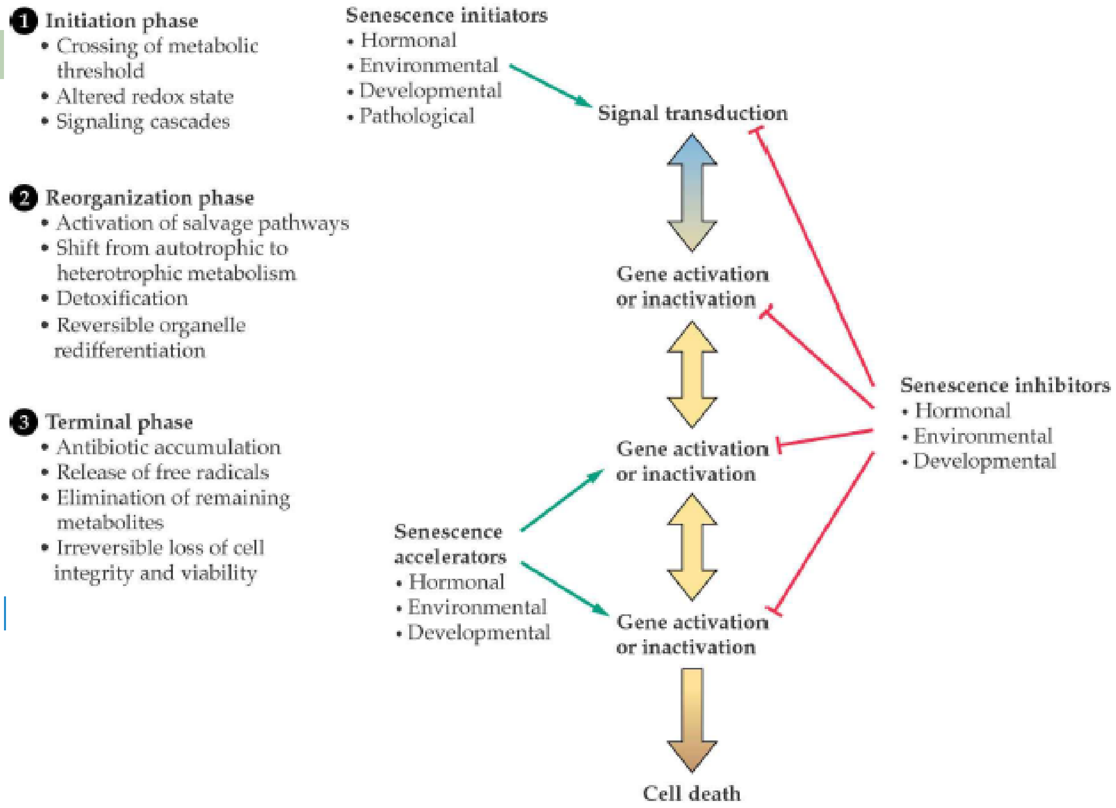


Drought

Senescence is a Regulated Process

Anabolic processes slowed down

Increased breakdown processes: Chlorophyll-breakdown, protein degradation…



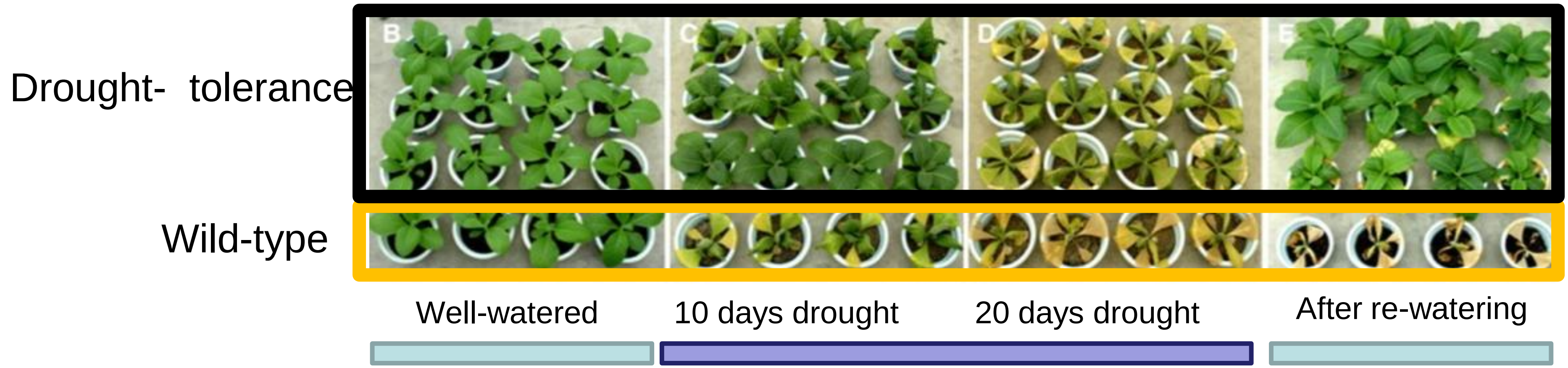
Partitioning: Nutrients like N, S, P are converted into transportable forms. Through phloem transport they reach the young leaves (at sequential senescense).

