

# Project Practical Course 300094 Proteomics in Systems Biology

Trainer: Stefanie Wienkoop

Tutor: Sebastian Schneider

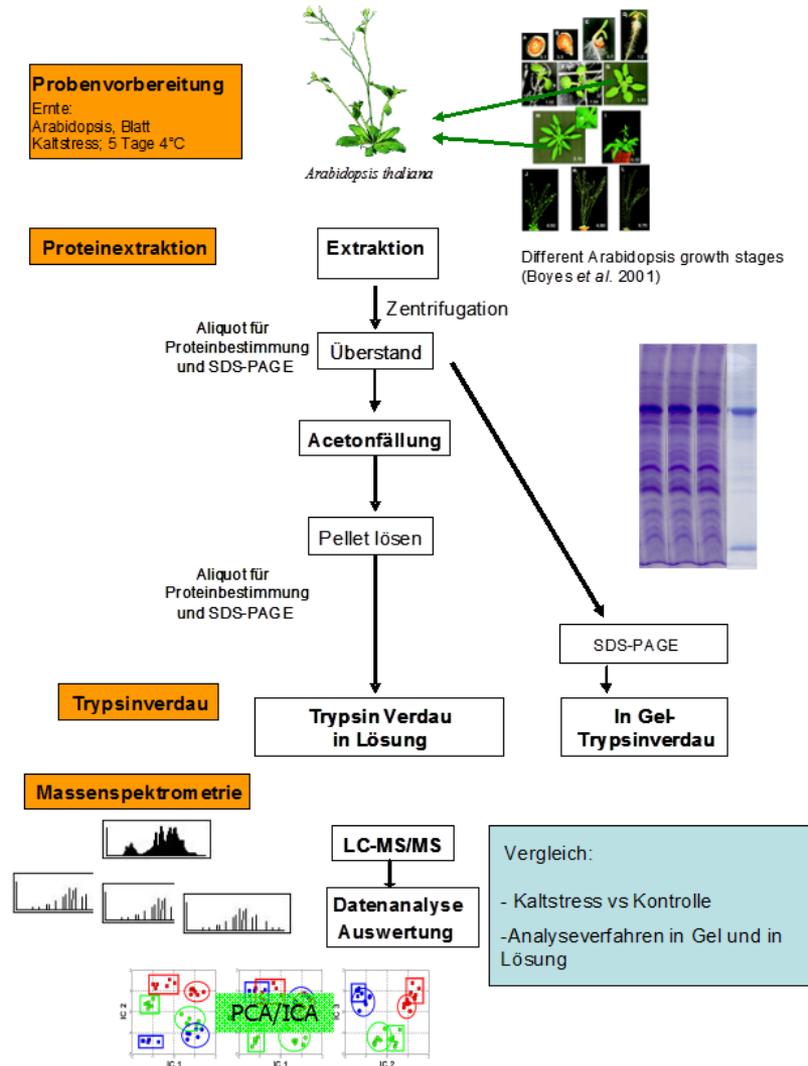
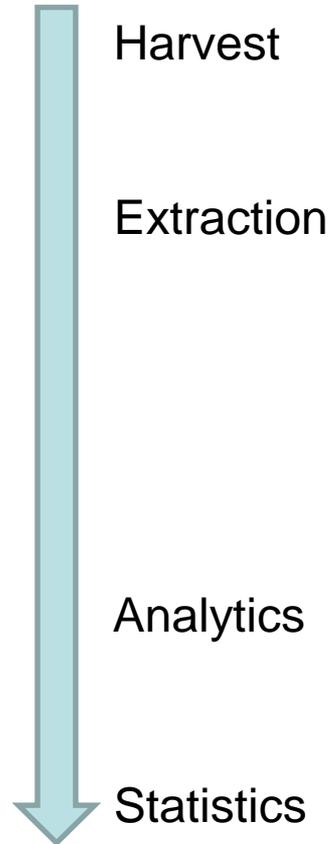
Additional lectures:

safety instructions – Lena Fragner

statistics – Gert Bachmann

systems biology – Wolfram Weckwerth

# Full Scientific Experiment !



## *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

## 4 Groups a 3 persons)

### Material – Extraction – GC-MS – Data mining:

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

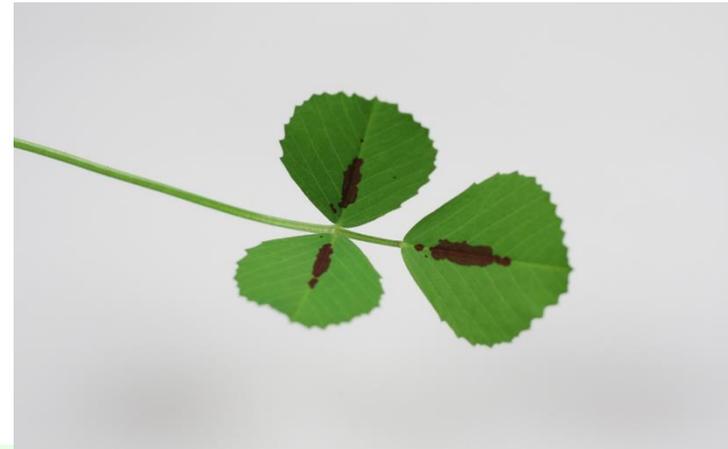
Group 1) wt

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

Group 3) NF11301 (ferritin F3)

Group 4) NF9644 (ferritin F2)

each: **control** and **drought stress** (7 days)



# PPMetabolomics – Model Plants

Stefanie Wienkoop - PPProteomics

## 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%

# PPPteomics – Technical Strategies and Model Plants

University Vienna - Stefanie Wienkoop – Plant Systems Interaction

## 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

Dept. Molecular Systems Biology

## **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



#ecology learning

login



Archiv: Semester vor WS09

Willkommen im eLearning Bereich des **Fakultätszentrums Ökologie Wien (VEC - Vienna Ecology Center)** In der untenstehenden Liste finden Sie Lehrveranstaltungen, welche freie online Inhalte anbieten. Für die Nutzung der meisten LV-websites ist (außer für Upload oder Wiki) **keine Authentifizierung nötig**. Um Zugang zu urheberrechtlich geschütztem Material zu bekommen oder eine Prüfung ablegen zu können, sind sowohl die **Registrierung per UNIVIS online** als auch der Besuch der Vorbesprechungen unerlässlich. Weiteres Lernmaterial ist am **Scriptenserver** verfügbar. Bitte besuchen Sie auch das **Vorlesungsverzeichnis der Universität Wien** für jene Lehrveranstaltungen welche eine andere Plattform (eGate: Fronter, Moodle) verwenden.

Das Lehrangebot ist **noch unvollständig** und wird laufend ergänzt.



