



Project Practical Course 300094

Proteomics in Systems Biology

Trainer: Stefanie Wienkoop

Tutor: Sebastian Schneider

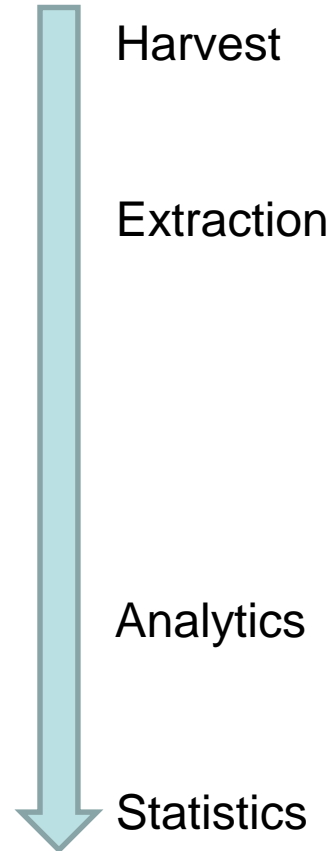
Additional lectures:

safety instructions – Lena Fragner

statistics – Gert Bachmann

systems biology – Wolfram Weckwerth

Full Scientific Experiment !

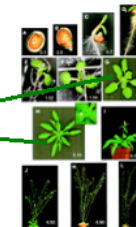


Probenvorbereitung

Ernte:
Arabidopsis, Blatt
Kaltstress, 5 Tage 4°C



Arabidopsis thaliana



Different Arabidopsis growth stages
(Boyes et al. 2001)

Proteinextraktion

Extraktion

Aliquot für
Proteinbestimmung
und SDS-PAGE

Überstand

Zentrifugation

Acetonfällung

Pellet lösen

Aliquot für
Proteinbestimmung
und SDS-PAGE

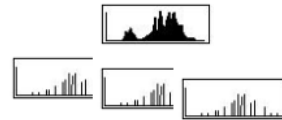
Trypsinverdau

Trypsin Verdau in Lösung

SDS-PAGE

In Gel- Trypsinverdau

Massenspektrometrie



LC-MS/MS

Datenanalyse Auswertung



Vergleich:

- Kaltstress vs Kontrolle
- Analyseverfahren in Gel und in Lösung



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**

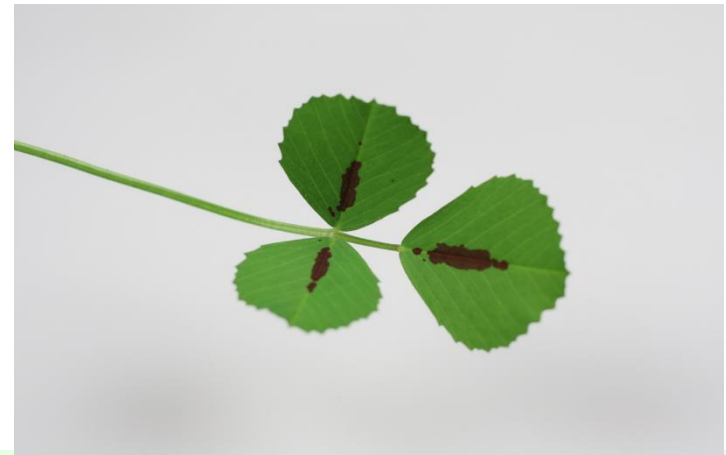
Group 1) wt

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%

PPProteomics – 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

131.130.57.230/clarotest190/index.php?category=MOSYSWS13 chlamydomonas mutant collection

Meistbesucht Home - PubMed - NCBI Web of Knowledge [v...] Promex dict.leo.org - Deutsch-... STRING Legume Information S... AmiGO: Advanced Sea... DFCI - Plant Gene Indi... Scopus SysPTM Stefanie Wienkoop - G... ResearchGate



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

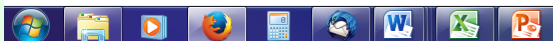
Liste der Kurse

- 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 Fagner, Wolfram Weckwerth
- 300606WS13 - Wachstum und Stoffwechsel der Pflanzen
checo eleamfix

Suche nach Schlagwort :

Suchen

<< Eine Ebene höher



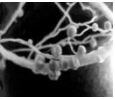
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Stefanie Wienkoop – Plant Systems Interaction

Dept. Molecular Systems Biology

University Vienna

For private use
only!



3 students per group!

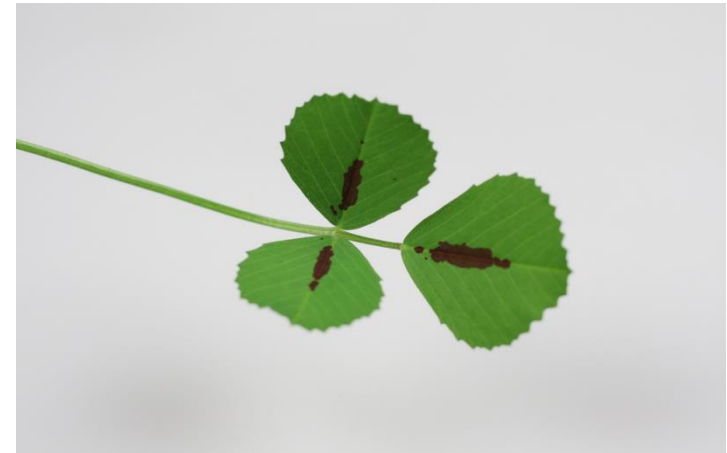
Medicago truncatula (*Rhizobium* inoculated) : **leaves**

Group 1) wt

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

Group 3) NF11301 (ferritin F3)