Drought

Plant Stress Recovery Capacity

Staygreen (SG) Effect
reduced leaf senescence

Recovery plays an important role in understanding SG



Yu, H., Chen, X., Hong, Y.-Y., Wang, Y., Xu, P., Ke, S.-D., Liu, H.-Y., Zhu, J.-K., Oliver, D.J., Xiang, C.-B. (2008) Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. Plant Cell 20:1134-1151.

Staygreen normally gene regulated was also found symbiont induced (SISG)!

Drought

Symbiotic induced StayGreen Effect - SISG

N-fix N-fed

Stress response

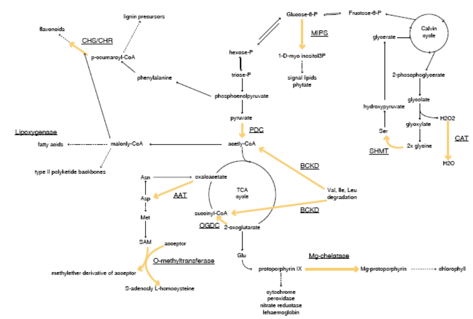
Recovery phase

12 days of
drought stress

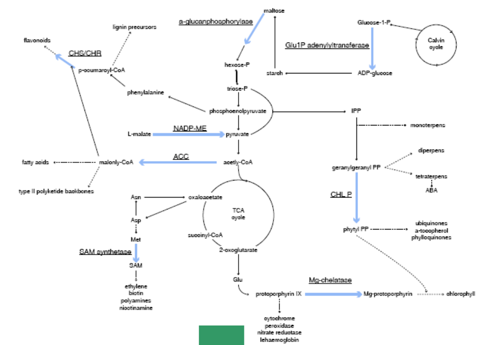
2 days after
rewatering

LC-MS/MS

N-fed



N-fix



Metabolomics /Proteomics profiling

Pattern recognition

Marker detection

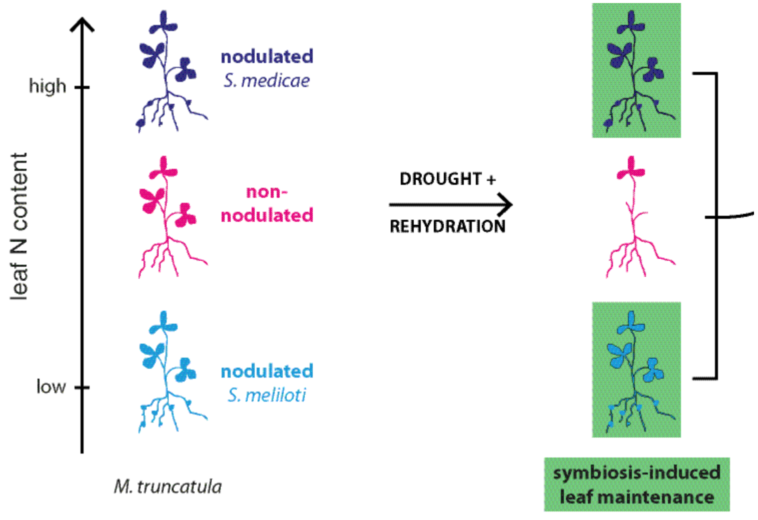
Higher stress tolerance - symbiotic interaction?