German

Authentifizierung :

Benutzername

Passwort

Eintreten

Passwort vergessen

Wollen Sie sich für eine Lehrveranstaltung anmelden? Bitte zuerst die **Anleitung** lesen!

<< Eine Ebene höher

## ► Molekulare Systembiologie WS13

Liste der Kurse

- 300029WS13 - UE Pflanzenanatomie Übungen - (in Parallelen)  
Wolfgang Postl, Doris Engelmeier, Judith Haumann
- 300132WS13 - UE Ökologie der Pflanzen im globalen Kontext - Modellierung, Ökophysiologie, Photosynthese, Globales Klima und Kohlenstoffhaushalt, Biodiversität, Natürliche genetische Variabilität, Ökologie und Evolution, Genotyp-Phenotyp Zusammenhang, Sequencing  
Wolfram Weckwerth, Wolfgang Postl, Stefanie Wienkoop
- 300152WS13 - PP Bodenbiologie - Der Boden als Lebensraum, Einführung in die Ökologie des Bodens und Taxonomie ausgewählter Bodenorganismen  
checo eleamfix
- 300193WS13 - Planung und Auswertung multifaktorieller Experimente in der Ökologie SE + UE  
checo eleamfix
- 300264WS13 - VO Bodenökologie (interdisziplinäre LV)  
checo eleamfix
- 300358WS13 - PP Metabolomics  
Stefanie Wienkoop, Lena Fragner, Wolfram Weckwerth
- 300606WS13 - Wachstum und Stoffwechsel der Pflanzen  
checo eleamfix

Suche nach Schlagwort :

Suchen

<< Eine Ebene höher





3 students per group!

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

Group 1) wt

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

Group 3) NF11301 (ferritin F3)

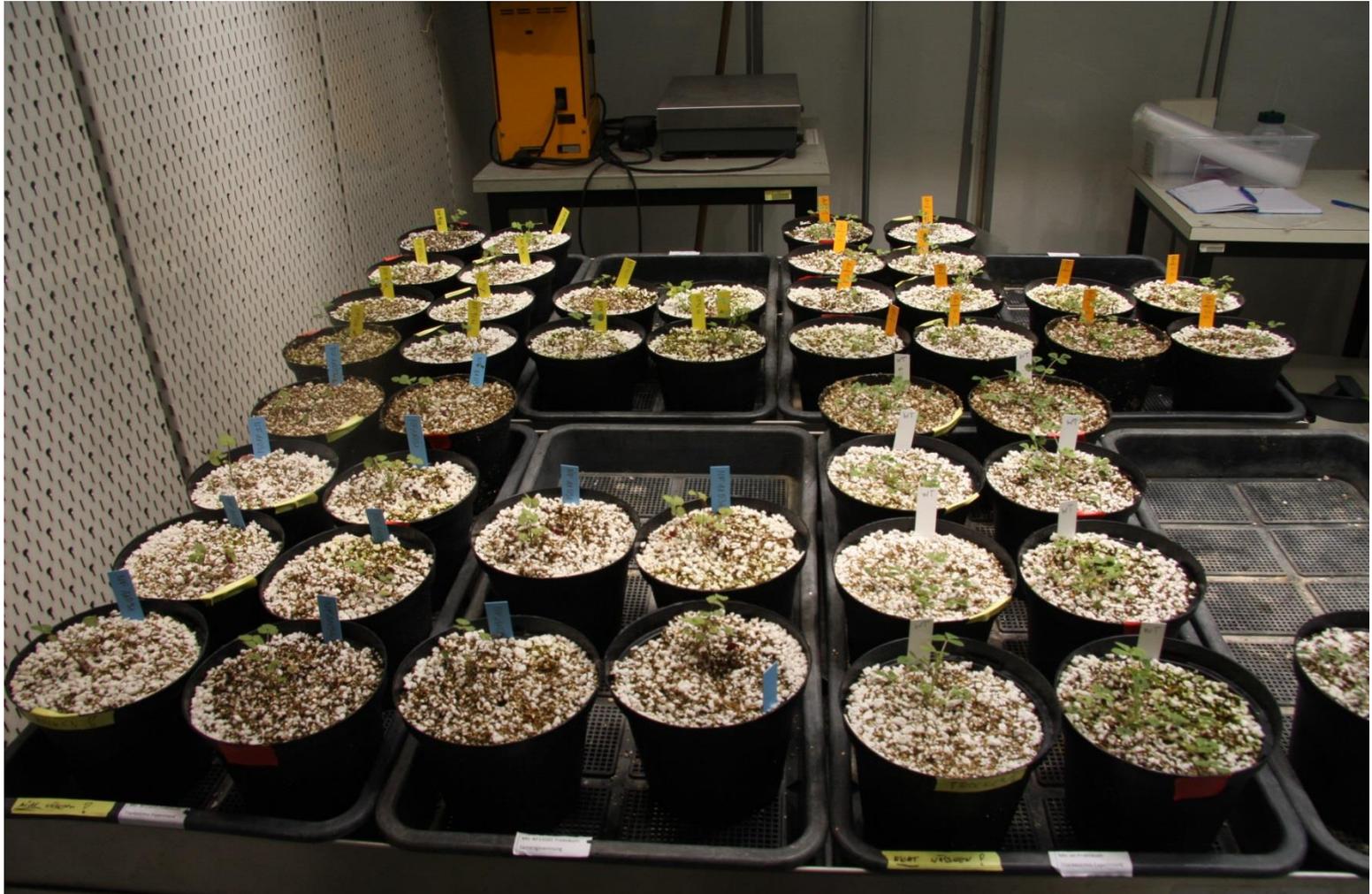
Group 4) NF9644 (ferritin F2)



each: **control** and **drought stress** (8 days water withhold)

# Proteomics

## Drought Experiment *Medicago truncatula*







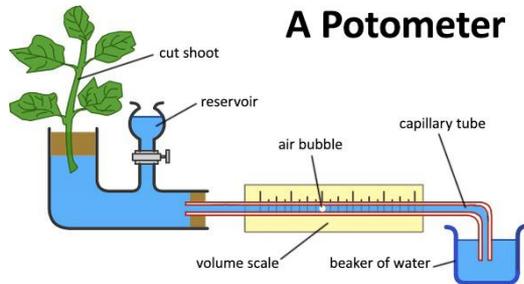
What is drought stress?

How to define drought stress?