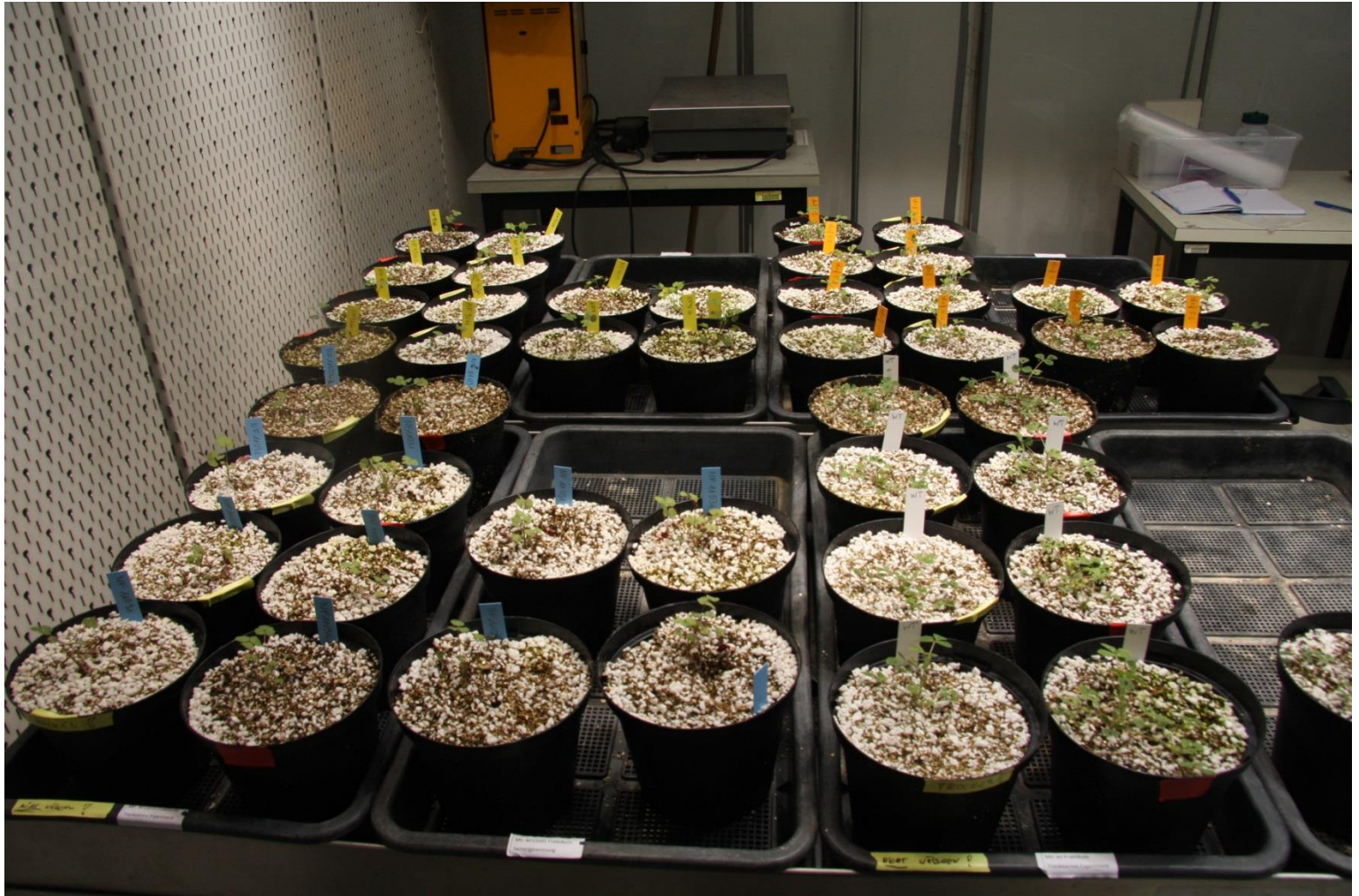
Group 4) NF9644 (ferritin F2)



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

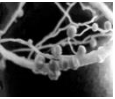
Proteomics

Drought Experiment *Medicago truncatula*



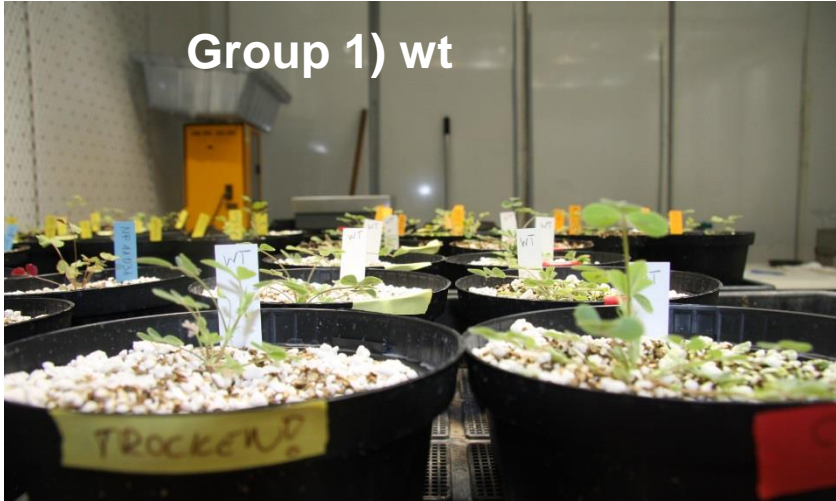
Proteomics

Drought Experiment *Medicago truncatula*



Plant-Systems
Interaction

Group 1) wt



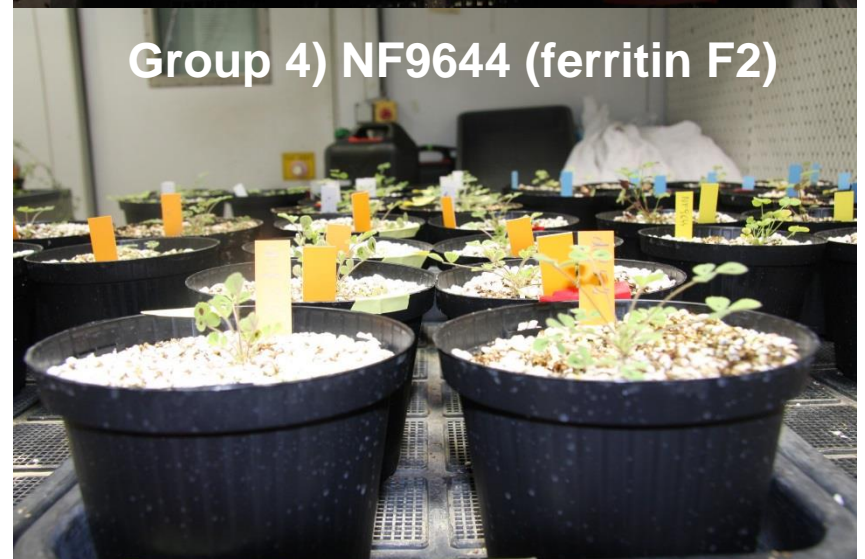
Group 2) dnf5-2 (N-fixation deficient)

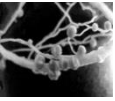


Group 3) NF11301 (ferritin F3)



Group 4) NF9644 (ferritin F2)





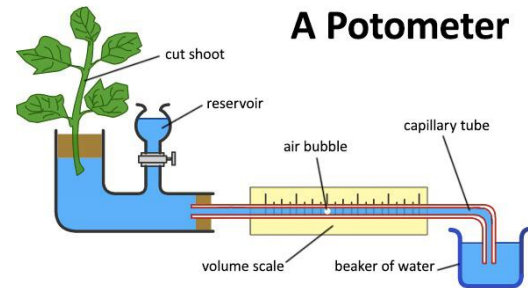
What is drought stress?

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. Dolferus / Plant Science 229 (2014) 247–261

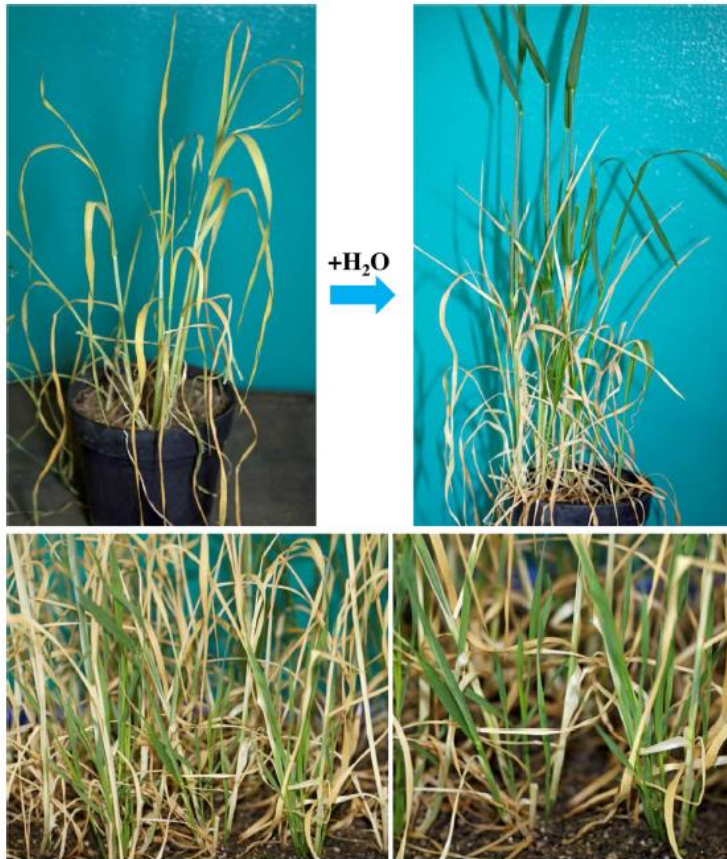


Fig. 3. Effect of drought stress at the reproductive stage in wheat. Drought stress leads to extensive leaf senescence in wheat (top left). Re-watering results in the development of new freshly green tillers that will flower and produce grains, while grain development in the older stressed tillers is 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

