Understanding Adaptive Responses to Drought Stress in Alfalfa

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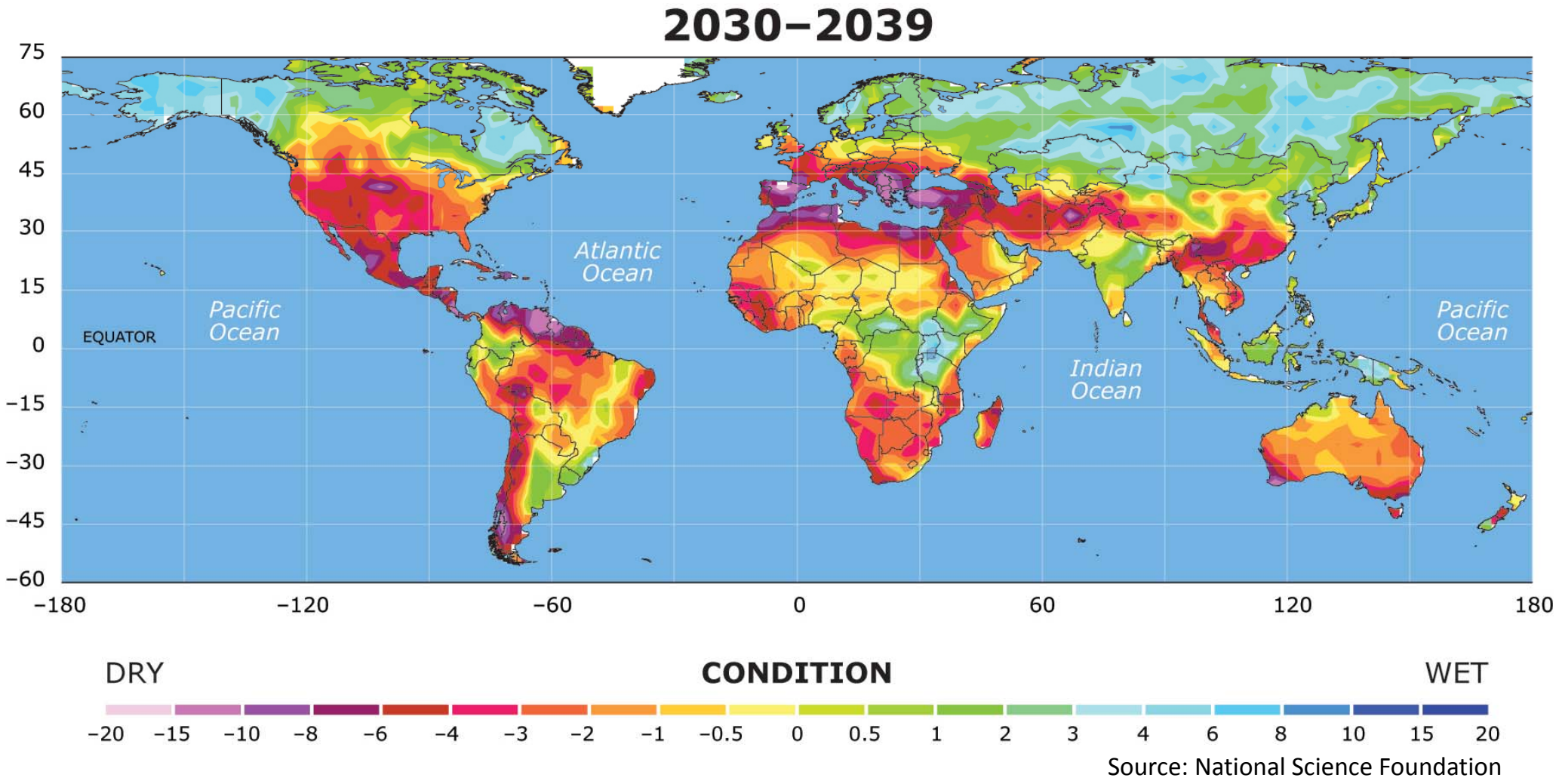
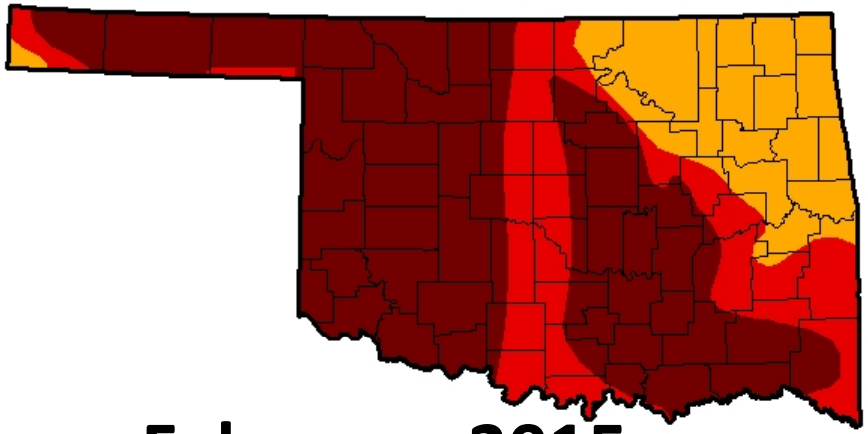
Anticipation of Continued Drought Worldwide



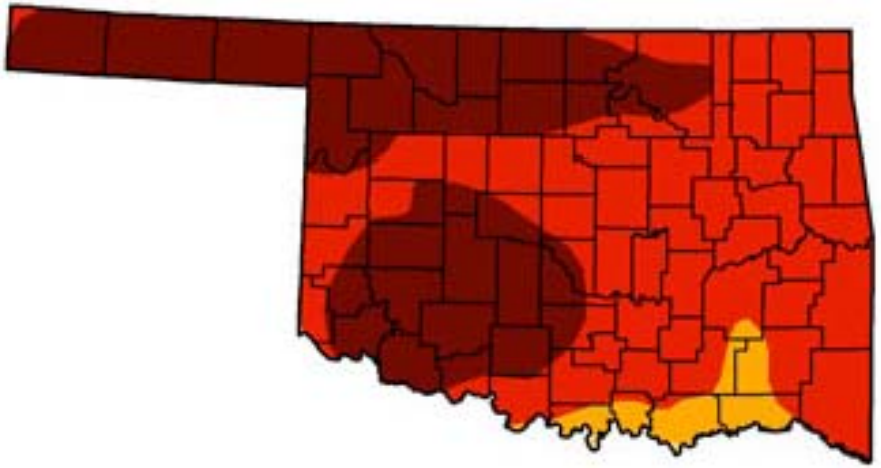
Source: National Science Foundation

Drought Stress in Oklahoma

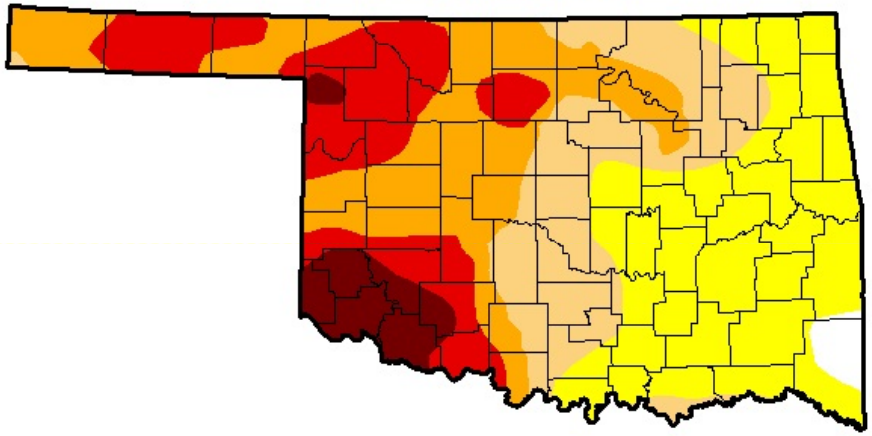
October, 2011



September, 2012



February, 2015



Drought Impact

- abnormally dry
- moderate drought
- severe drought
- extreme drought
- exceptional drought

Source: Oklahoma Climatological Survey | U.S. Drought Monitor - Oklahoma

Value of Alfalfa in Agriculture

Biomass Yield

Average: 3 to 4 ton/acre

Maximum: 10 to 24 ton/acre

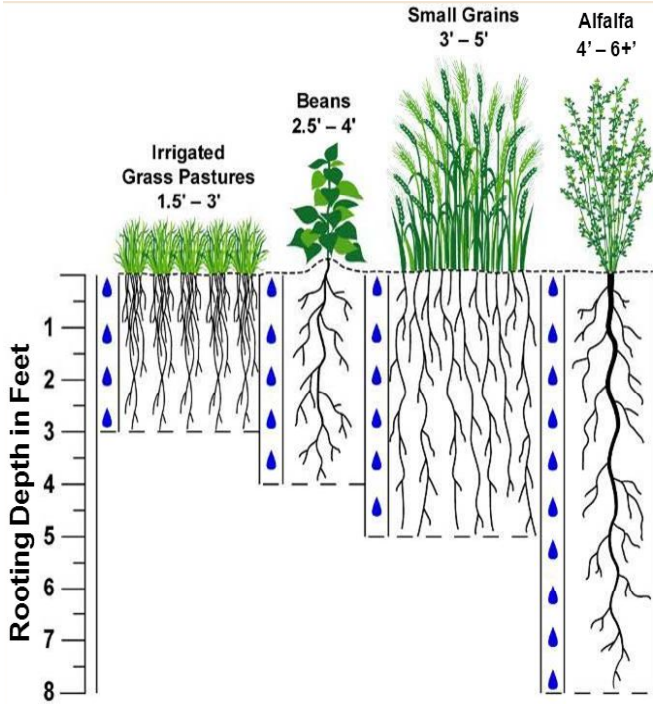
Symbiotic N Fixation

Contribute ~150 lbs/acre of N

Sustainable production

Environmental Benefits

- ⬇ Surface erosion
- ⬇ Fertilizer use
- ⬆ Improve soil tilth
- ⬆ Carbon sequestration