Staudinger *et al.* 2015

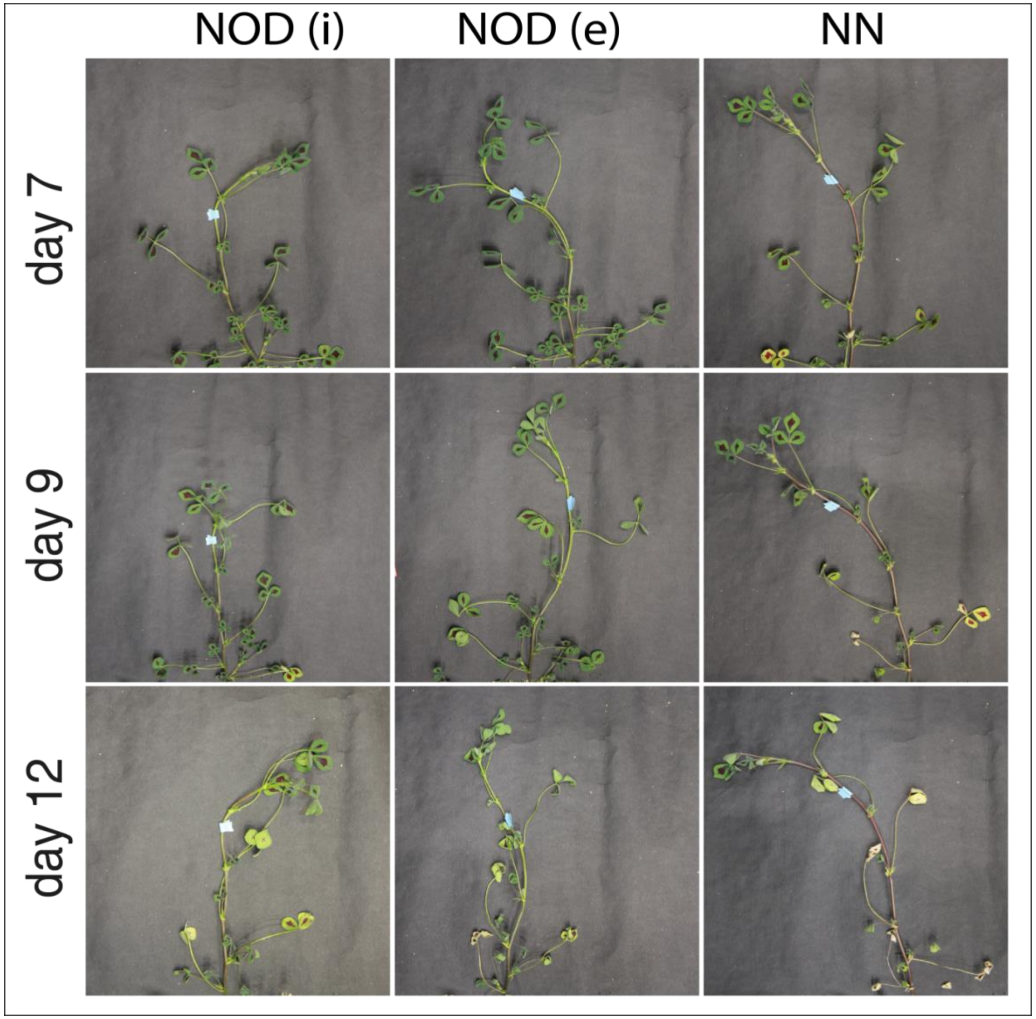
Drought

SISG is independent of N-fixation efficiency

Symbiotic Rhizobia Interaction induces a staygreen (SISG) phenotype in *Medicago truncatula* upon drought