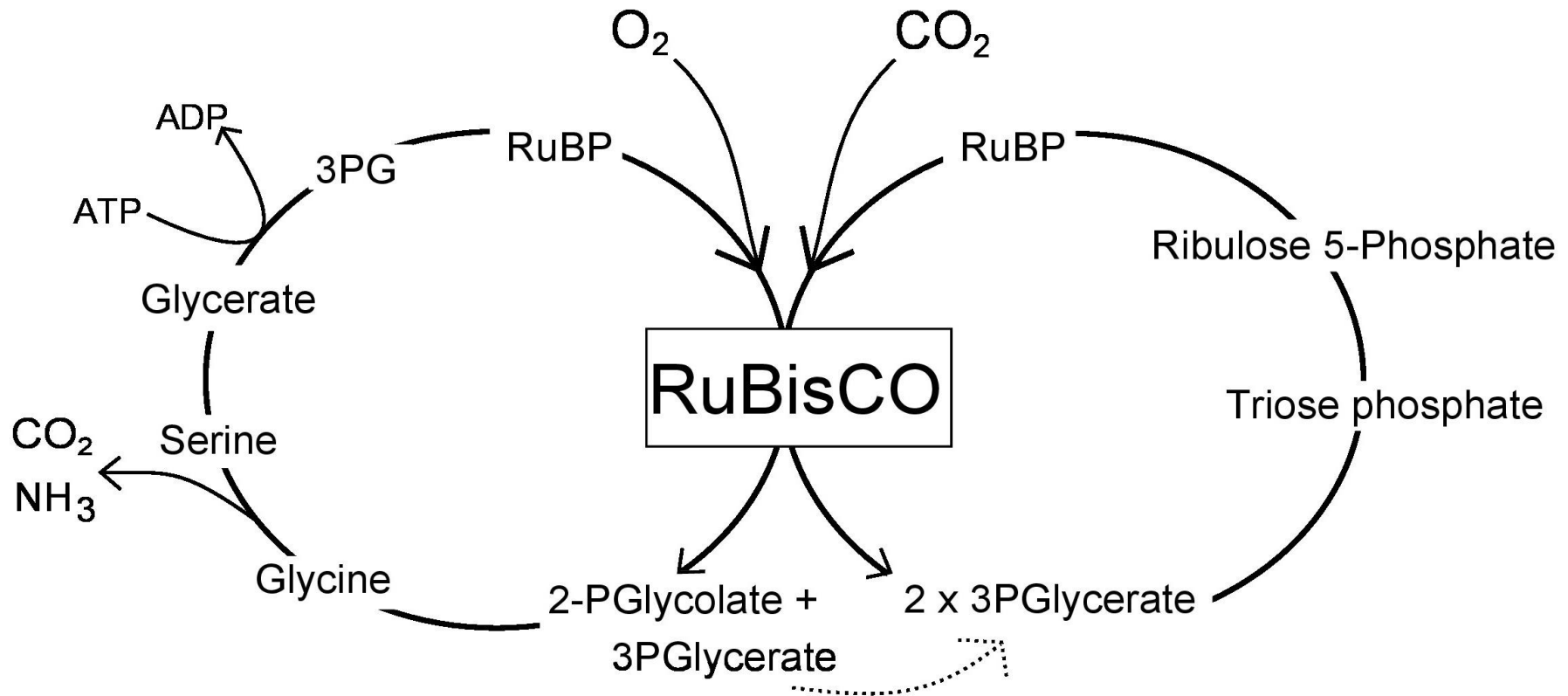
Drought

Pathways involved in drought stress adaptation

?

Drought

Photorespiration



Photorespiration

Calvin Cycle

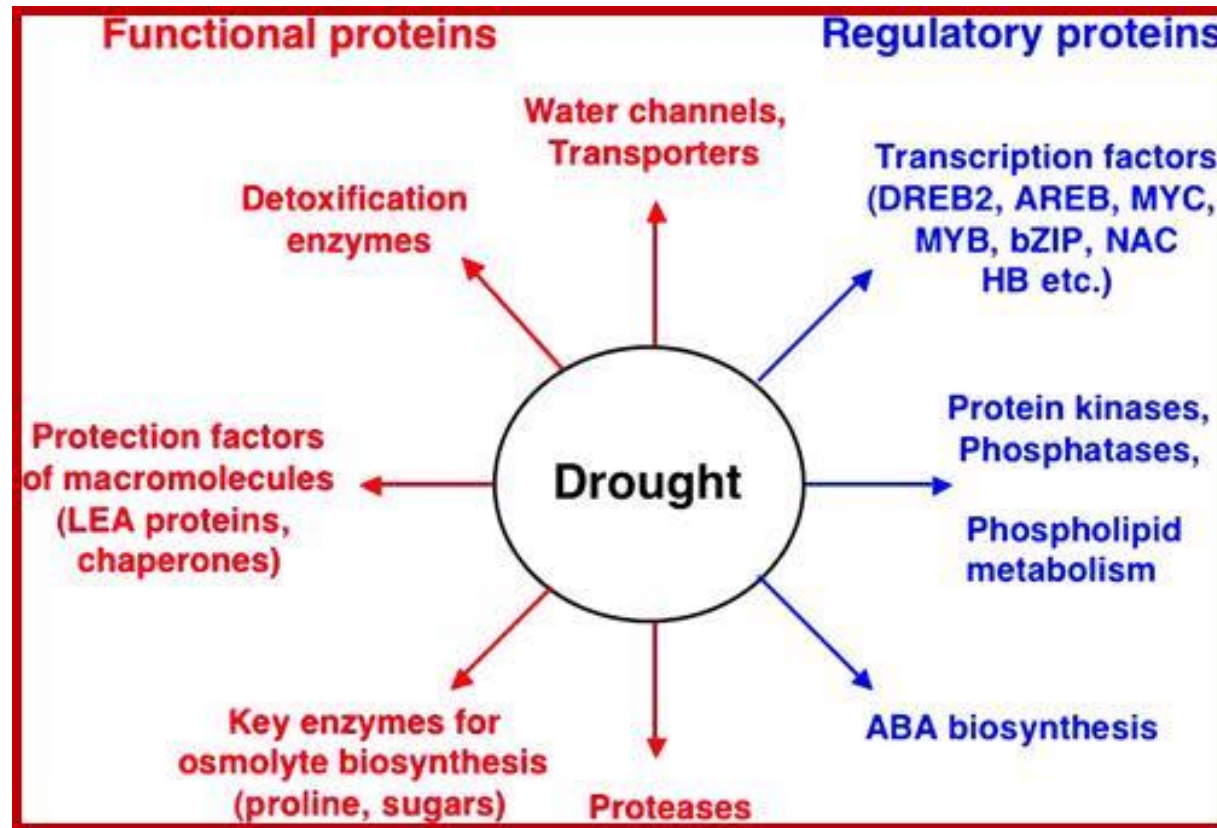
Drought

Proteins involved in drought stress adaptation

?