Source: University of Nevada Extension

Impact of Drought Stress on Alfalfa

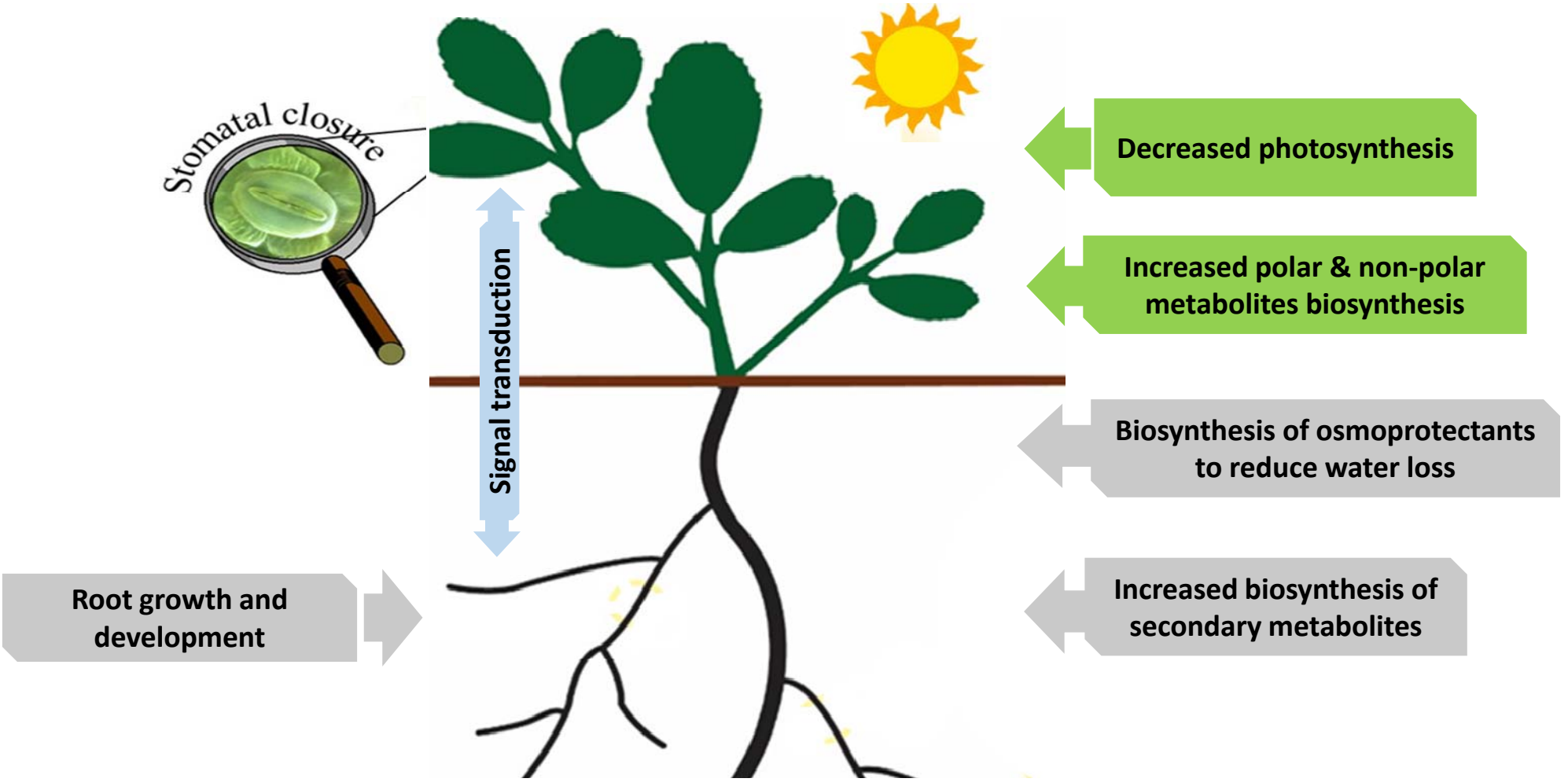
Reduced productivity under drought stress

Target more efficient water use

Better conversion of biomass per unit of water

Source: IPCC 2015; MSU Extension

Complex Plant Responses to Drought Stress



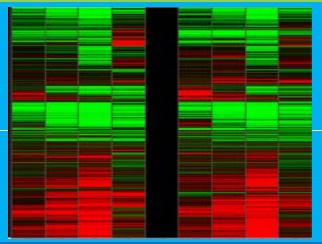
Adapted from Chaves et al. 2007. Funct.Plant Biol. 30:239-264.

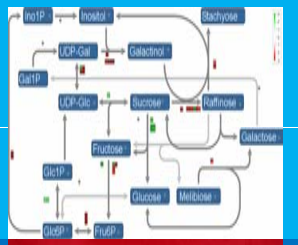
Outline of Research Approaches

A. Field evaluation and selection

B. Greenhouse evaluations

C. Drought tolerance mechanisms





D. Develop populations

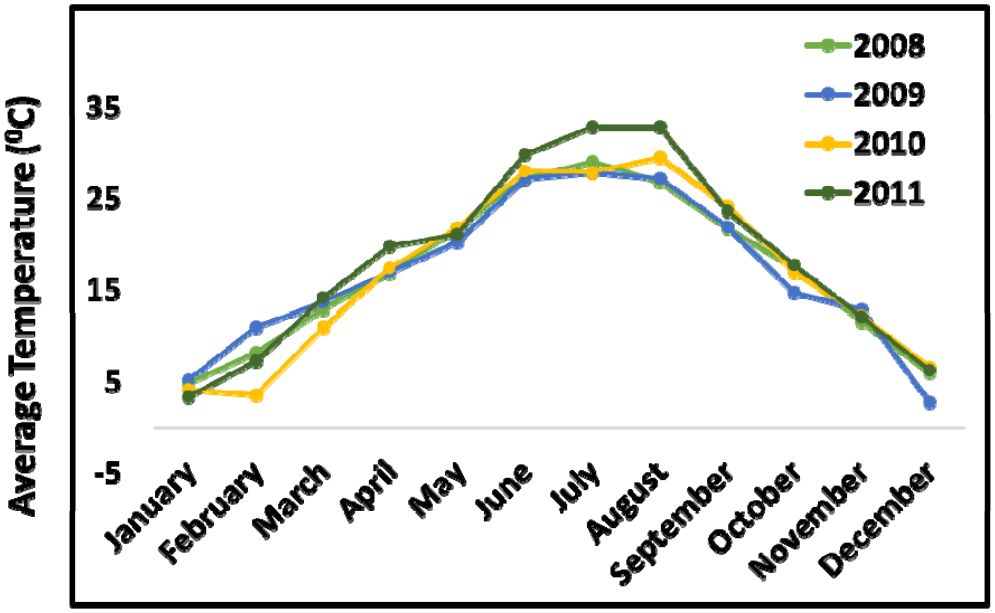
Phenotyping Diverse Alfalfa Germplasm in the Field

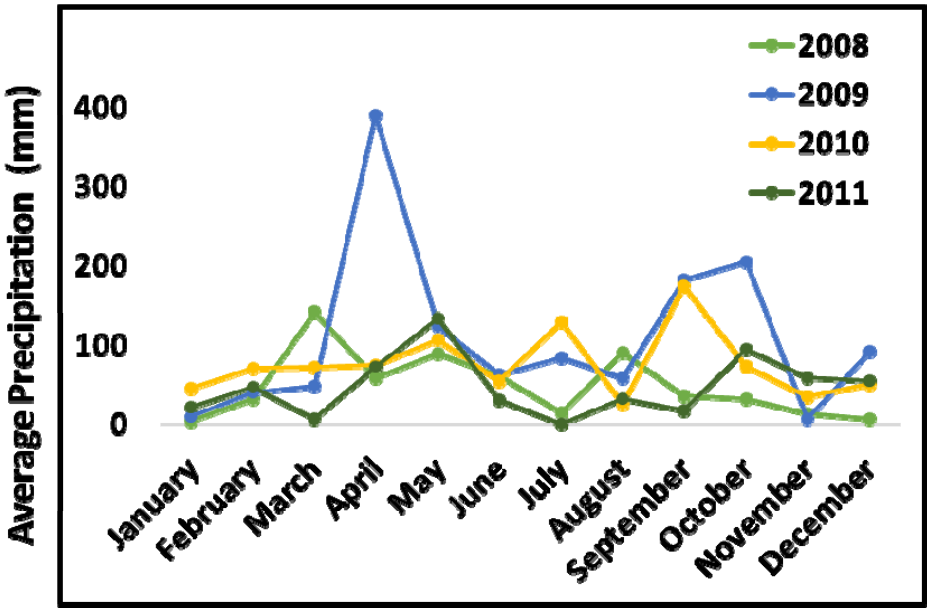
Red River Farm (Burneyville, OK)

- 233 accessions plus 11 checks
- 10 individual plants per accession
- 4 replications, RCBD