Staudinger *et al*. 2016



Drought

Phenotyping

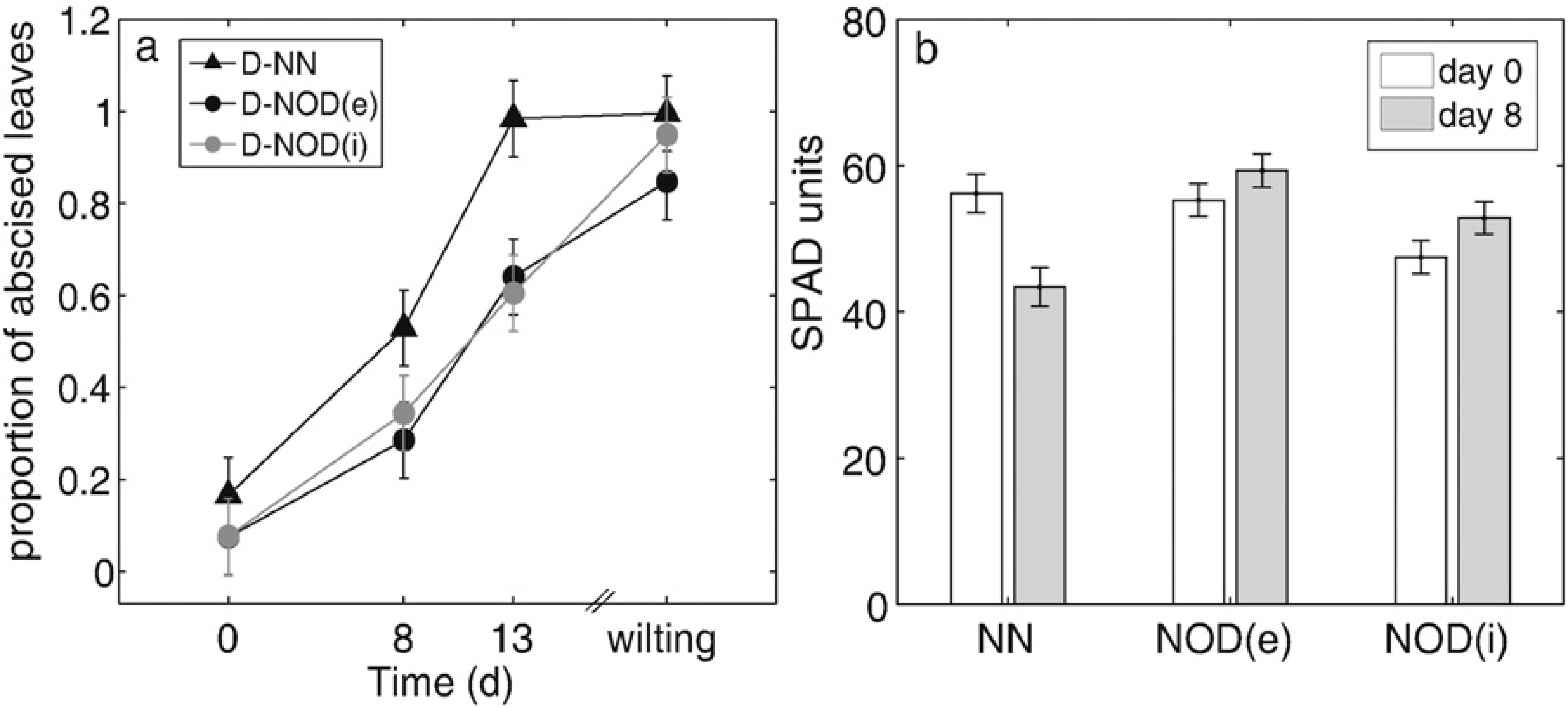


Fig. 2. Leaf senescence symptoms inM. truncatula induced by waterwithholding. (a) Leaf abscission rate. The day ofwilting was ~day 15 in all conditions. (b) Leaf chlorophyll index at the start of the desiccation period (day zero, white bars) and after eight days of water withholding (gray bars). Values are means; error bars indicate 95% LSD confidence intervals; n=5. D: drought treated, NN: non-nodulated, NOD(e): S. medicae nodulated.

Staudinger *et al*. 2016

Drought

Phenotyping

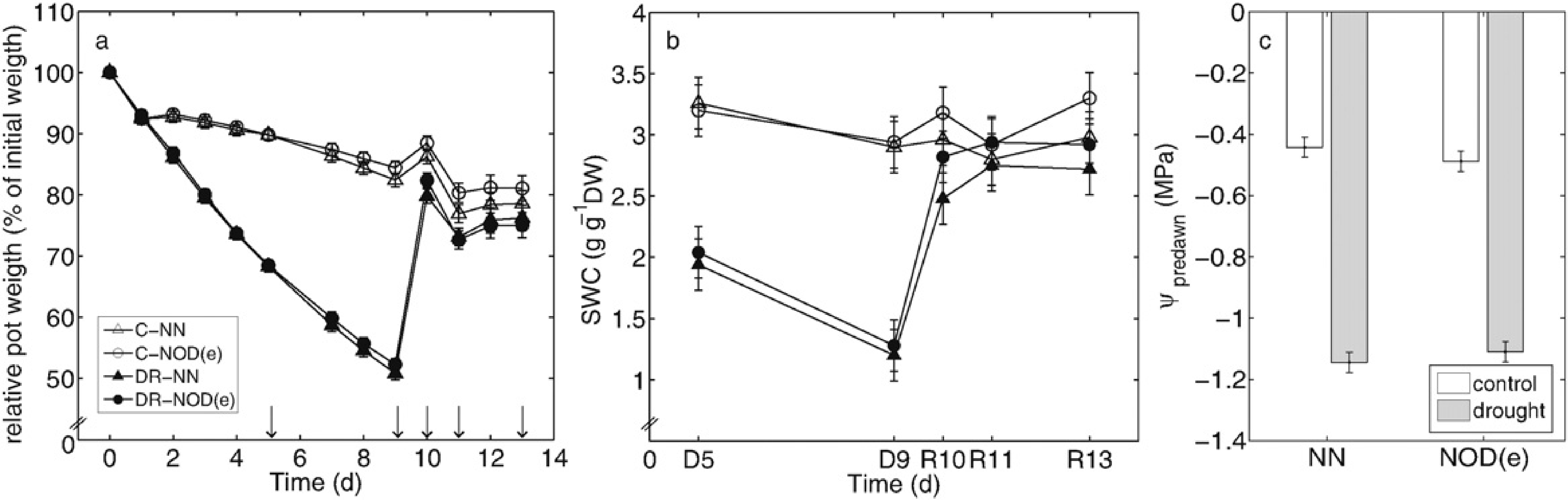
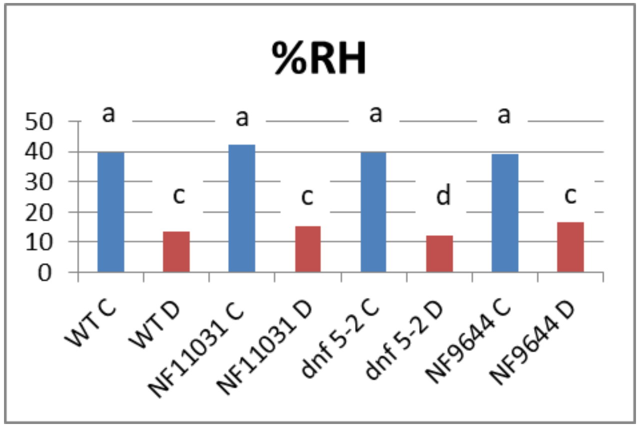
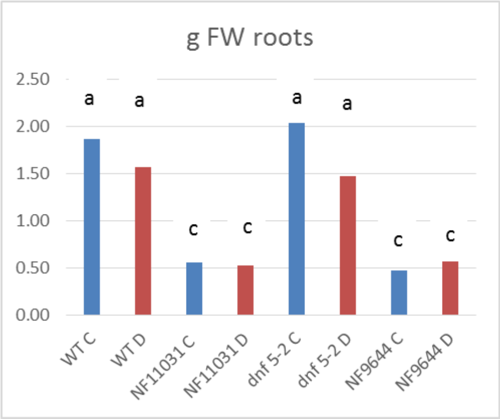