Drought

Proteins involved in drought stress adaptation

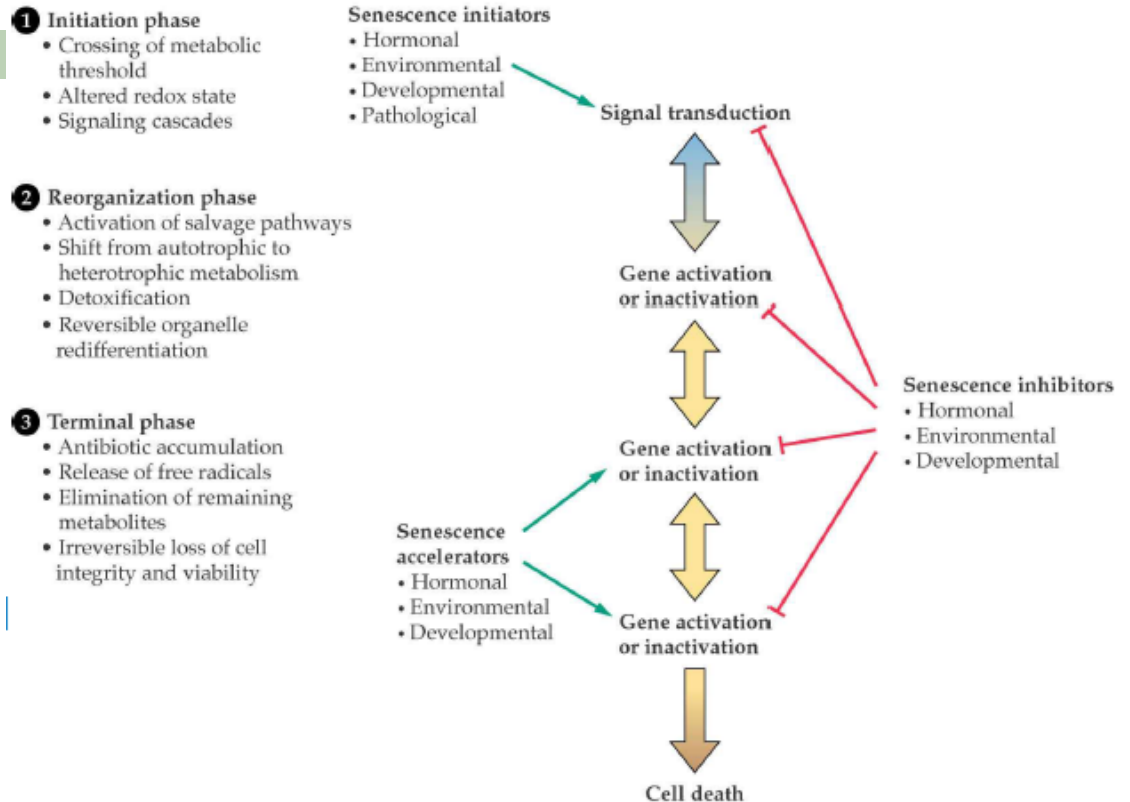


Drought

Senescence is a Regulated Process

Anabolic processes slowed down

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



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

Drought

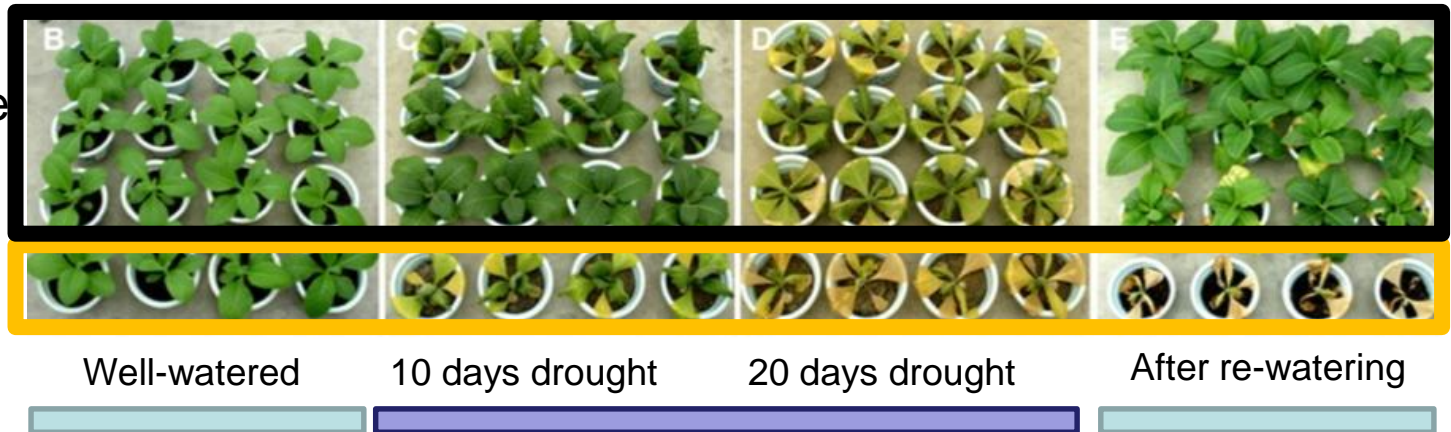
Plant Stress Recovery Capacity

Staygreen (SG) Effect
reduced leaf senescence

Recovery plays an important role in understanding SG

Drought- tolerance

Wild-type

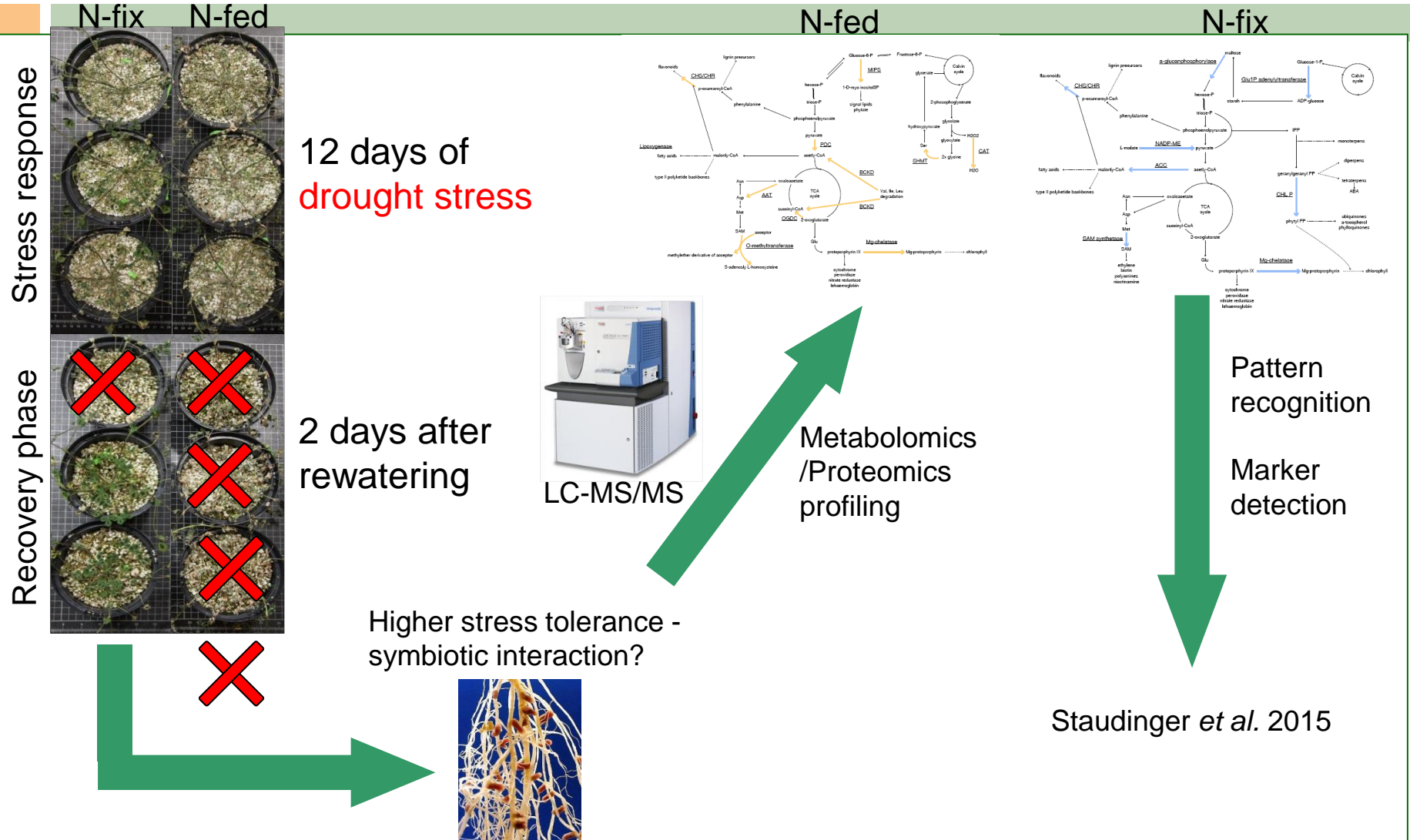


Yu, H., Chen, X., Hong, Y.-Y., Wang, Y., Xu, P., Ke, S.-D., Liu, H.-Y., Zhu, J.-K., Oliver, D.J., Xiang, C.-B. (2008)
Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. *Plant Cell* 20:[1134-1151](#).

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

Drought

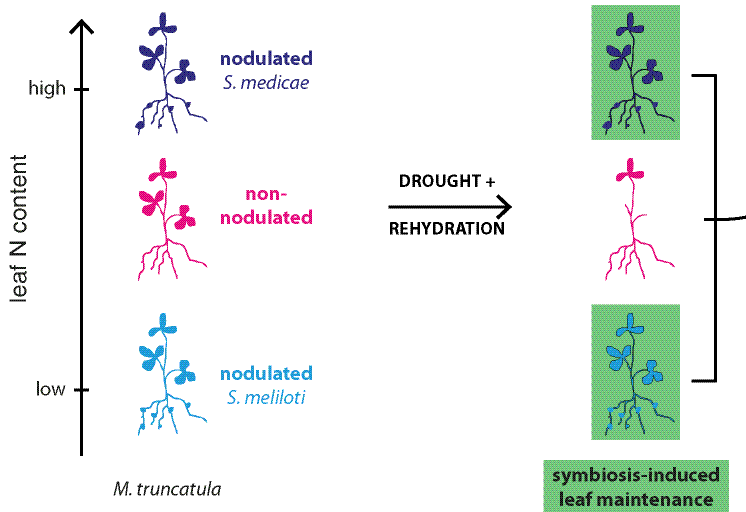
Symbiotic induced StayGreen Effect - SISG