Transplanted seedlings 3/12-3/13/08

Field Conditions at the Red River Farm





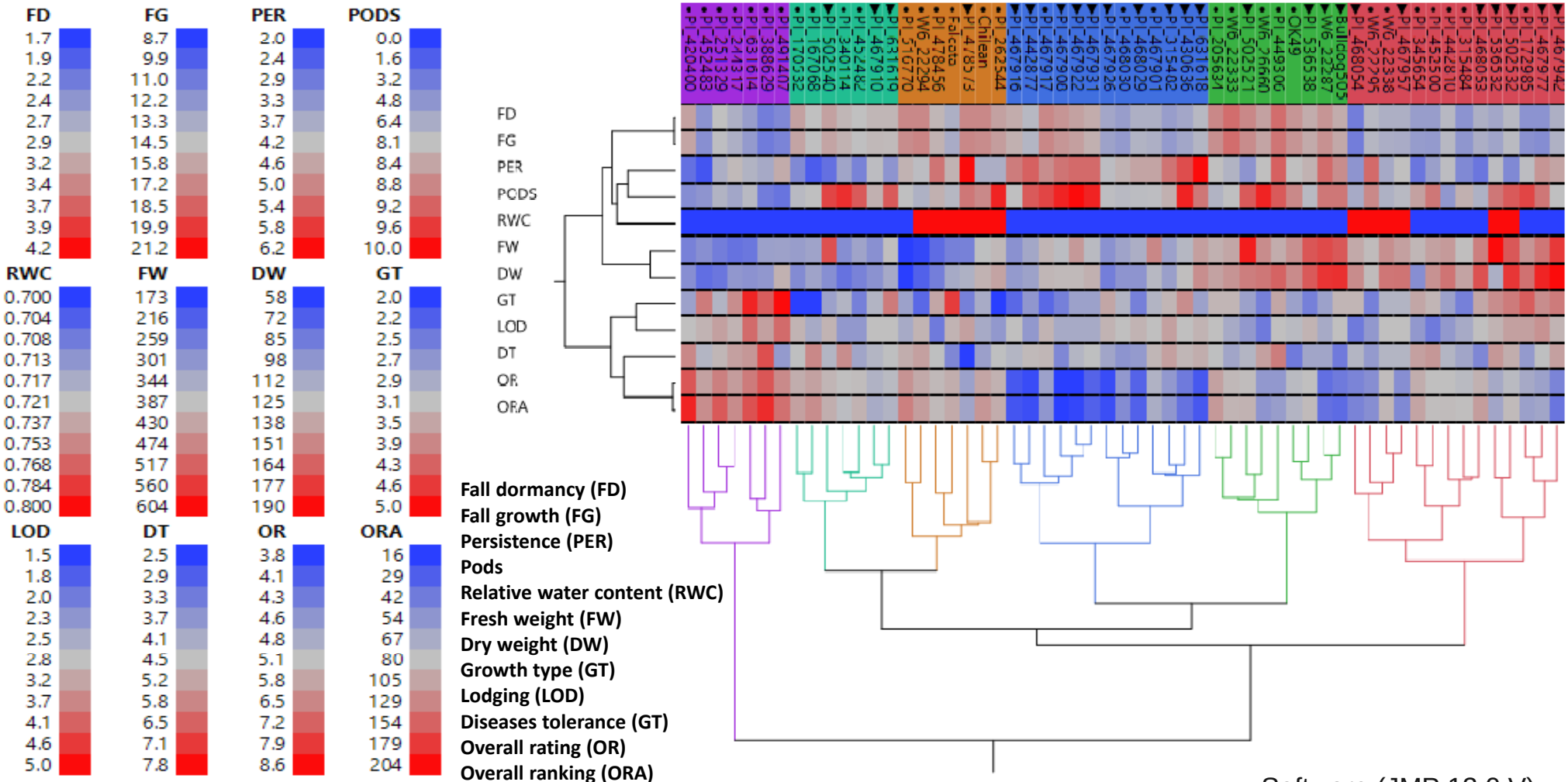
Source: Mesonet, Oklahoma

Phenotypic Data Collected from Field Trial

Agronomical	Morphological	Physiological	Forage Quality
• Yield • Fall dormancy • Fall growth • Field persistence • Disease response	• Flower • Pods • Growth type • Lodging	• Relative water content	• Dry matter • Crude protein • ADF • NDF • TDN • Lignin

- ADF: Acid Detergent Fiber
- NDF: Neutral Detergent Fiber
- TDN: Total Digestible Nutrients

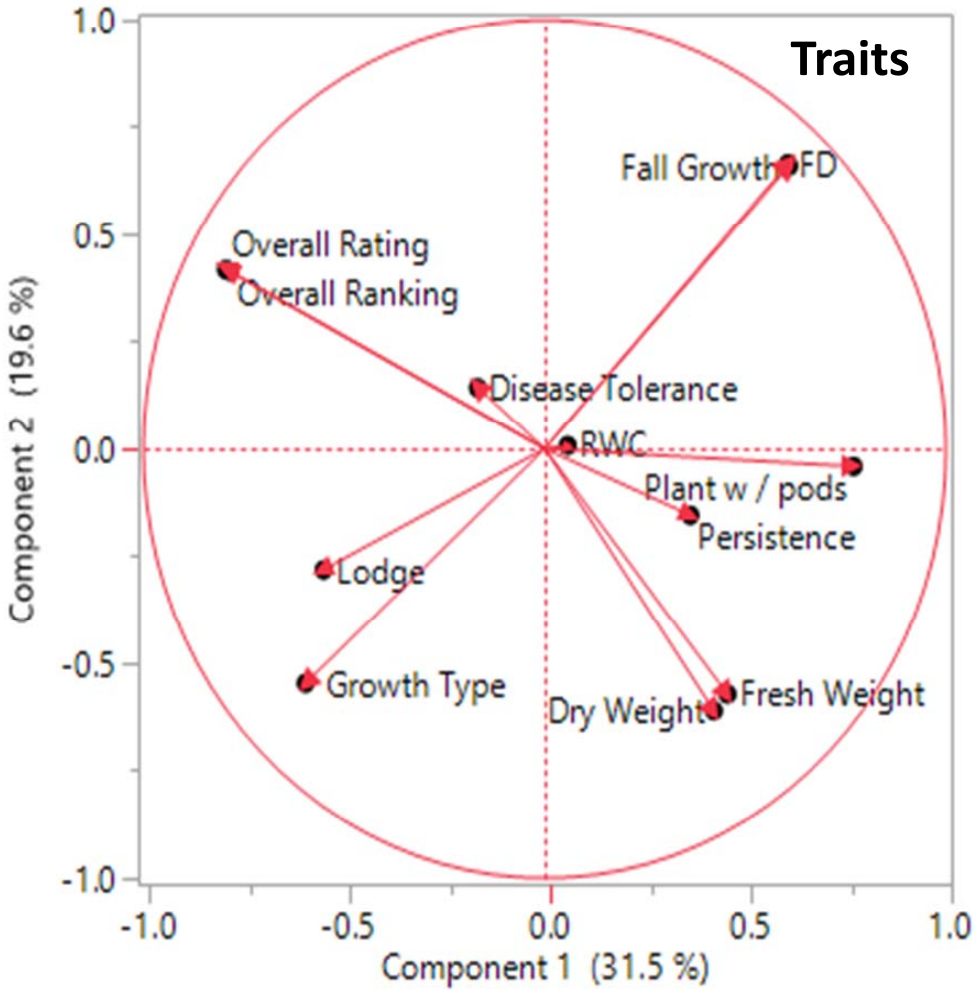
Cluster Analysis of Genetic Variation in Alfalfa

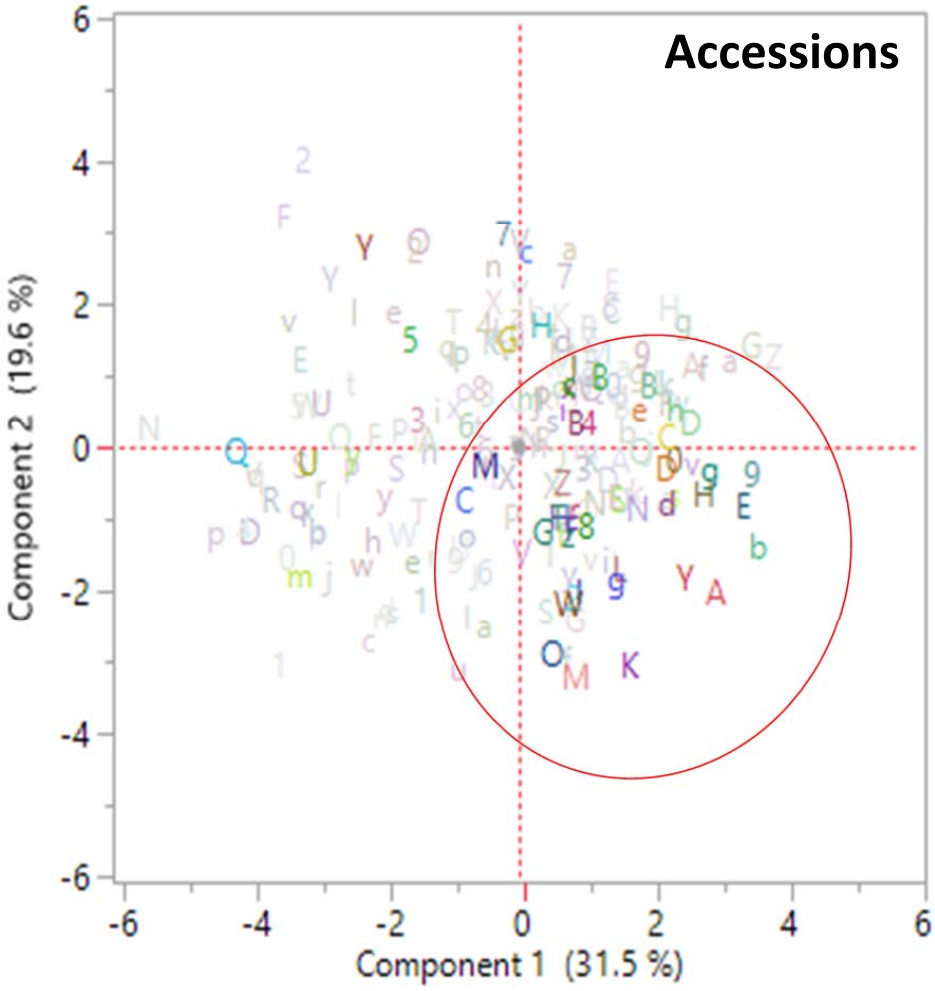


Fall dormancy (FD)
Fall growth (FG)
Persistence (PER)
Pods
Relative water content (RWC)
Fresh weight (FW)
Dry weight (DW)
Growth type (GT)
Lodging (LOD)
Diseases tolerance (GT)
Overall rating (OR)
Overall ranking (ORA)