Fig. 3.Water status during drought and rehydration. Estimated (a) and absolute (b) SWC and D9 predawn leaf xylem water potential (c).Well-watered (open symbols, C) and treated Medicago truncatula (closed symbols, DR) was grown in a vermiculite/perlite mixture. Arrows in (a) designate the sampling time points shown in (b), ψ was measured on day nine. Values are means; error bars indicate 95% LSD confidence intervals; n = 5–10 in (a), n = 5 in (b). NN: non-nodulated, NOD(e): S. medicae nodulated and NOD(i): S. meliloti nodulated.

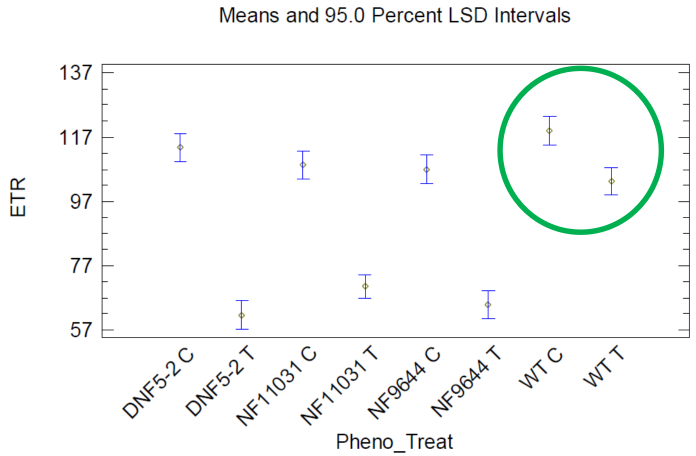
Staudinger *et al*. 2016

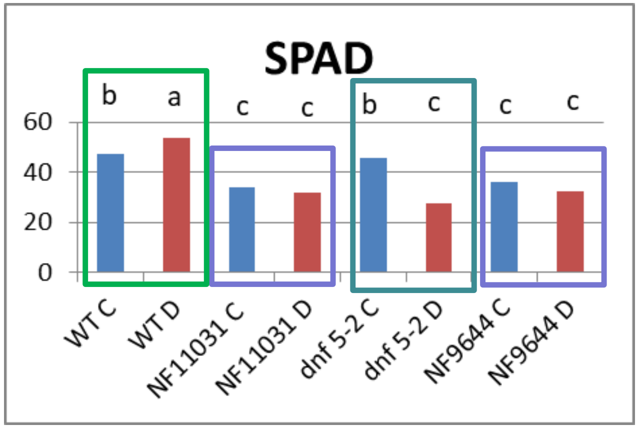
Drought Phenotyping





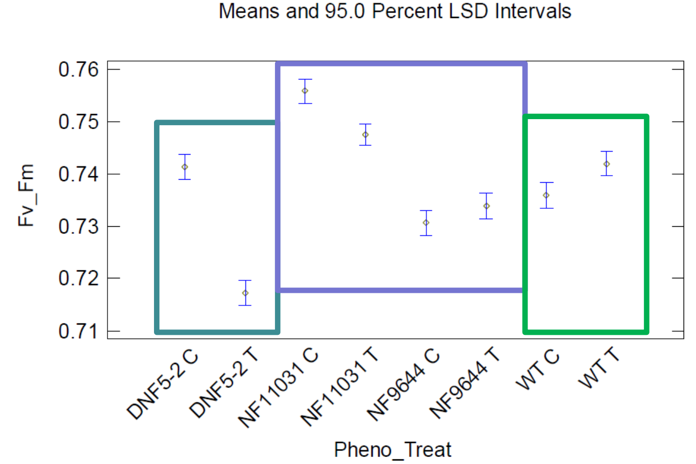
ETR = Electron Transfer Rate





no N-fixation
=
N-deficient!
=
lower chlorophyll content?