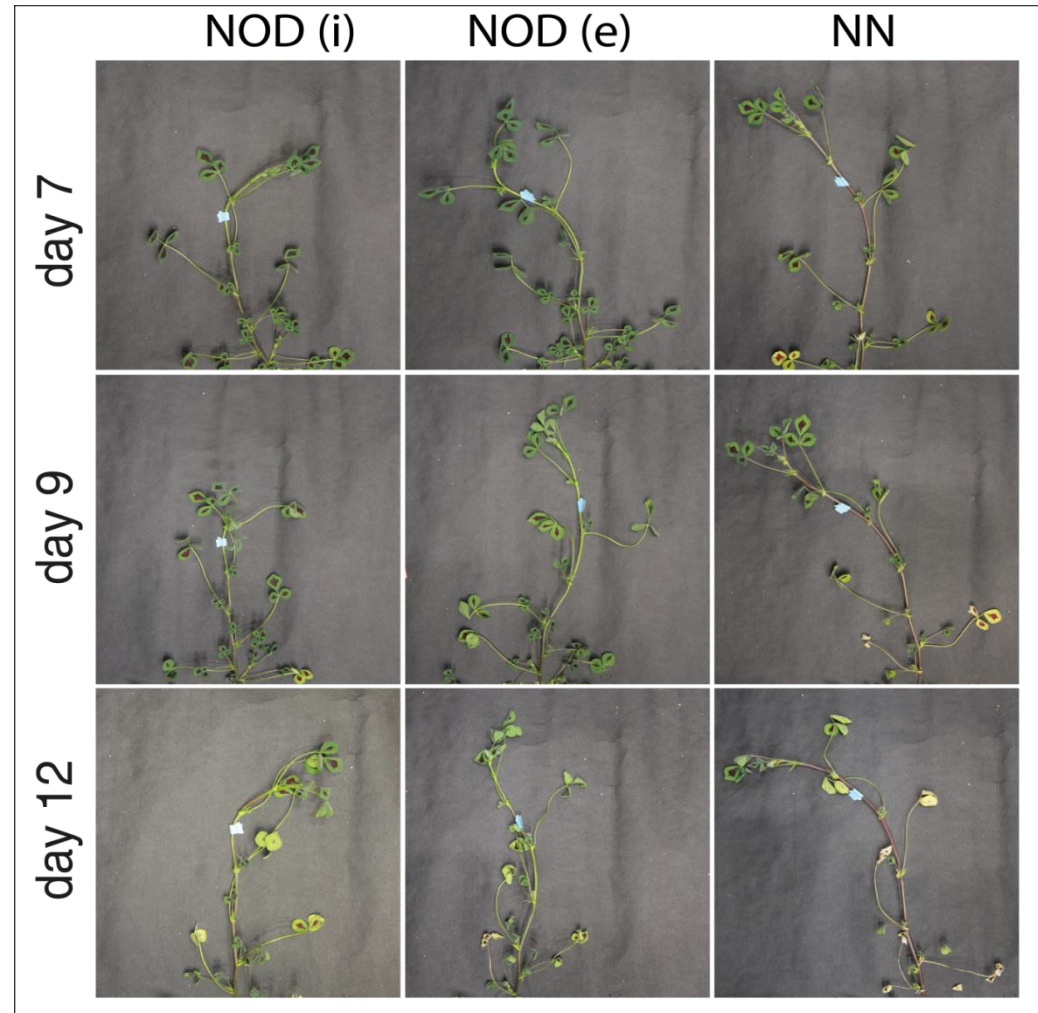
Drought

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

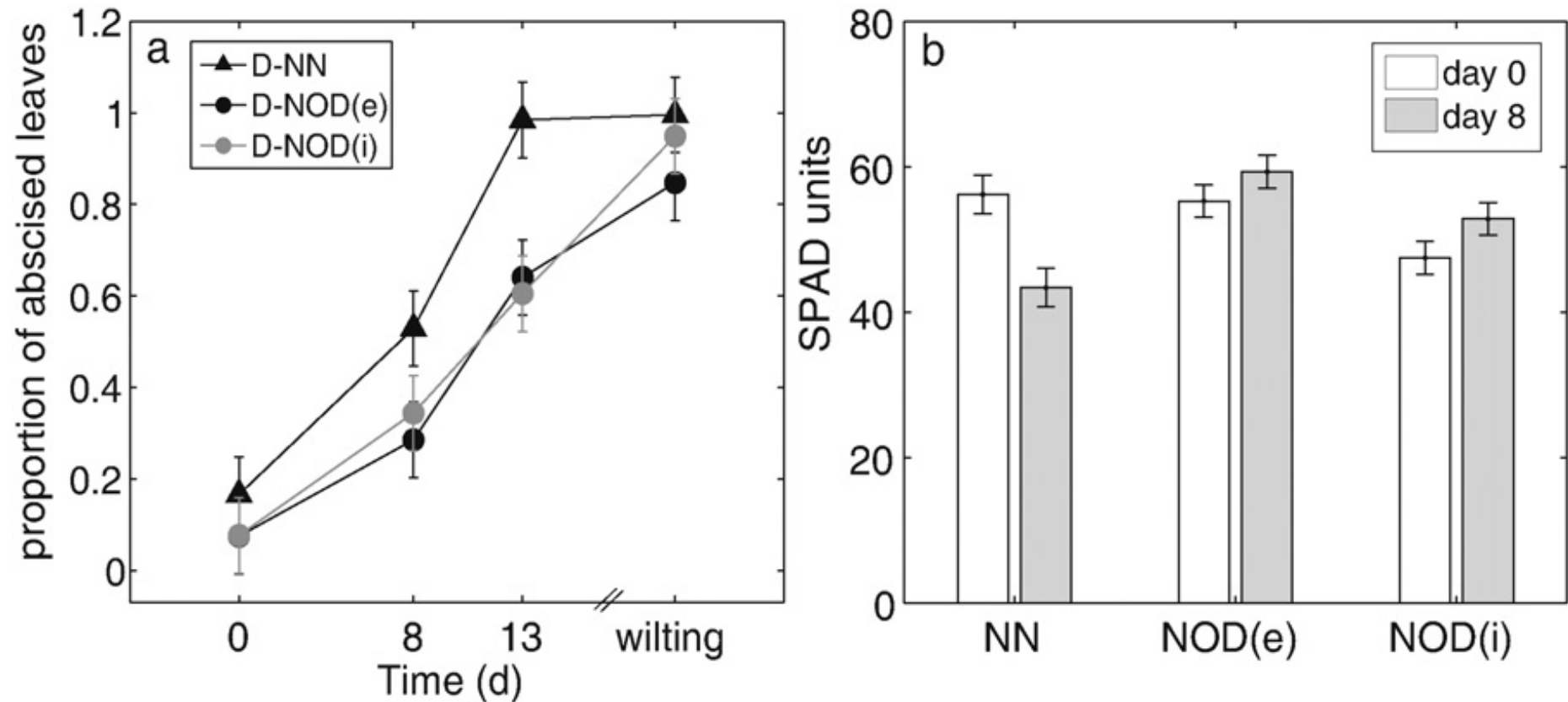


Fig. 2. Leaf senescence symptoms in *M. truncatula* induced by water withholding. (a) Leaf abscission rate. The day of wilting was ~day 15 in all conditions. (b) Leaf chlorophyll index at the start of the desiccation period (day zero, white bars) and after eight days of water withholding (gray bars).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