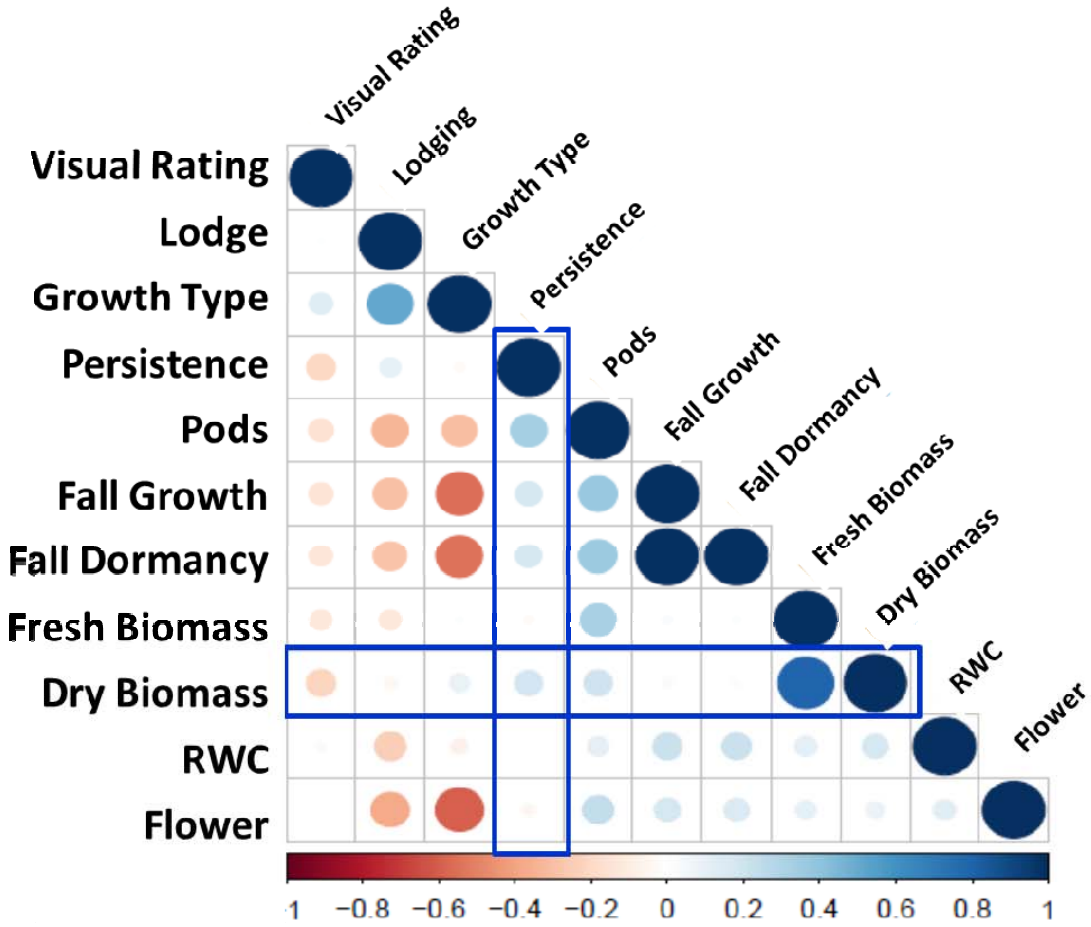
Software (JMP 13.0 V)

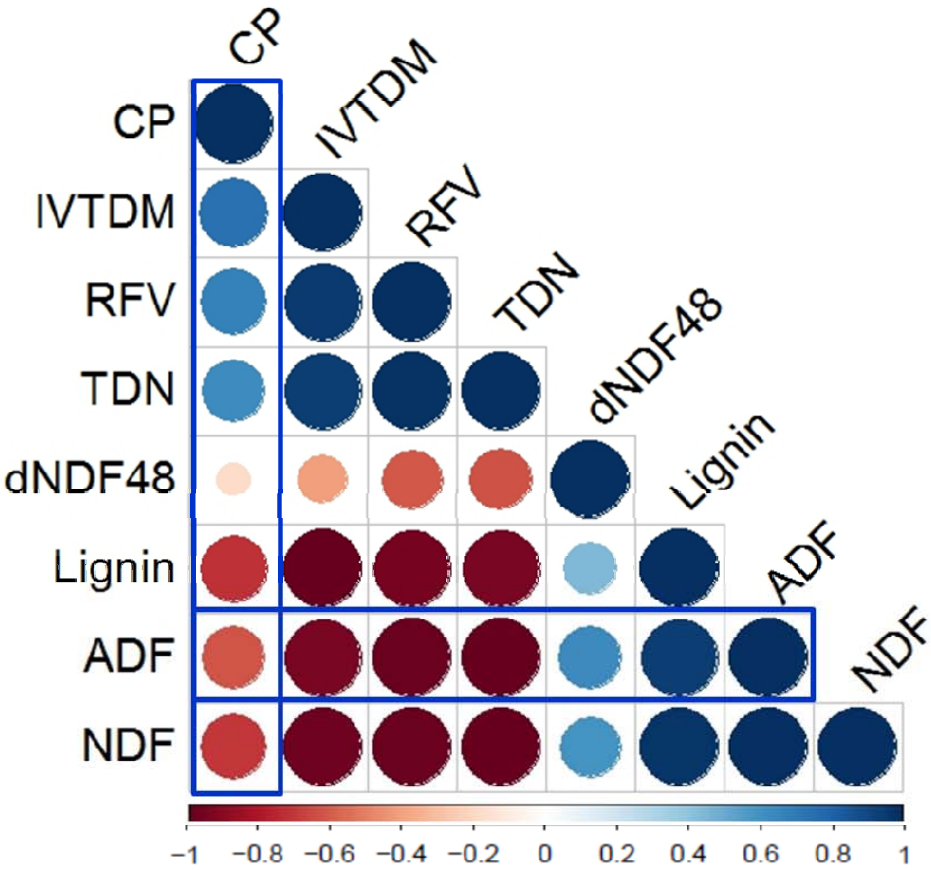
PCA - Agronomic and Morphological Responses





Correlation Between Agronomic and Forage Quality Traits





Selected Alfalfa Accessions From the Field

PI	Species	Origin
PI_478573	sativa	Peru
PI_467926	sativa	United States
PI_631618	falcata	Nepal
PI_467908	sativa	United States
PI_468054	varia	Canada
PI_430636	sativa	China
PI_467922	sativa	United States
PI_468029	varia	Canada
PI_467916	sativa	United States
PI_516895	sativa	Morocco
PI_442877	sativa	China
PI_467957	sativa	United States
PI_467931	sativa	United States
PI_467942	sativa	United States
PI_478448	sativa	Bolivia
PI_536532	sativa	United States
PI_502532	varia	Former Soviet Union
PI_502521	varia	Former Soviet Union
PI_536538	sativa	United States
PI_502540	varia	Former Soviet Union
PI_468053	varia	Canada

Persistence 50-65%

20-30%

Greenhouse evaluation

Outline of Research Approaches

A. Field evaluation and selection

B. Greenhouse evaluations

Greenhouse Drought Screening Assay Development

- **Soil (6 types)**
  - Metro-Mix 350 & 830
  - Quikrete all-purpose sand
  - Redi-earth
  - Course perlite & Vermiculite
- **Growth container (type and size)**
  - **Treepots**
  - Deepots
  - PVC mesocosms
  - Containers
- **Monitor soil moisture levels**
  - CR1000 and EM50 dataloggers
  - GS1 and EC5 VWC sensors
- **Vegetative clones or seedlings**
- **Length and severity of drought**

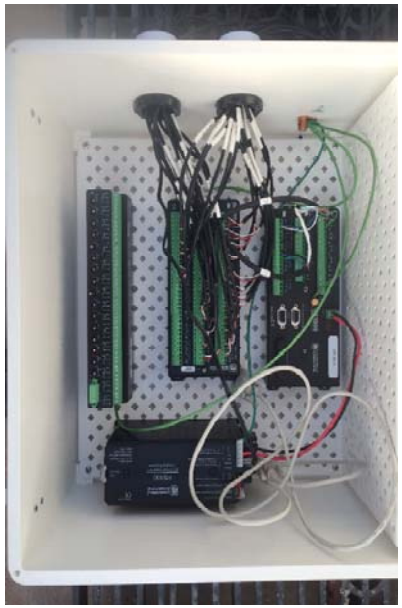
Greenhouse Drought Screening Assay

- **Soil substrate**
  - Quikrete all-purpose sand
  - Course perlite
  - 2:1 (perlite:sand)
- **Growth container**
  - Square treepots
  - 32 cm x 14 cm x 14 cm (L x W x D)
- **Monitor soil moisture levels**
  - CR1000 dataloggers
  - GS1 VWC sensors
- **Vegetative clones**
- **Eight genotypes**
- **RCBD Experimental design**
- **Treatments**
  - Well watered
  - Water stress