# Proteomics

# Defining Drought Stress

**A Potometer**



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 senescence

256

R. Doberus / Plant Science 229 (2014) 247–261



**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

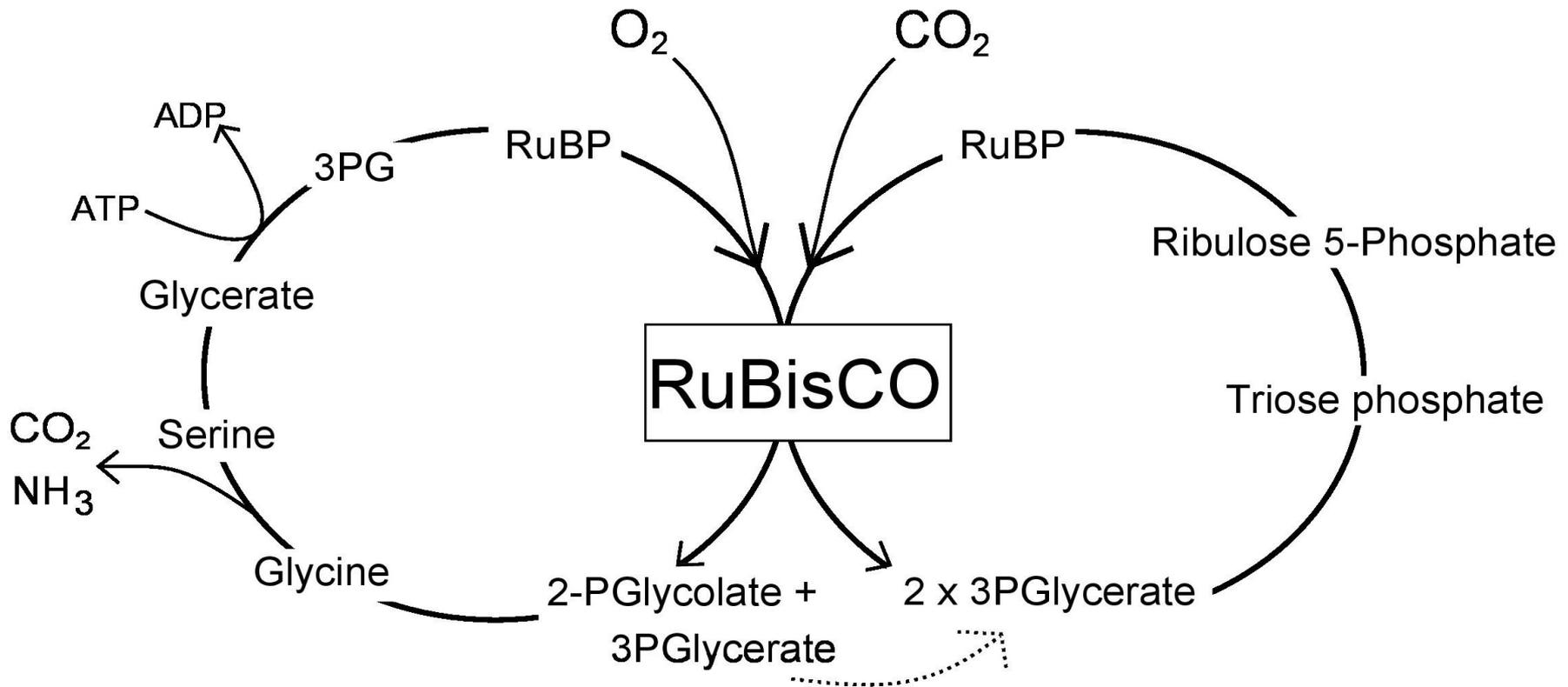
# Drought

## Pathways involved in drought stress adaptation



# Drought

# Photorespiration



## Photorespiration

## Calvin Cycle

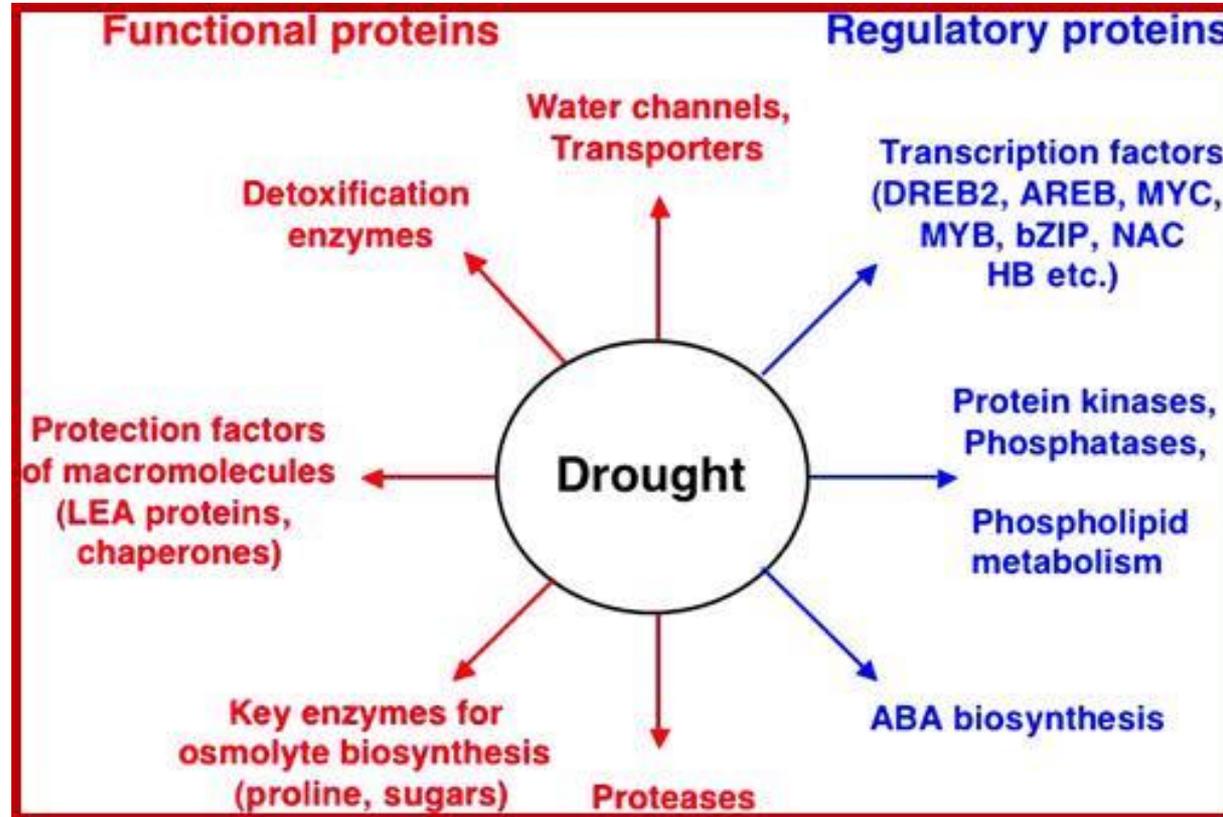
# Drought

## Proteins involved in drought stress adaptation



# Drought

## Proteins involved in drought stress adaptation



# Drought

## Senescence is a Regulated Process

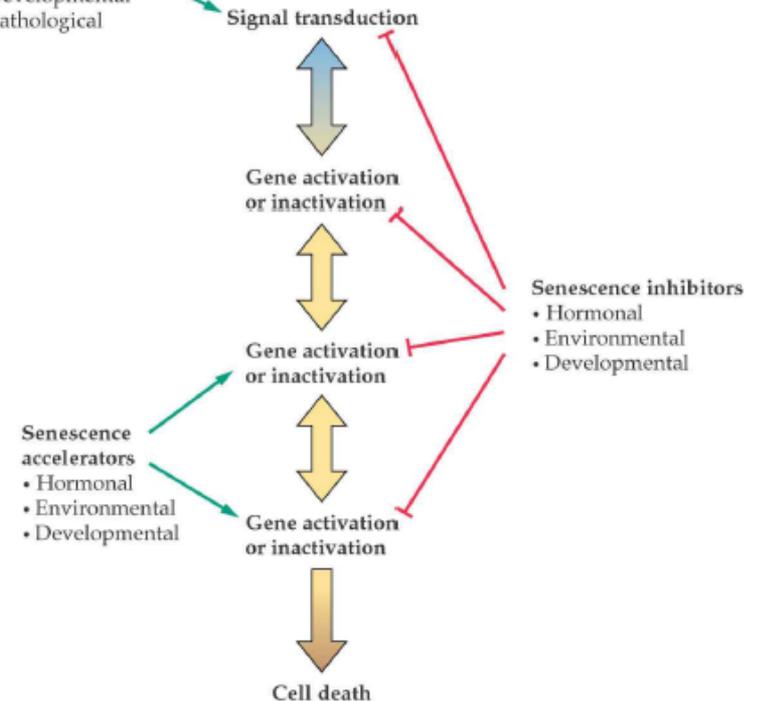
Anabolic processes slowed down

Increased breakdown processes: Chlorophyll-breakdown, protein degradation...

- 1 Initiation phase**
  - Crossing of metabolic threshold
  - Altered redox state