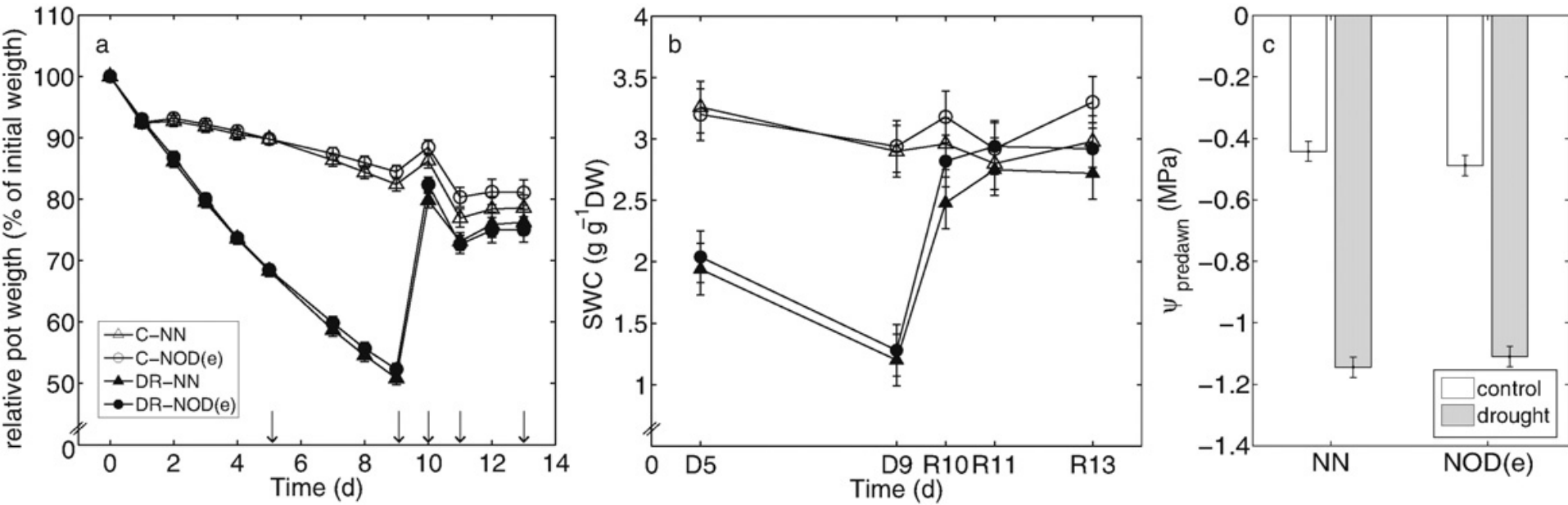
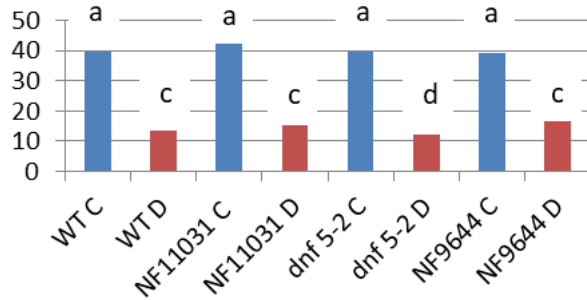


Fig. 3. Water status during drought and rehydration. Estimated (a) and absolute (b) SWC and D9 predawn leaf xylem water potential (c). Well-watered (open symbols, C) and treated *Medicago truncatula* (closed symbols, DR) was grown in a vermiculite/perlite mixture. Arrows in (a) designate the sampling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