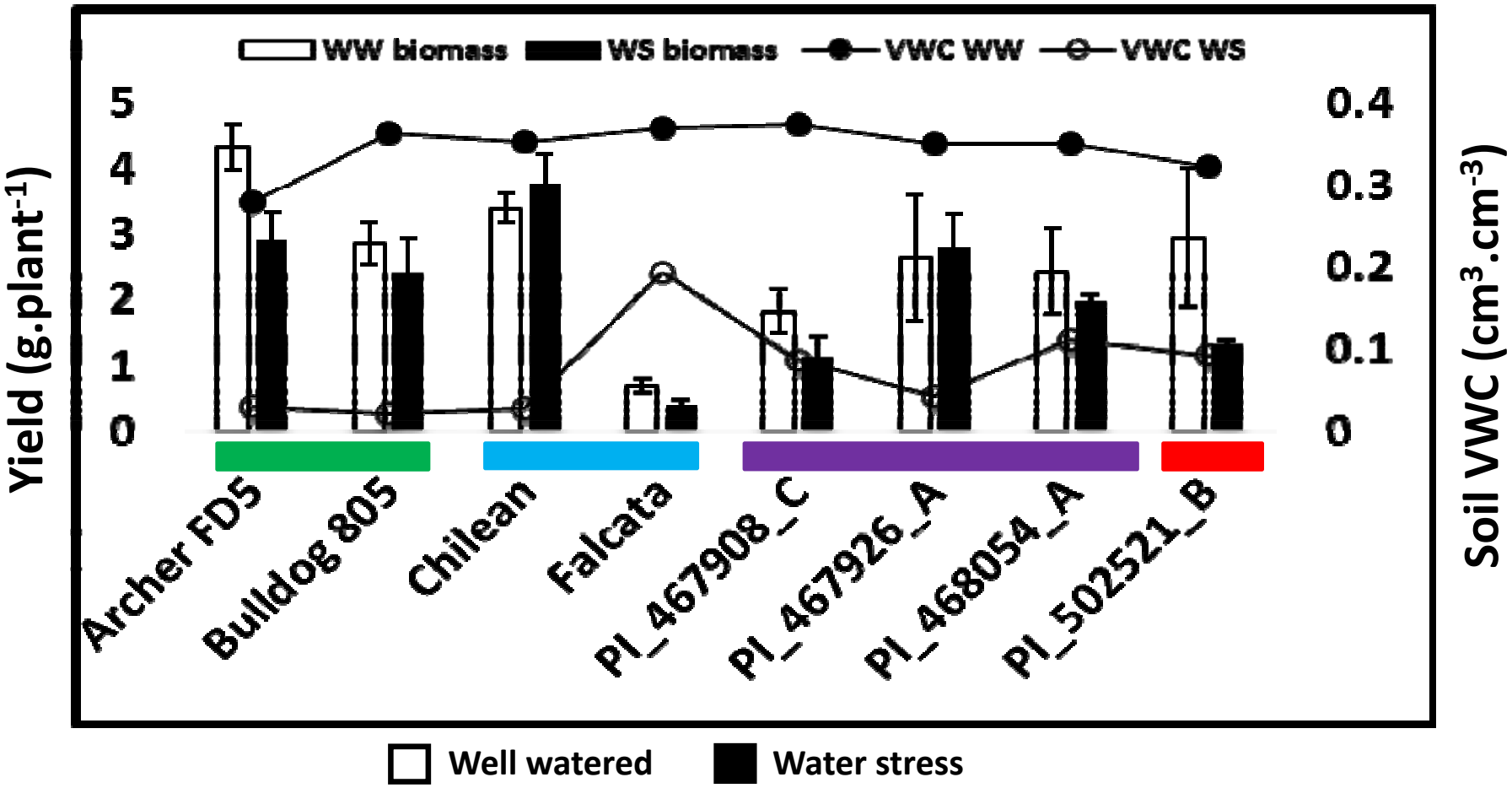
Use of Sensors to Monitor Drought in Real-time

Sensors	Dataloggers	Experimental setup
GS-1 (VWC)	CR1000, Campbell Scientific	One sensor per treepot

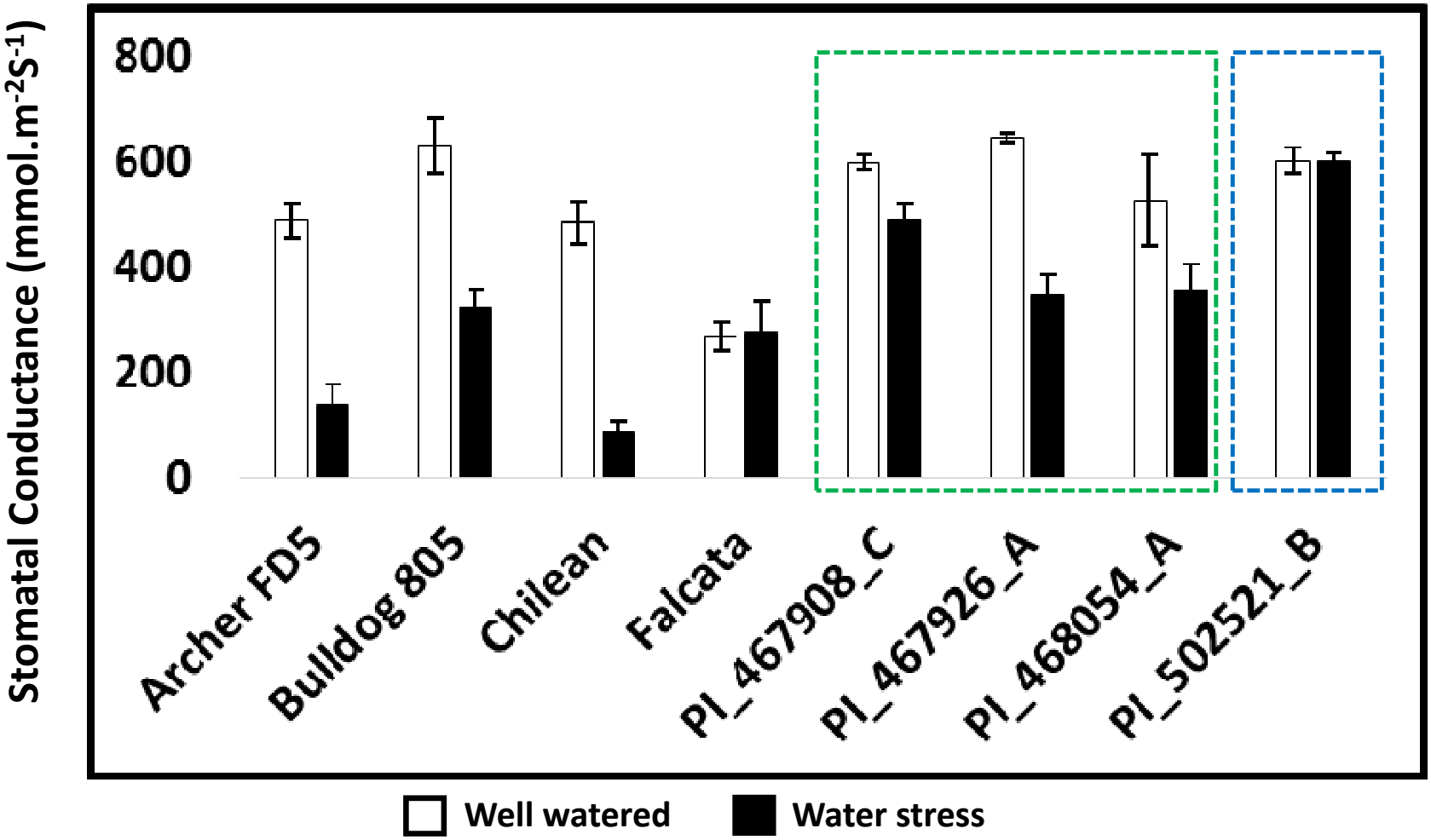
One sensor per treepot



Variation for Biomass Yield of Selected Alfalfa Genotypes



Differences in Stomatal Conductance under Drought Stress



Outline of Research Approaches

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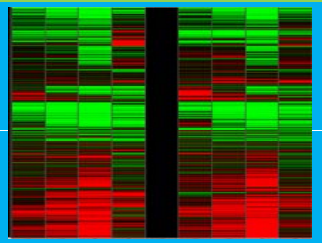
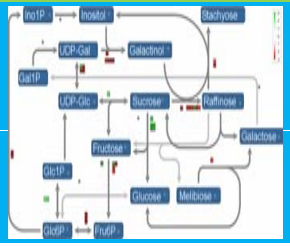
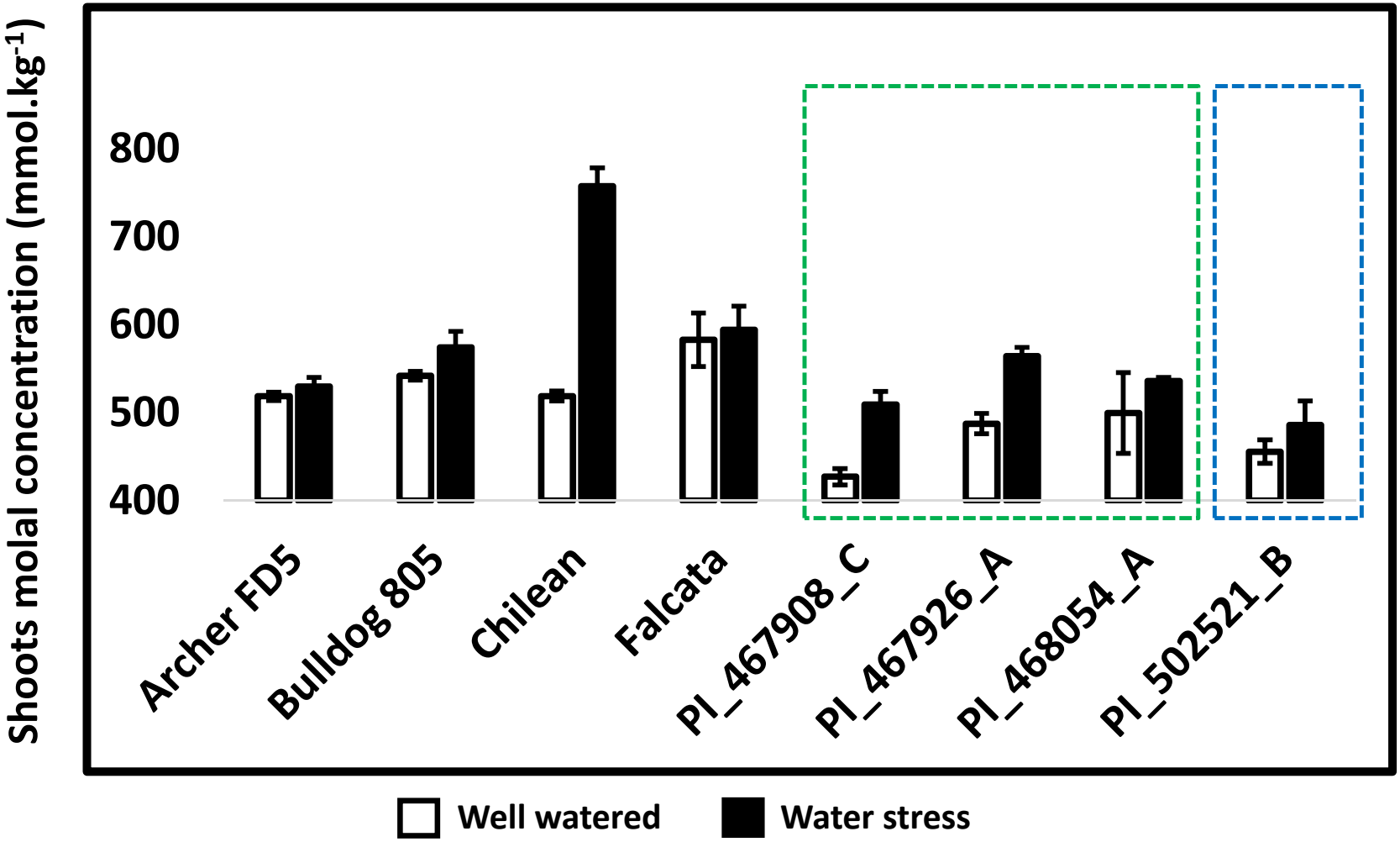
C. Drought tolerance mechanisms