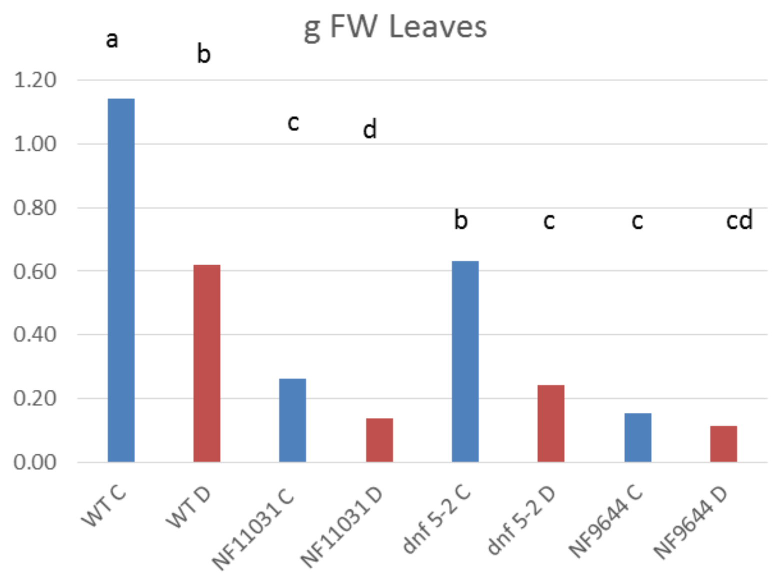
PSII efficiency



SPAD=Soil Plant Analysis Development

Drought

Phenotyping



Drought

Osmotic Adjustment - Osmolytes &

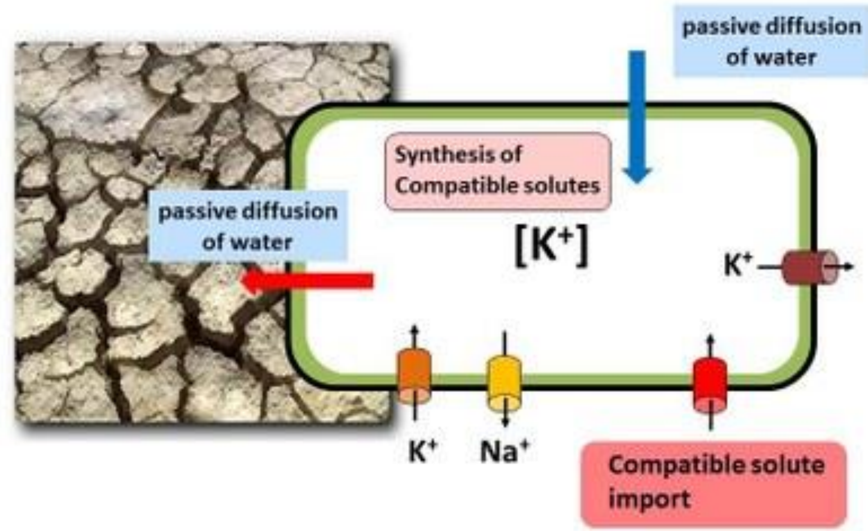
- Osmotic adjustment occurs when the concentrations of solutes within a plant cell increases to maintain a positive turgor pressure within the cell
- The cell actively accumulates solutes and as a result the solute potential (Ψs) drops, promoting the flow of water into the cell

- **Compatible solutes reduce ROS-induced potassium efflux.**

SISG: Symbiosis increased K^+ accumulation

Reminder: role of **potassium**!

- Maintaining adequate plant K is, critical for plant drought resistance
- K^+ has root growth promoting effect
- K^+ can enhance the total dry mass accumulation.
- stomatal regulation by K^+
- improved water retention in plant tissues



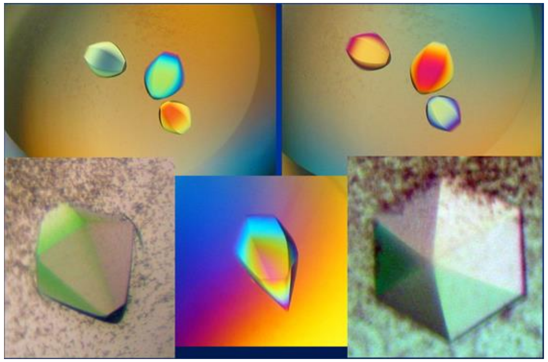
Drought

Synthesis of compatible solutes

- Almost all organisms, ranging from microbes to animals and plants, synthesize compatible solutes in response to osmotic stress.