  - Signaling cascades
- 2 Reorganization phase**
  - Activation of salvage pathways
  - Shift from autotrophic to heterotrophic metabolism
  - Detoxification
  - Reversible organelle redifferentiation
- 3 Terminal phase**
  - Antibiotic accumulation
  - Release of free radicals
  - Elimination of remaining metabolites
  - Irreversible loss of cell integrity and viability

### Senescence initiators

- Hormonal
- Environmental
- Developmental
- Pathological



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

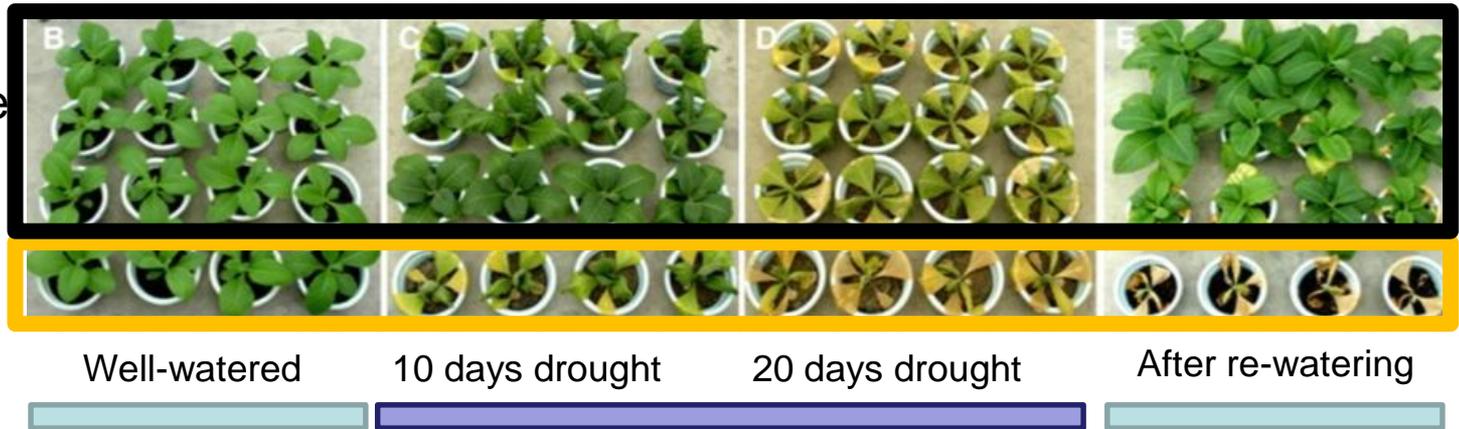
# Drought

## Plant Stress Recovery Capacity

**Staygreen (SG) Effect**  
reduced leaf senescence

Recovery plays an important role in understanding SG

Drought- tolerance



Wild-type

Well-watered

10 days drought

20 days drought

After re-watering

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](#).