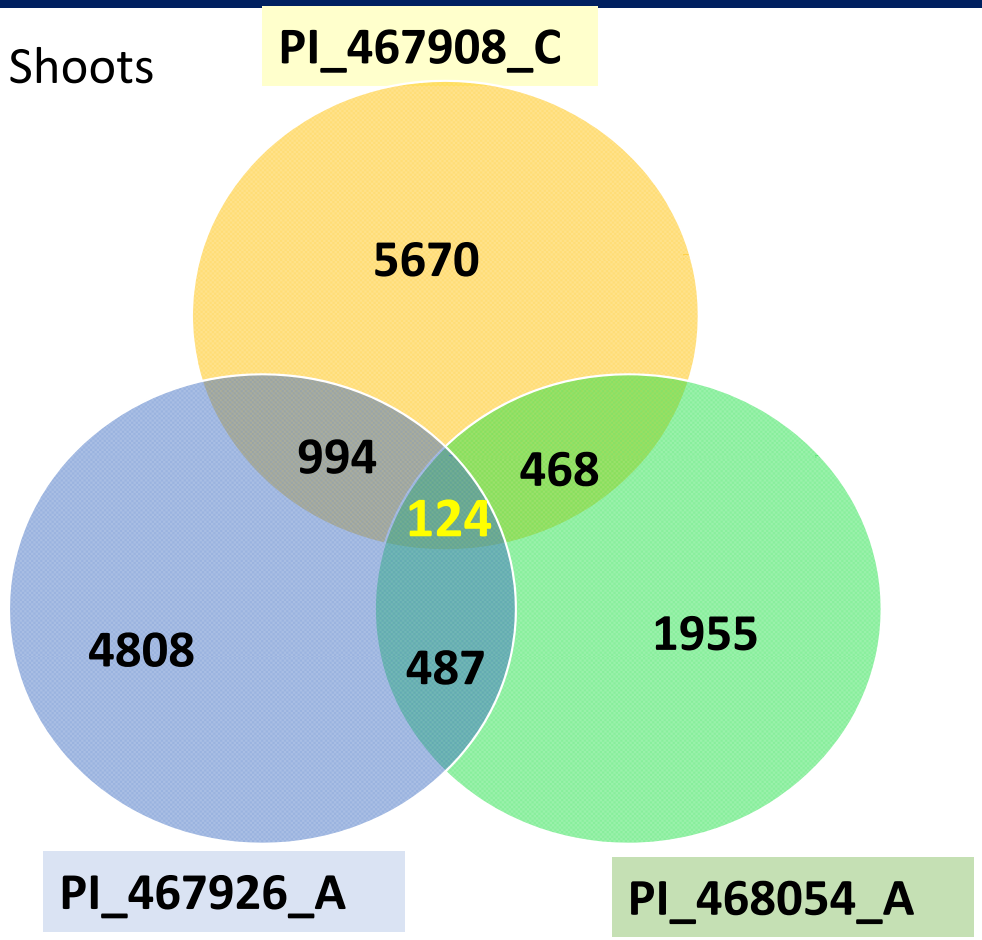


Synthesis of Osmoprotectants in Response to Drought



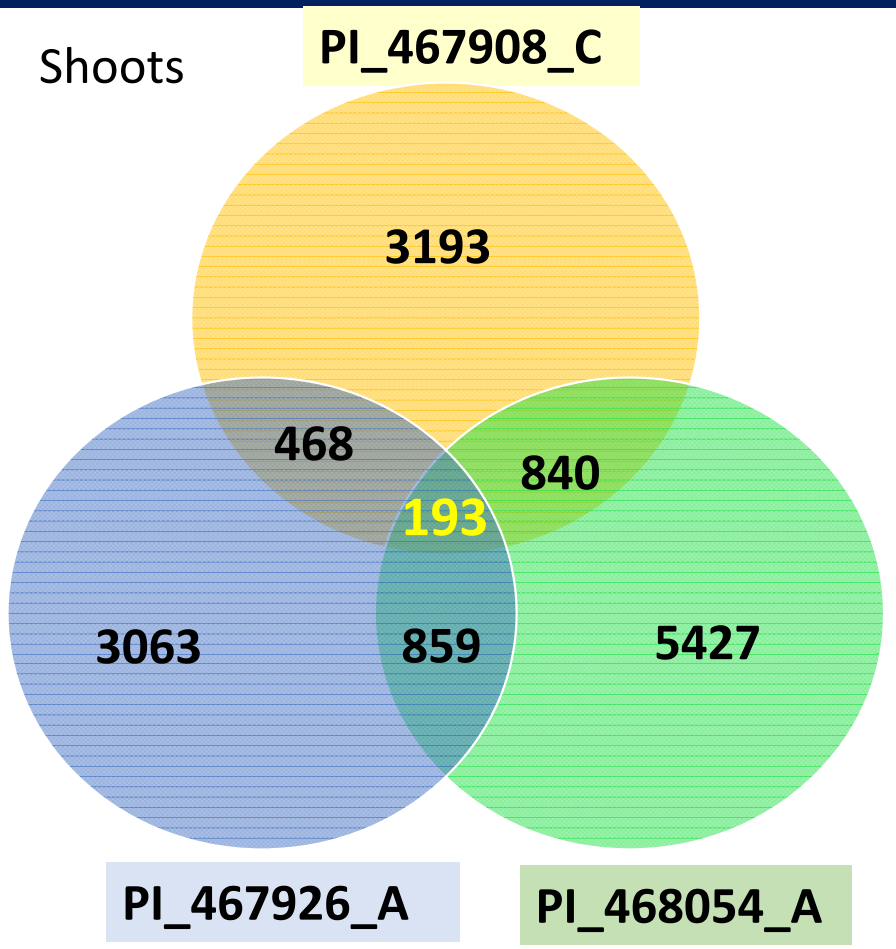
Differential Gene Expression to Drought Stress