- Compatible solutes are nontoxic molecules such as amino acids, glycine betaine, sugars, or sugar alcohols which can accumulate at high concentration without interfering with normal metabolism.

- They may have a role in osmotic adjustment, stabilizing proteins and cell structures, scavenging reactive oxygen species.



Crystals of the ectoine hydroxylase EctD from *Salibacillus salexigens*

(Picture provided by Dr. K. Reuter; University of Marburg)

SISG: Symbiosis reduced accumulation of starch & increased sugar accumulation

Questions

Do we see the SISG effect?

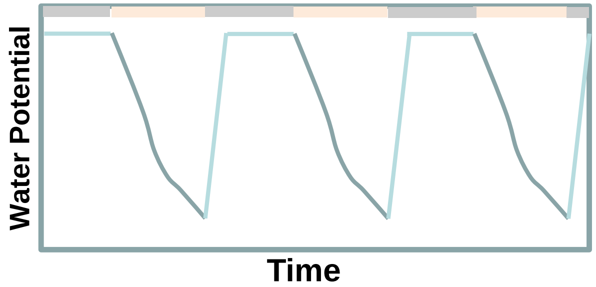
What is the proteome response of the leaves to adjust to drought stress?

Is it an active process?

What role plays Ferritin?

What role plays N-fixation?

Drought



Deacclimation Research – Why?

Plants are exposed to a continuously changing environment.

Extremes such as several weeks of drought are followed by rain.

This requires a molecular plasticity of the plant enabling drought acclimation and the necessity of deacclimation processes for recovery and continuous growth.

THE ABILITY OF PLANTS TO RECOVER FROM STRESS

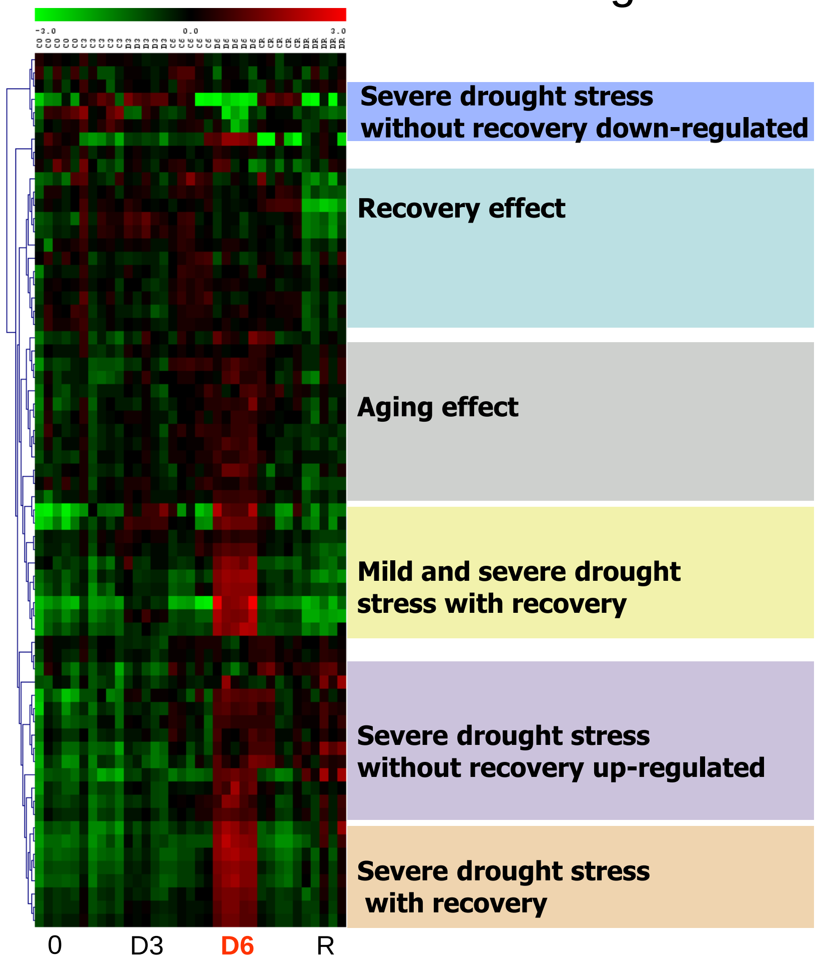
= IMPORTANT MECHANISM OF THE PLANTS TOLERANCE