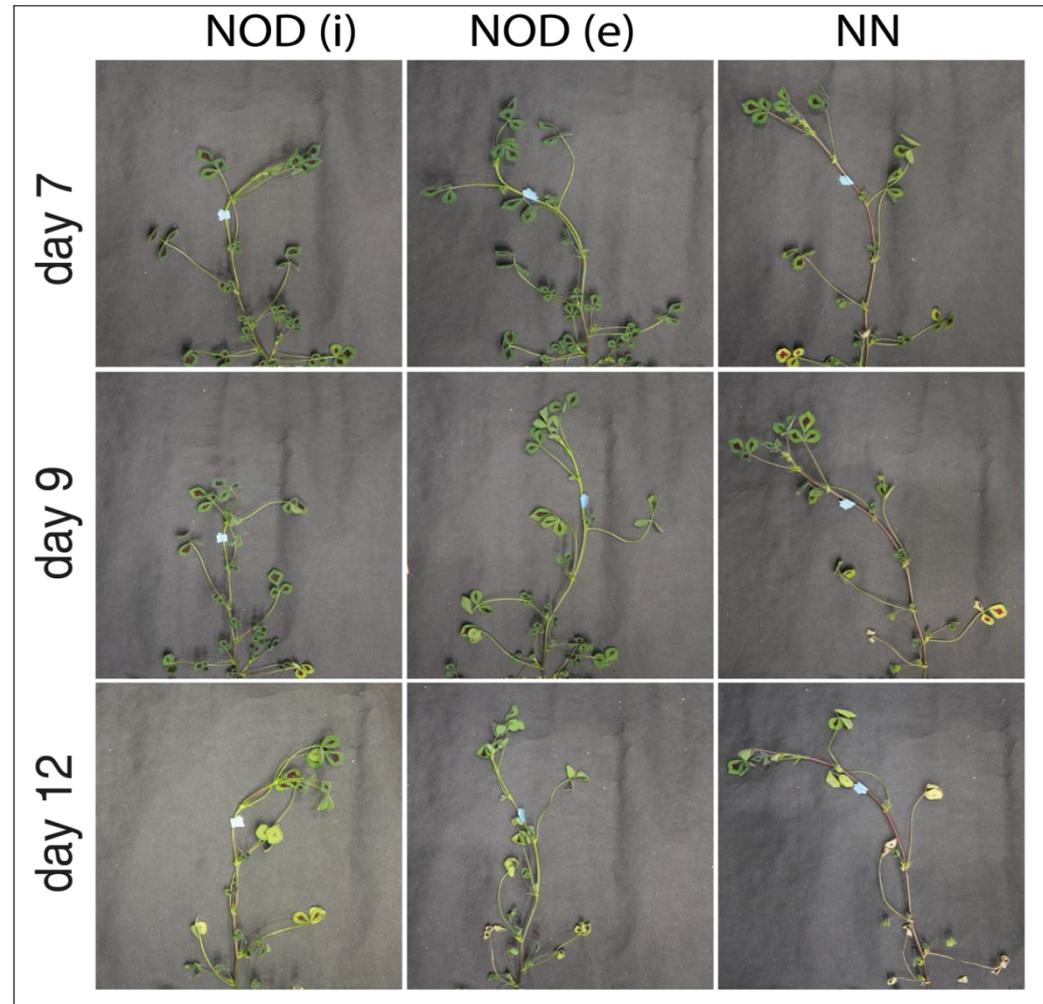
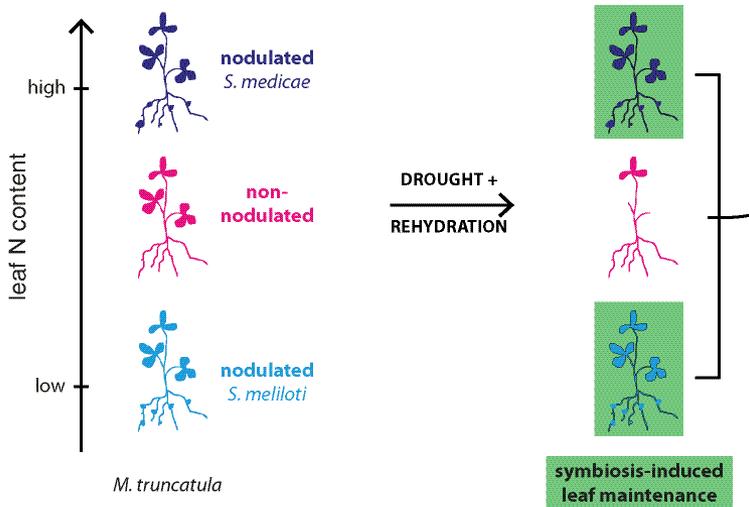
Staygreen normally gene regulated was also found symbiont induced (SISG)!



# Drought

## 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

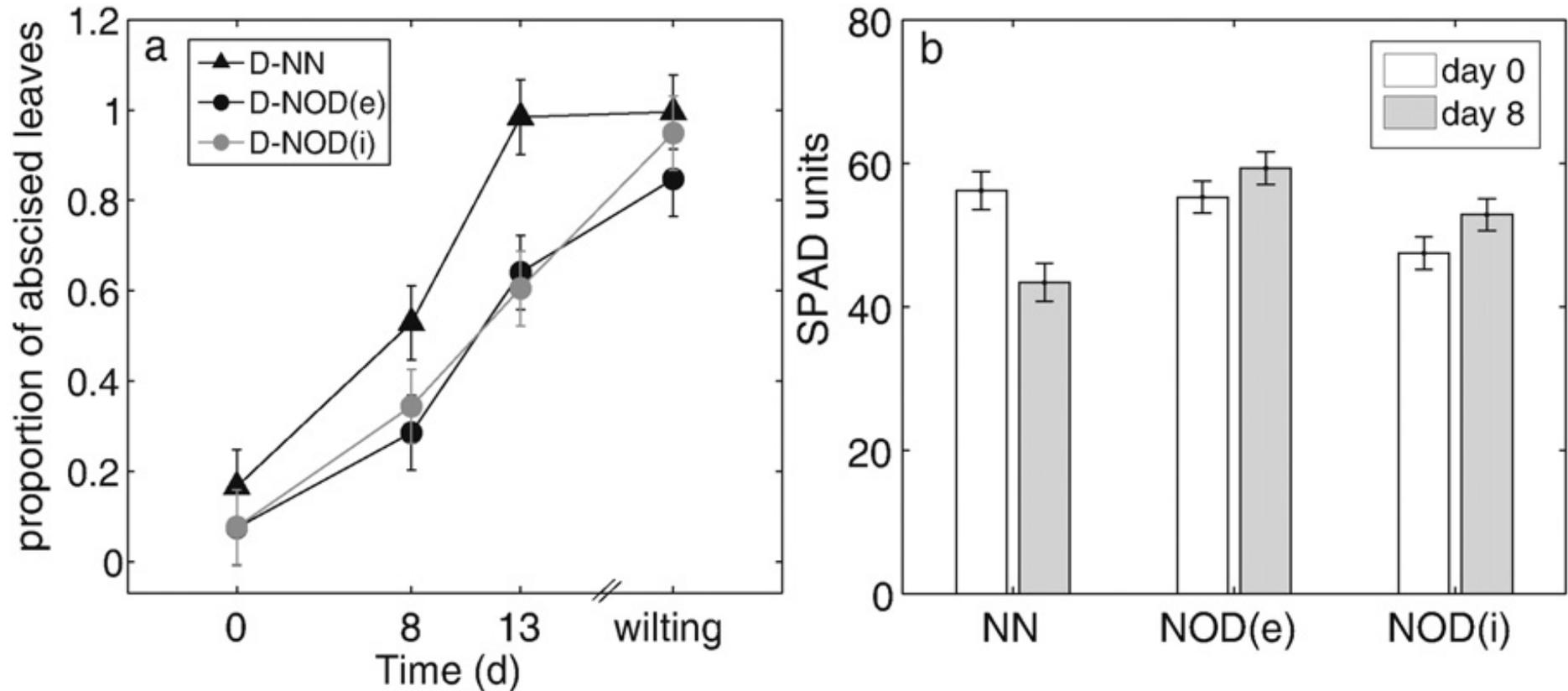


Fig. 2. Leaf senescence symptoms in *M. truncatula* induced by water withholding. (a) Leaf abscission rate. The day of wilting was ~day 15 in all conditions. (b) Leaf chlorophyll index at the start of the desiccation period (day zero, white bars) and after eight days of water withholding (gray bars). 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