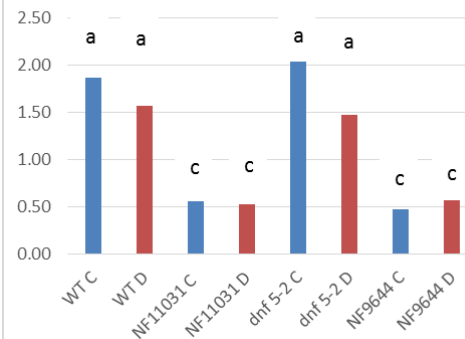
Drought

Phenotyping

%RH

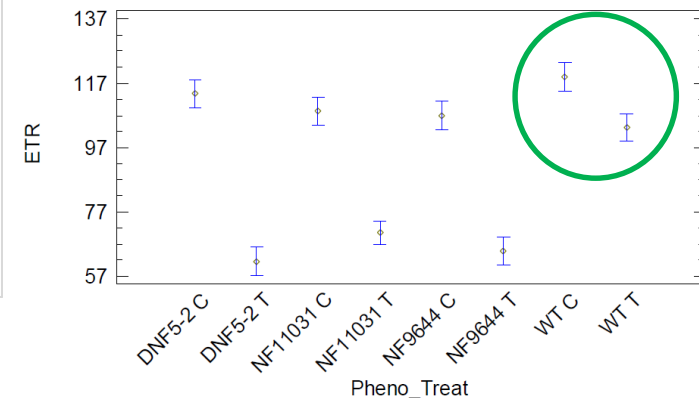


g FW roots

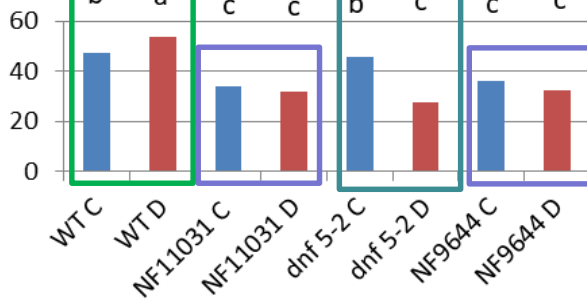


ETR = Electron Transfer Rate

Means and 95.0 Percent LSD Intervals



SPAD



no N-fixation

=

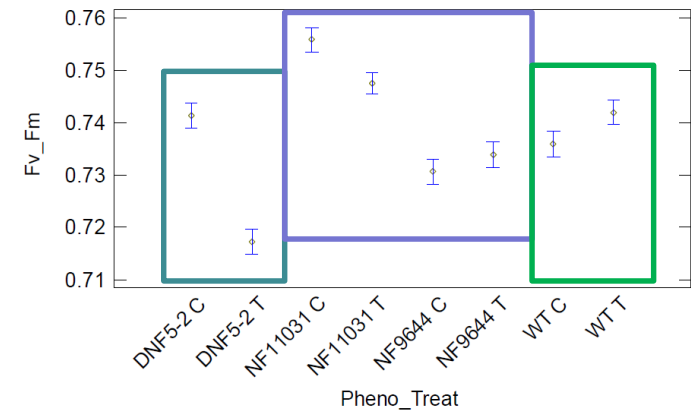
N-deficient!

=

lower
chlorophyll
content?

PSII
efficiency

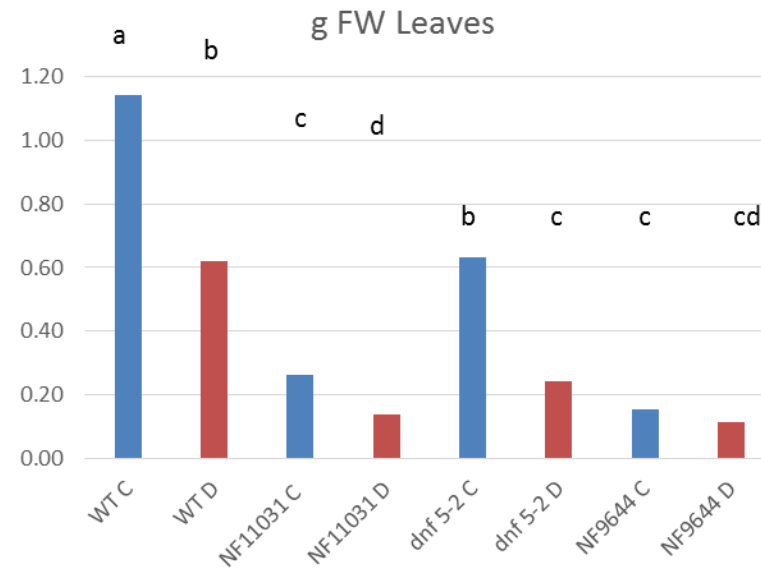
Means and 95.0 Percent LSD Intervals



SPAD=Soil Plant Analysis Development

Drought

Phenotyping



Drought

Osmotic Adjustment - Osmolytes &

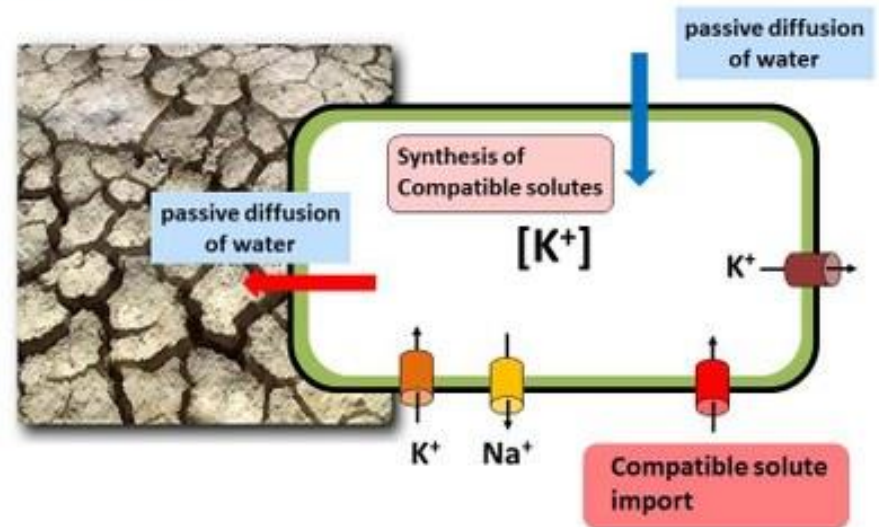
- Osmotic adjustment occurs when the concentrations of solutes within a plant cell increases to maintain a positive turgor pressure within the cell
- The cell actively accumulates solutes and as a result the solute potential (Ψ_s) drops, promoting the flow of water into the cell

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

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

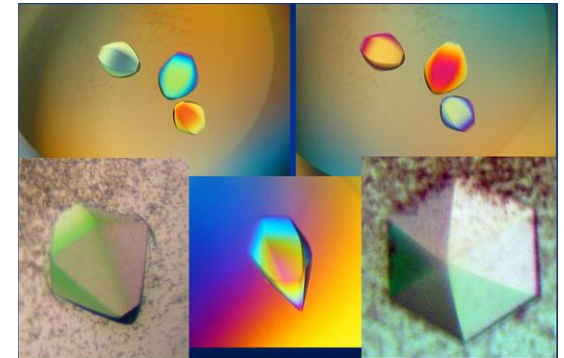
SISG:
Symbiosis
increased
K⁺ accumulation



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?**

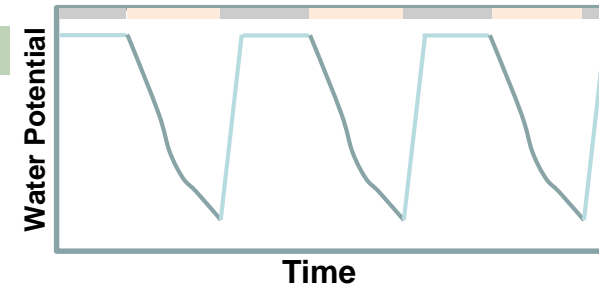
Is it an active process?

What role plays Ferritin?

What role plays N-fixation?

Drought

Drought Recovery



Deacclimation Research – Why?

Plants are exposed to a continuously changing environment.