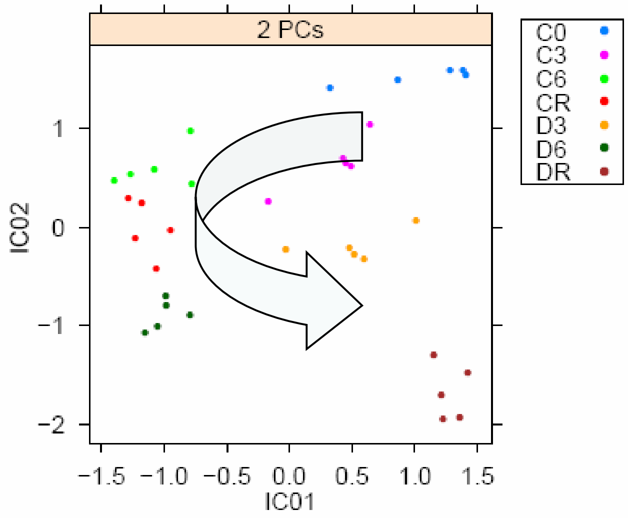
Drought

How to find Molecular Mechanisms and „Stress Marker“

HIC

Medicago root nodules



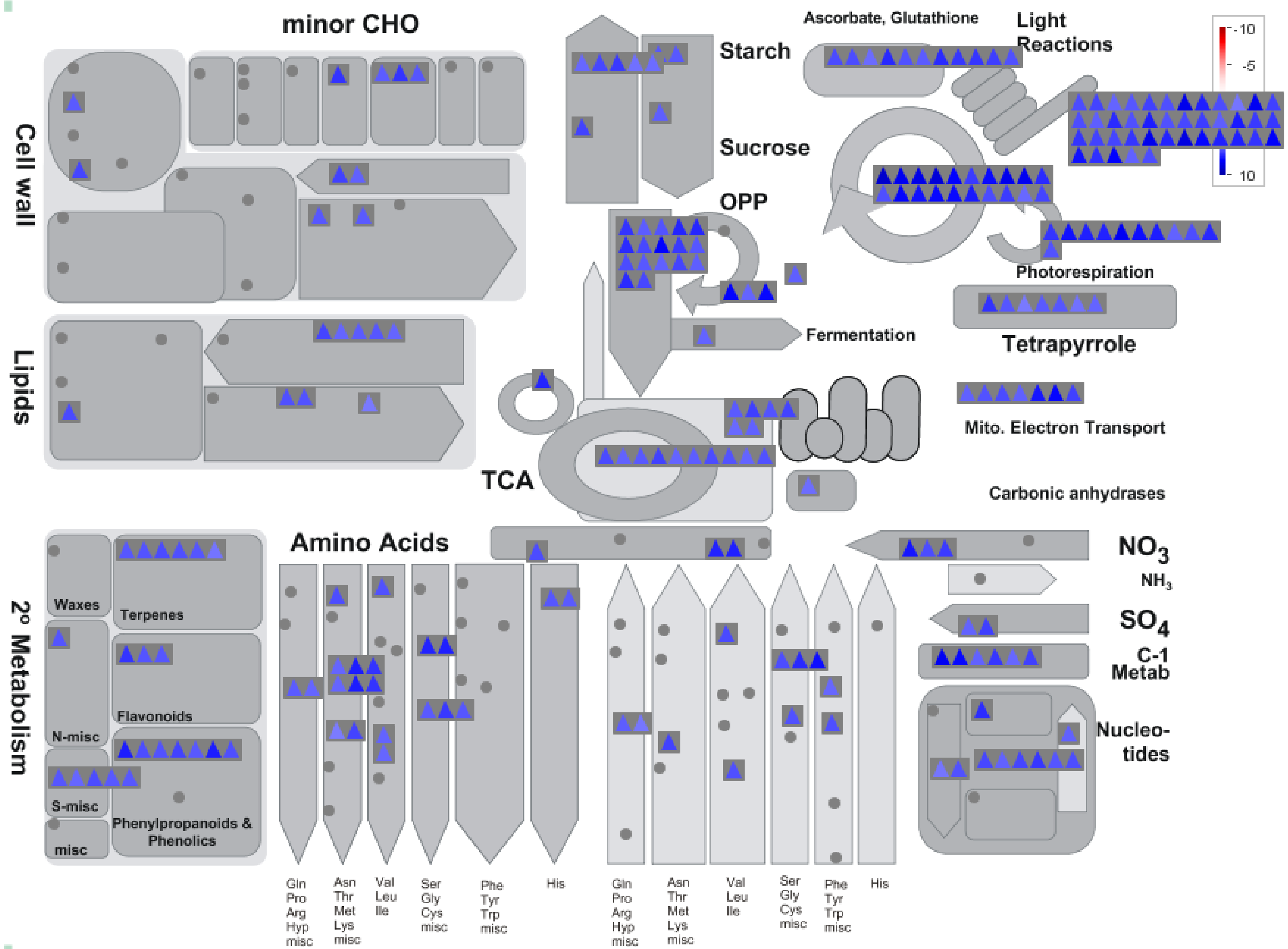


N-fixation strongly inhibited upon drought!

Larrainzar et al. MPMI 2009

Drought

MAPMAN
Output of Identified Proteins



Drought

MAPMAN
Output of Protein Ratios (D vs C)