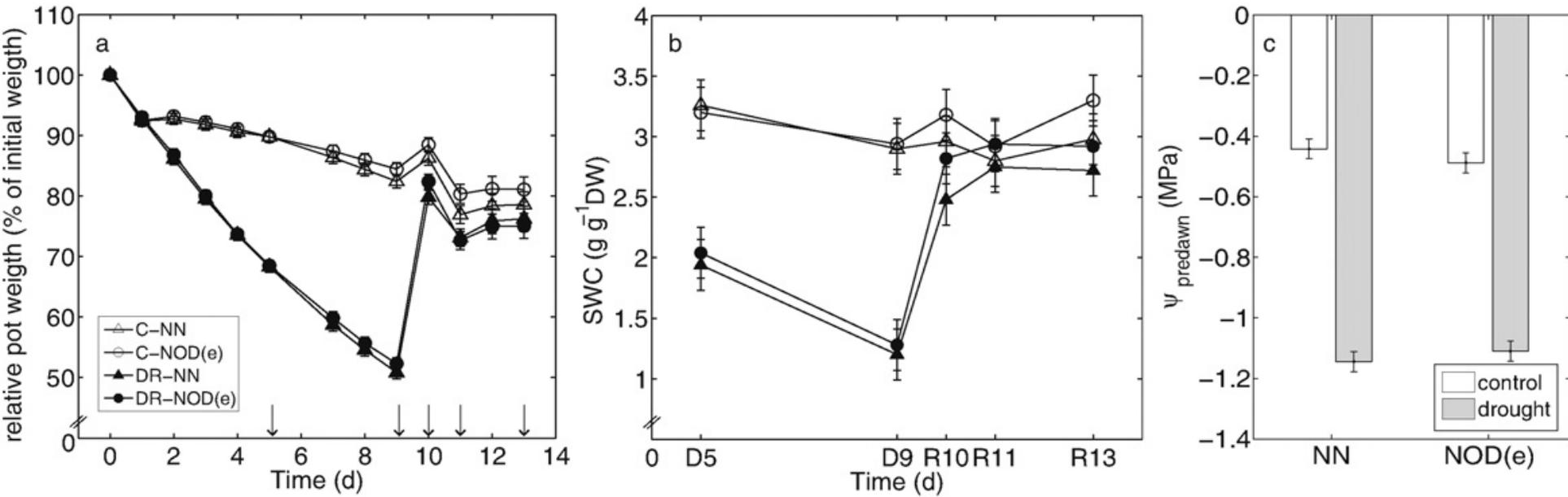
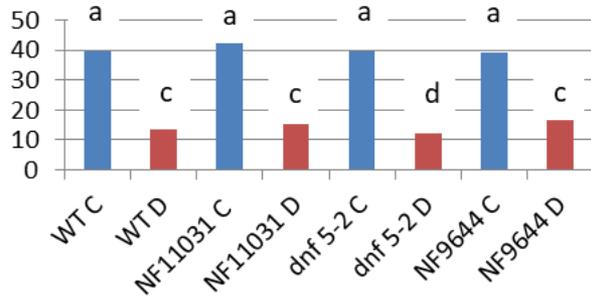


Fig. 3. Water status during drought and rehydration. Estimated (a) and absolute (b) SWC and D9 predawn leaf xylem water potential (c). Well-watered (open symbols, C) and treated *Medicago truncatula* (closed symbols, DR) was grown in a vermiculite/perlite mixture. Arrows in (a) designate the sampling time points shown in (b),  $\psi$  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.