Shoots



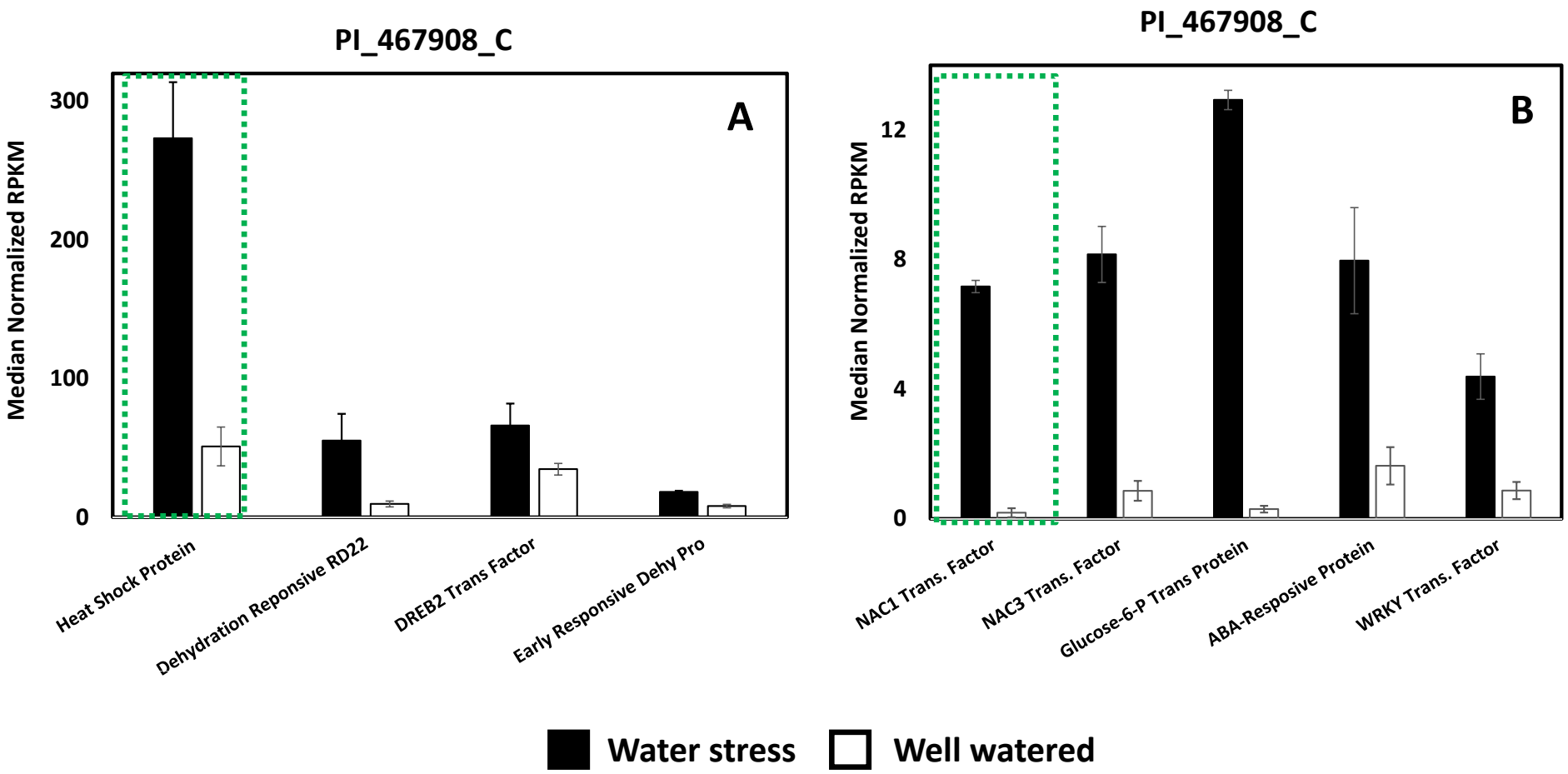
Upregulated > 2X

Shoots

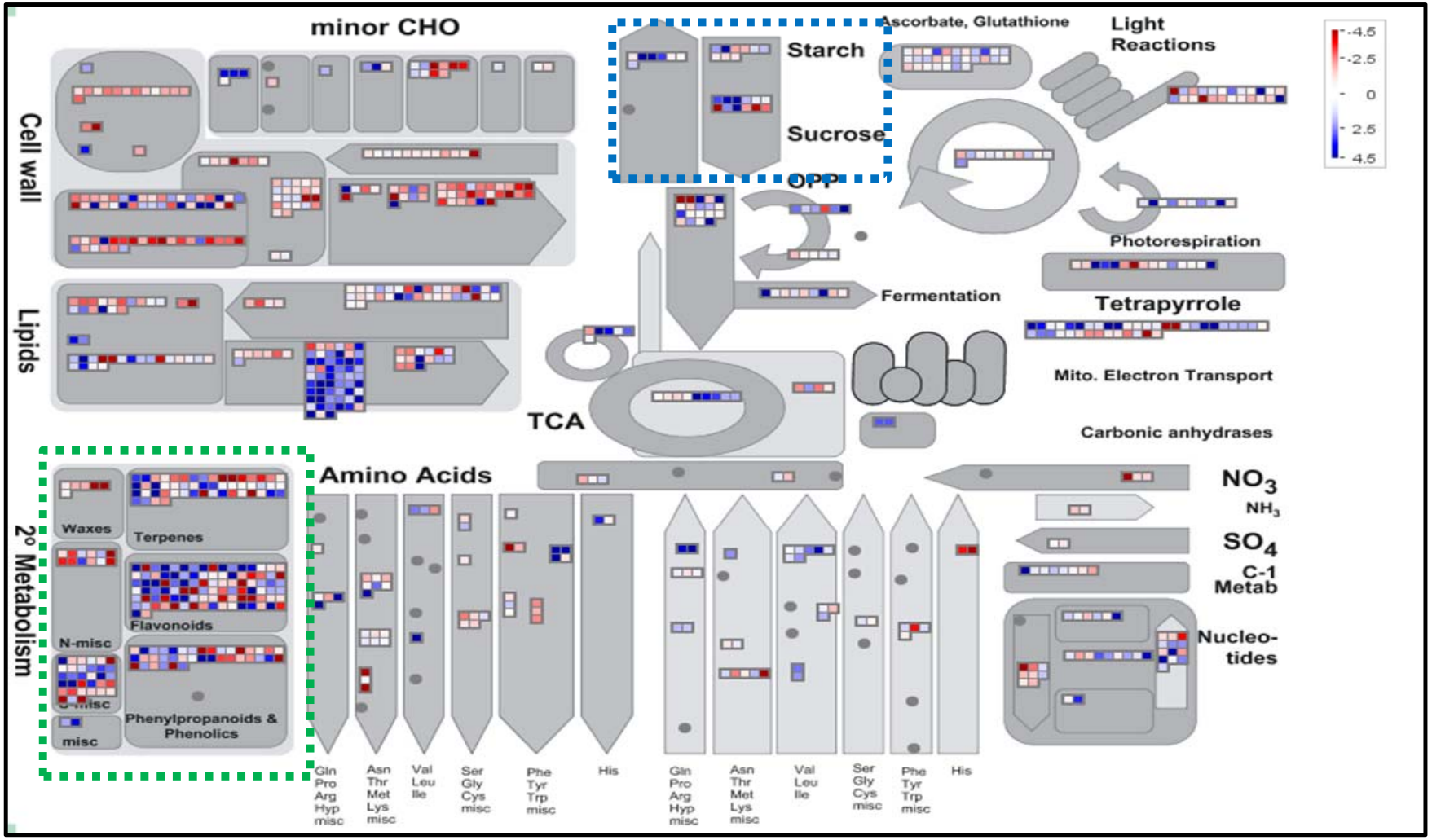


Downregulated < 2X

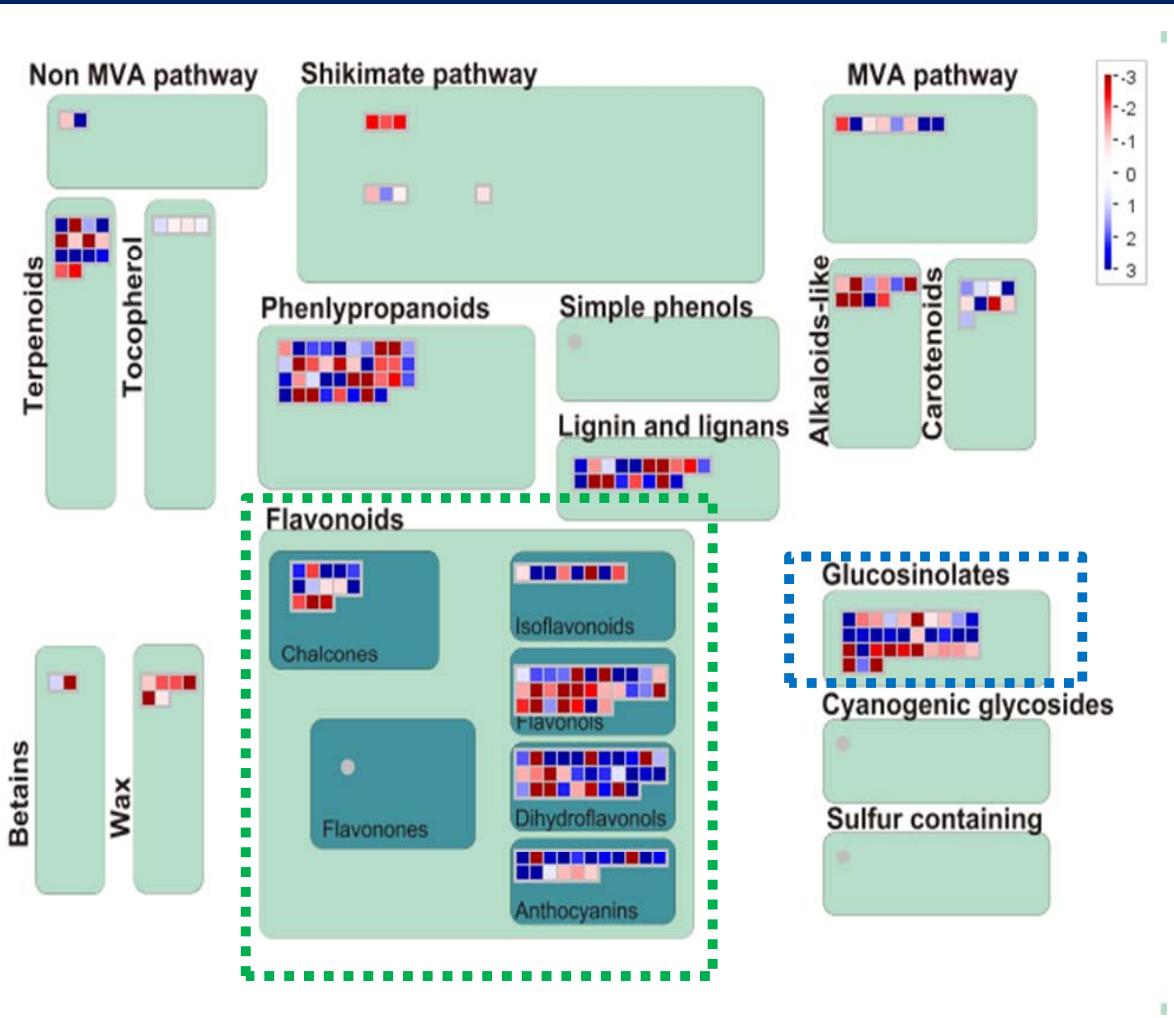
Differential Expression of Transcription Factors to Drought