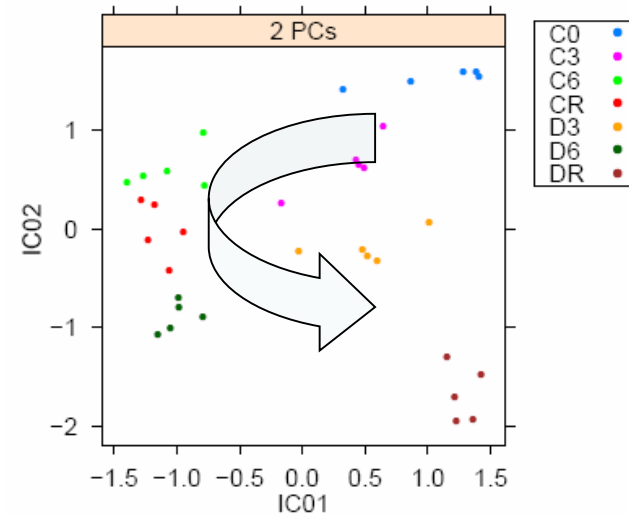
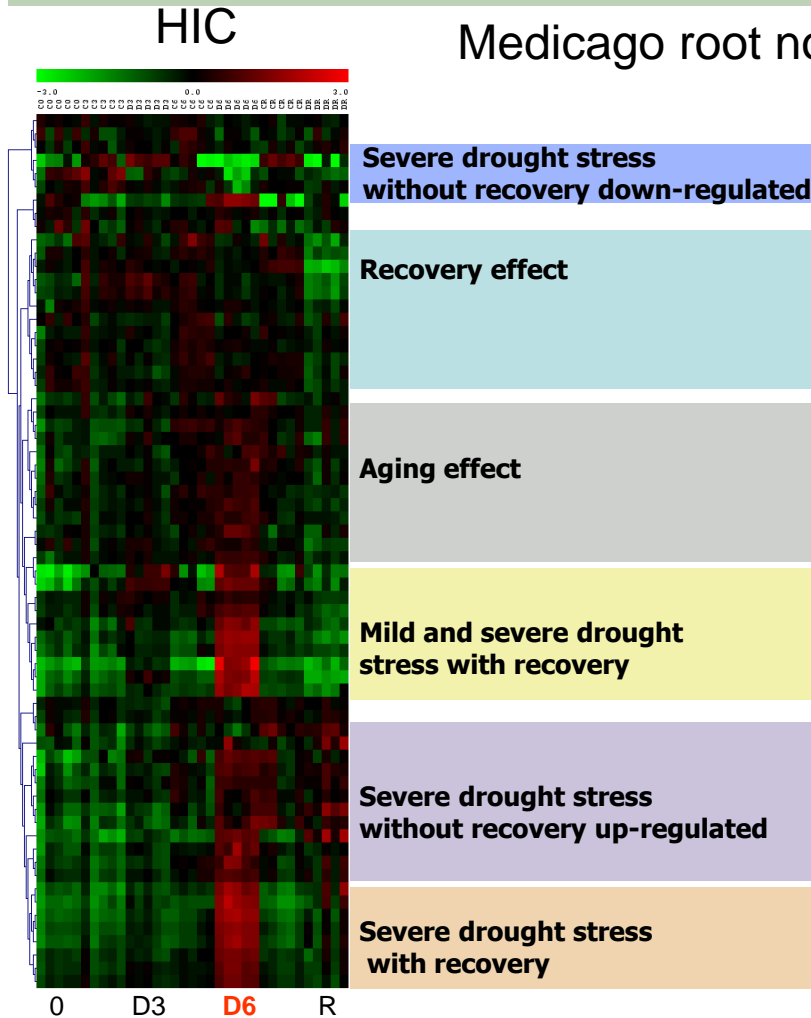
Extremes such as several weeks of drought are followed by rain.

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“

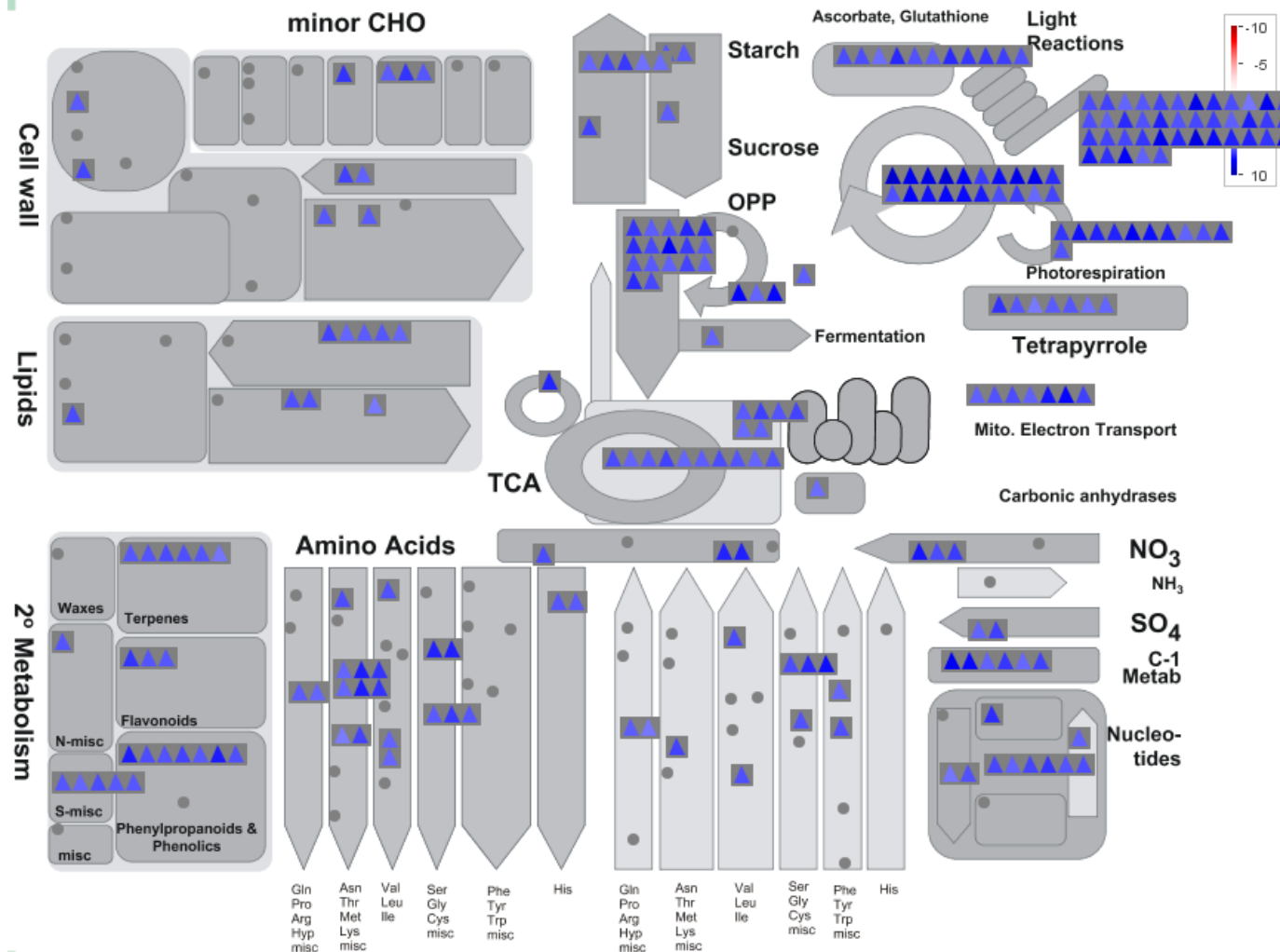


N-fixation strongly
inhibited upon
drought!

Larrainzar et al. MPMI 2009

Drought

MAPMAN Output of Identified Proteins



Drought

MAPMAN Output of Protein Ratios (D vs C)