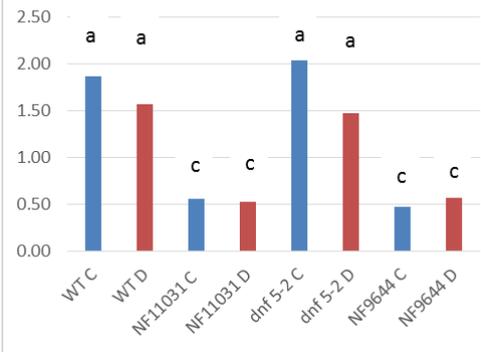
# Drought

# Phenotyping

**%RH**

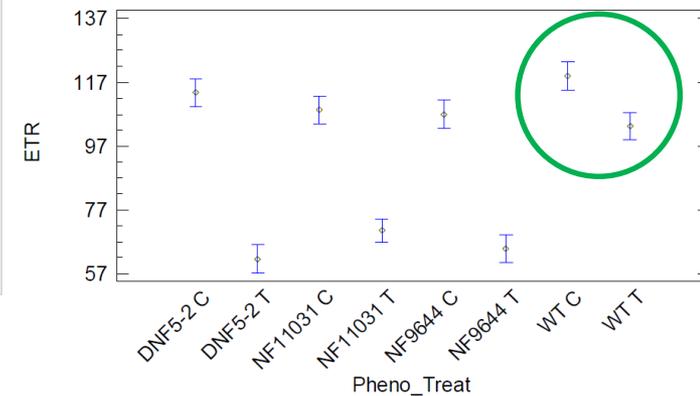


**g FW roots**

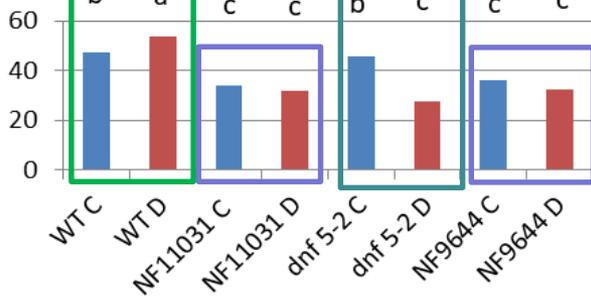


**ETR = Electron Transfer Rate**

Means and 95.0 Percent LSD Intervals



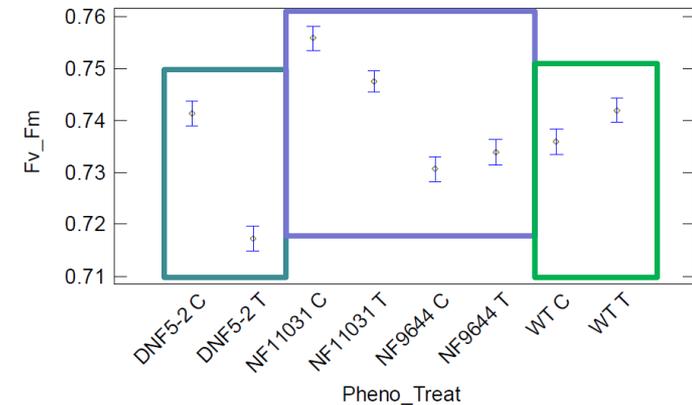
**SPAD**



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

**PSII efficiency**

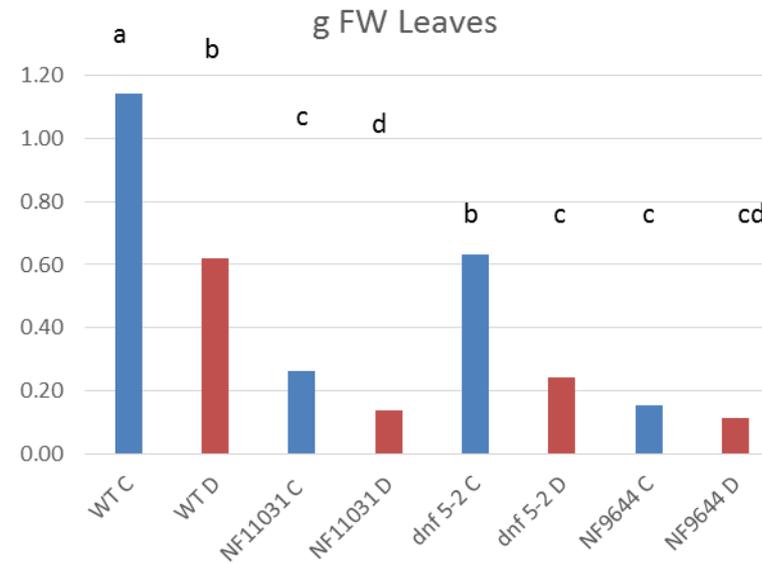
Means and 95.0 Percent LSD Intervals



SPAD=Soil Plant Analysis Development

# Drought

# Phenotyping



# Drought

## Osmotic Adjustment - Osmolytes &

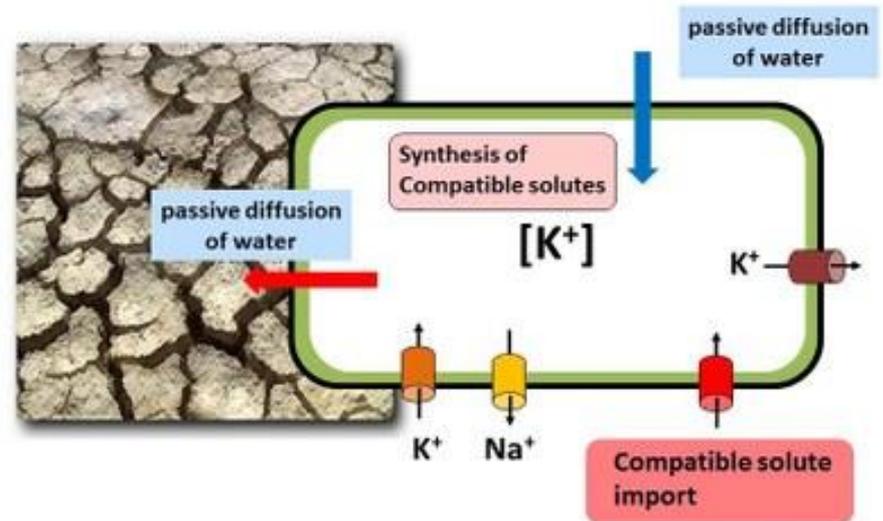
- Osmotic adjustment occurs when the concentrations of solutes within a plant cell increases to maintain a positive turgor pressure within the cell
- The cell actively accumulates solutes and as a result the solute potential ( $\Psi_s$ ) drops, promoting the flow of water into the cell

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

SISG:  
Symbiosis increased  
K<sup>+</sup> accumulation

### Reminder: role of **potassium!**

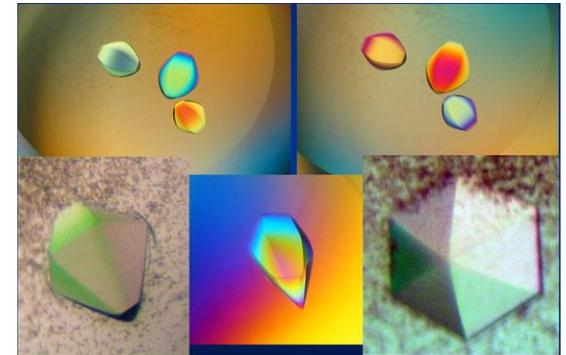
- Maintaining adequate plant K is, critical for plant drought
- resistance
- K<sup>+</sup> has root growth promoting effect
- K<sup>+</sup> can enhance the total dry mass accumulation.
- stomatal regulation by K<sup>+</sup>
- improved water retention in plant tissues



# 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

## Proteomic Drought Phenotyping

**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

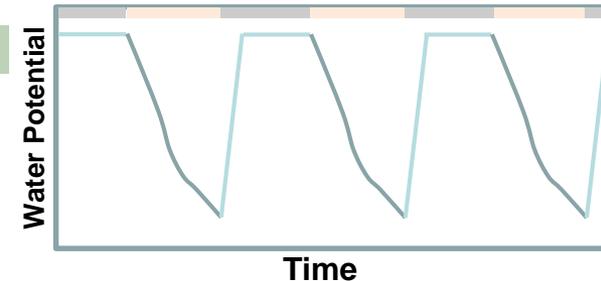
## Drought Recovery

### Deacclimation Research – Why?

Plants are exposed to a continuously changing environment.

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

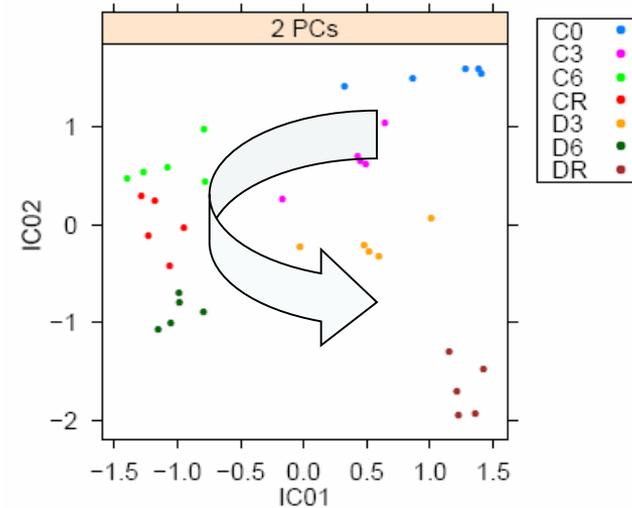
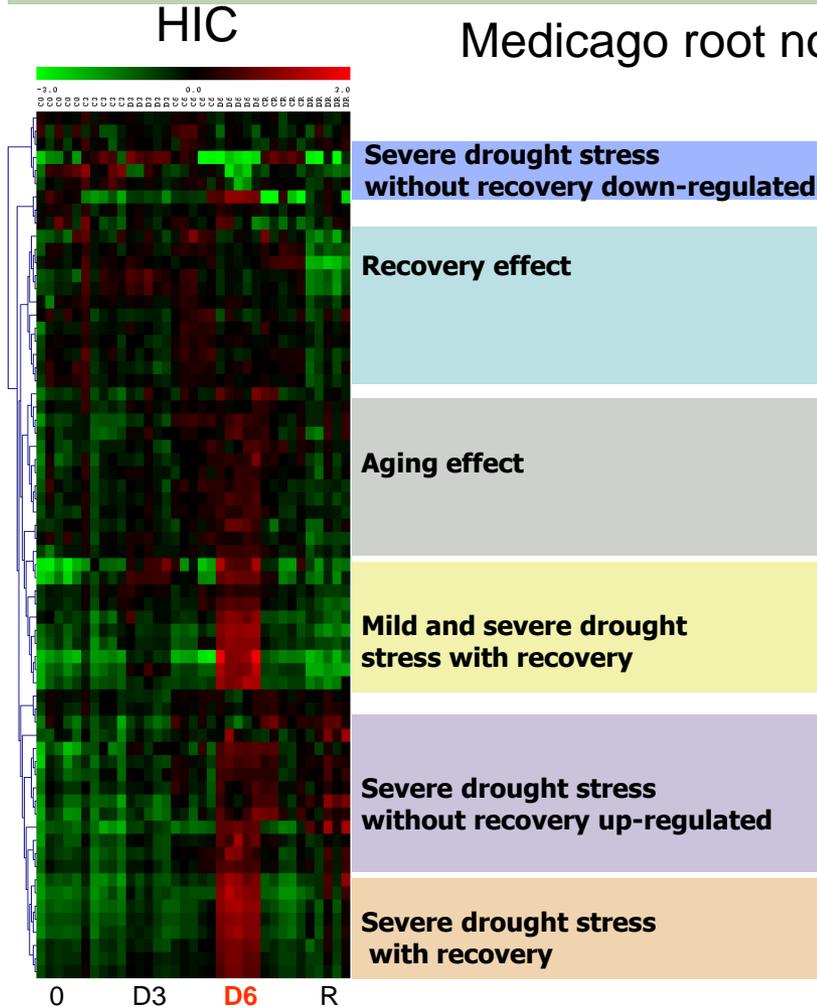
This requires a molecular plasticity of the plant enabling drought acclimation and the necessity of deacclimation processes for recovery and continuous growth.



**THE ABILITY OF PLANTS TO RECOVER FROM STRESS  
= IMPORTANT MECHANISM OF THE PLANTS TOLERANCE**

# Drought

## How to find Molecular Mechanisms and „Stress Marker“

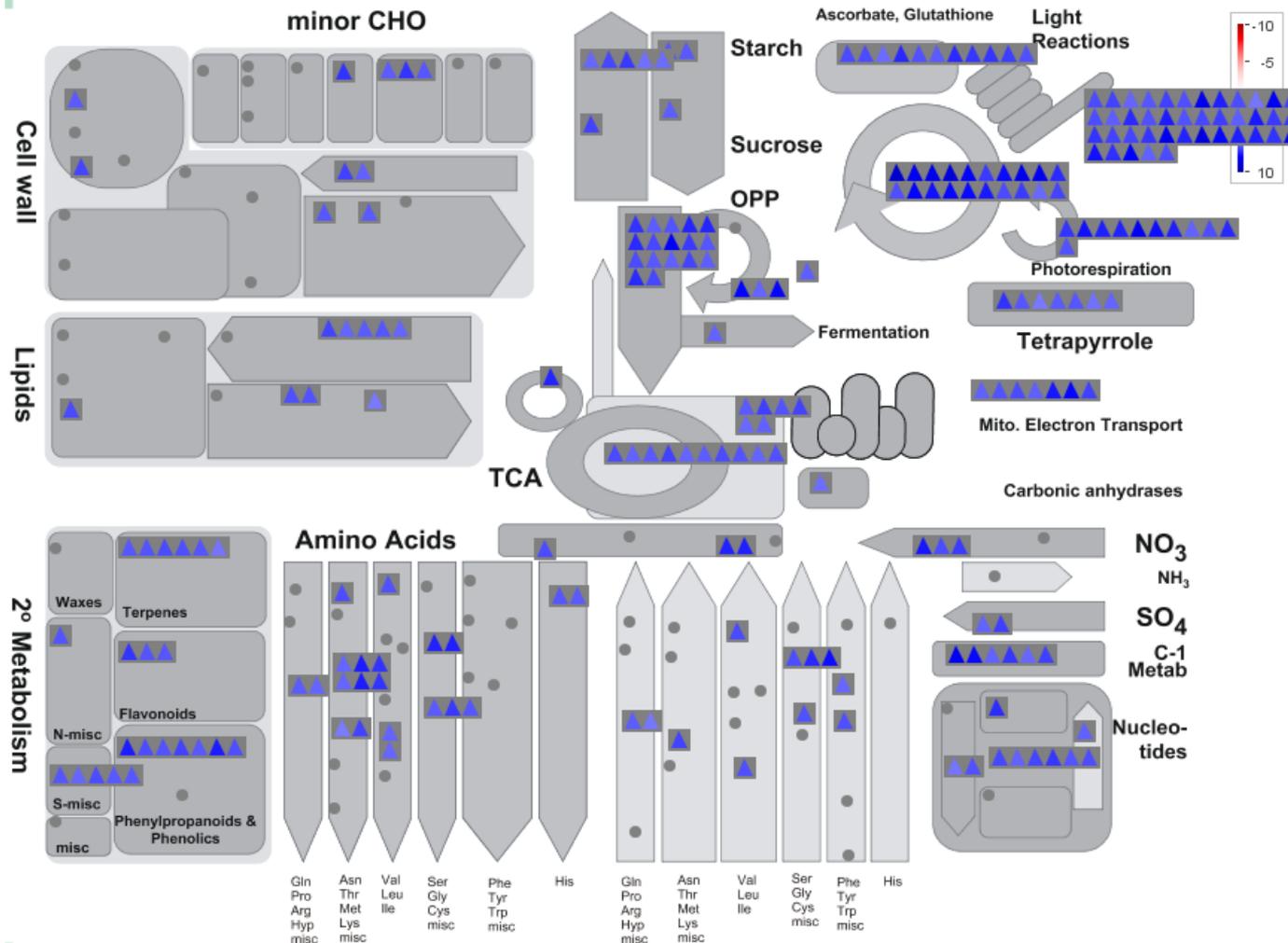


N-fixation strongly inhibited upon drought!

*Larrainzar et al. MPMI 2009*

# Drought

## MAPMAN Output of Identified Proteins



# Drought

## MAPMAN Output of Protein Ratios (D vs C)