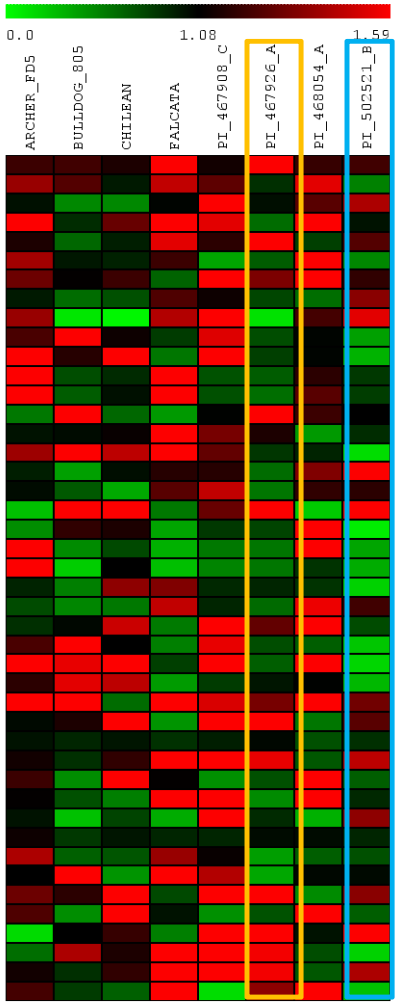


Changes in Gene Expression Associated with Plant Metabolism



Differential Gene Expression of Secondary Metabolites



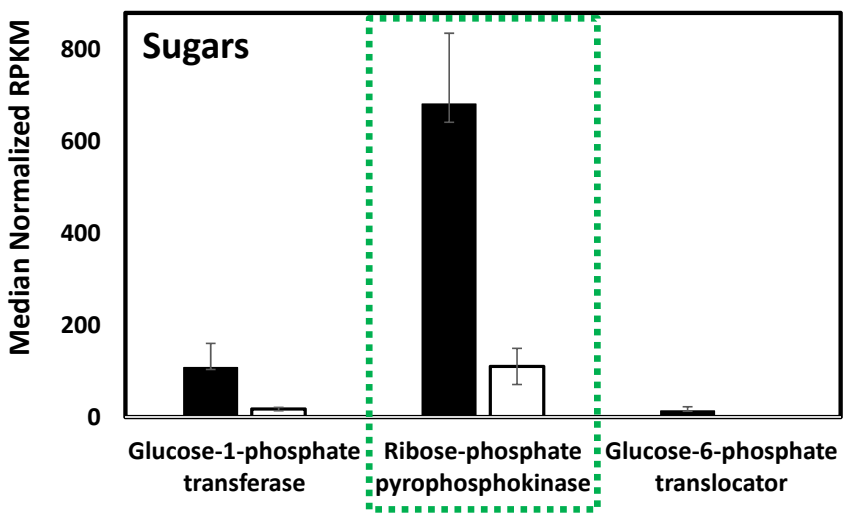


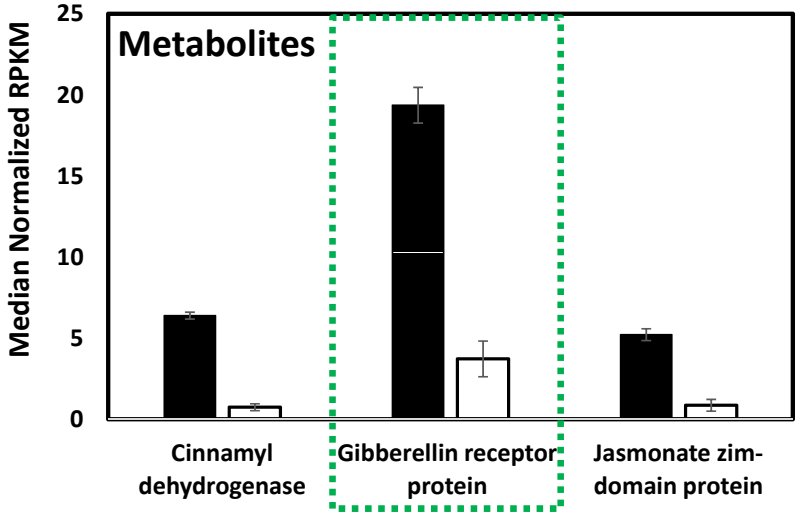
Trihydroxy Flavons

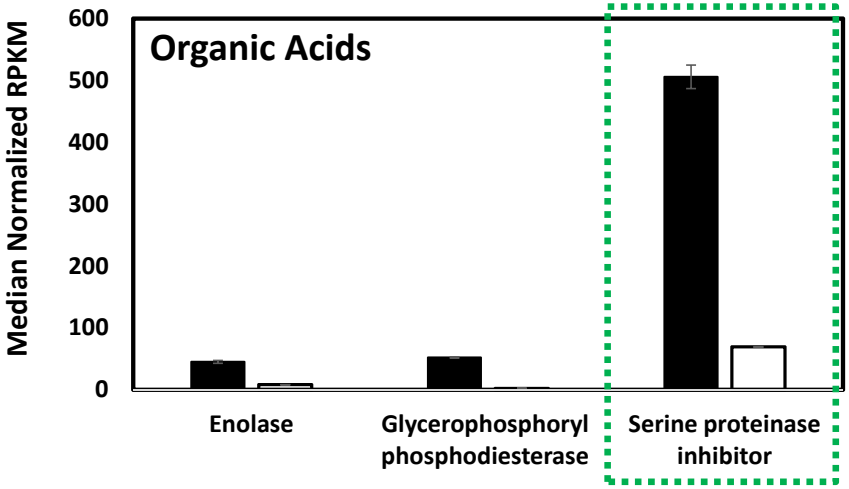
7-O-Glucoside

"3,7,4'-Trihydroxyflavone "
3-Rha-Gal-GlcA-Soyasapogenol B
"6,7,4'-Trihydroxyflavone "
"7,3',4',5'-Tetrahydroxyflavone "
"7,3',4'-Trihydroxyisoflavone Precursor "
7-Hydroxy-2'-methoxyflavone
7-Hydroxy-3-methoxyflavone
Apigenin
Apigenin-7-O-glucoside
Aralia-saponin VII
Bayogenin
Epicatechin
Epicatechin Pentose
Formononetin-7-O-glucoside
Galacturonic acid-glucose-glucose-Bayogenin
Gal-GlcA-SoyB
Genistein
Genistein 7-O-b-D-glucosidegenistin
GlcA-Glc-Bayogenin
Glu Glu Bayogenin Fragment
Glucose-glucose-Bayogenin
Gypsogenic acid
Gypsogenin_3' hexose
Hexose-hexoseA-Hederagenin
Hexose-hexose-bayogenin
hexose-hexose-hexose-Bayogenin
Hexose-hexose-hexose-Hederagenin
Hexose-hexose-Rha-Bayogenin
Isoferulic acid
Isoliquiritigenin
Isopimpinellin
Kaempferol Glucuronide Rhamnose
Kaempferol-3-O-glucoside
Leteolin 3'-methyl ether 7-glucoside
Medicagenic Acid
Myristic acid
Naringenin-7-O-glucoside - aglycone
p-Coumaric acid
Protocatechuic aldehyde
Quercitrin Quercetin 3-rhamnoside
Rha-hexose-hexose-Hederagenin
Rha-hexose-hexose-hexose-Quillaic acid
Rutin
Zhanic Acid Aglycone

Gene Expression Associated with Plant Metabolism

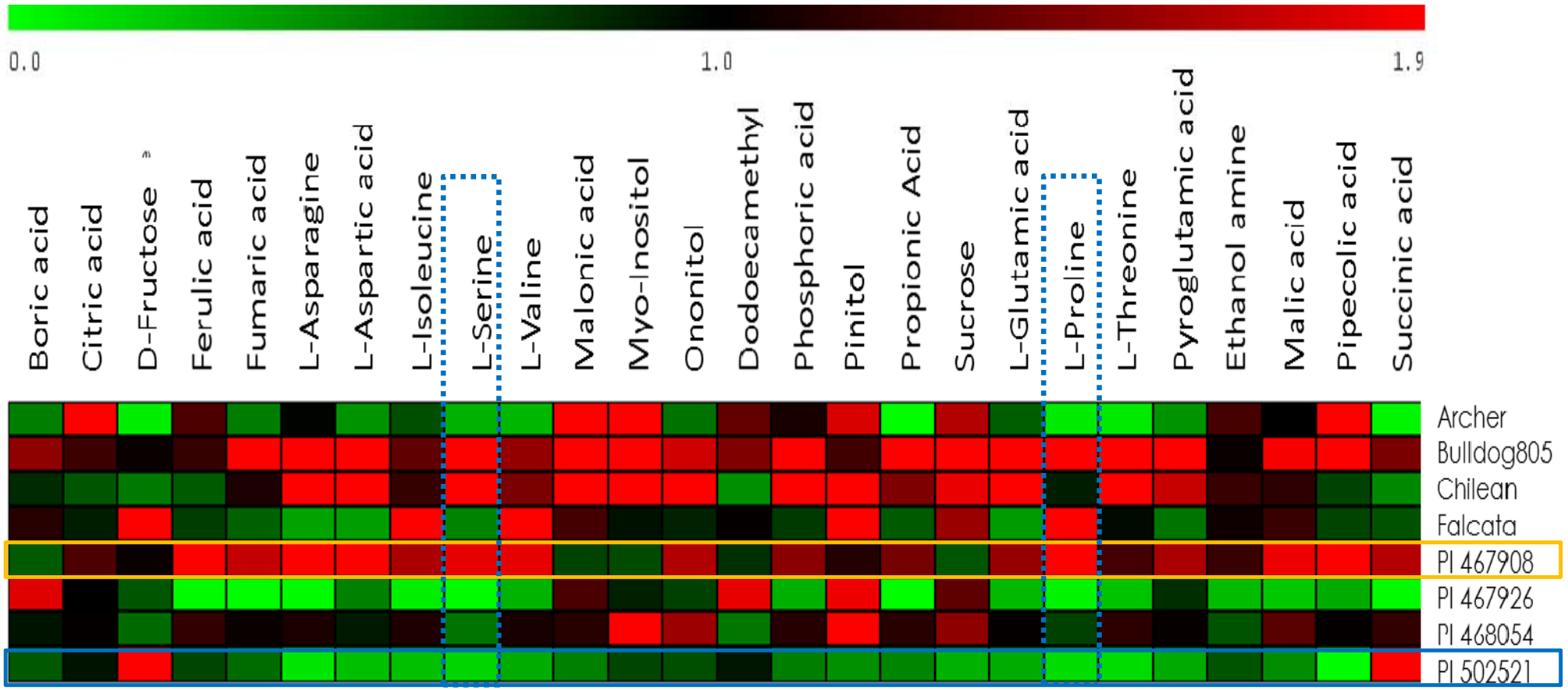




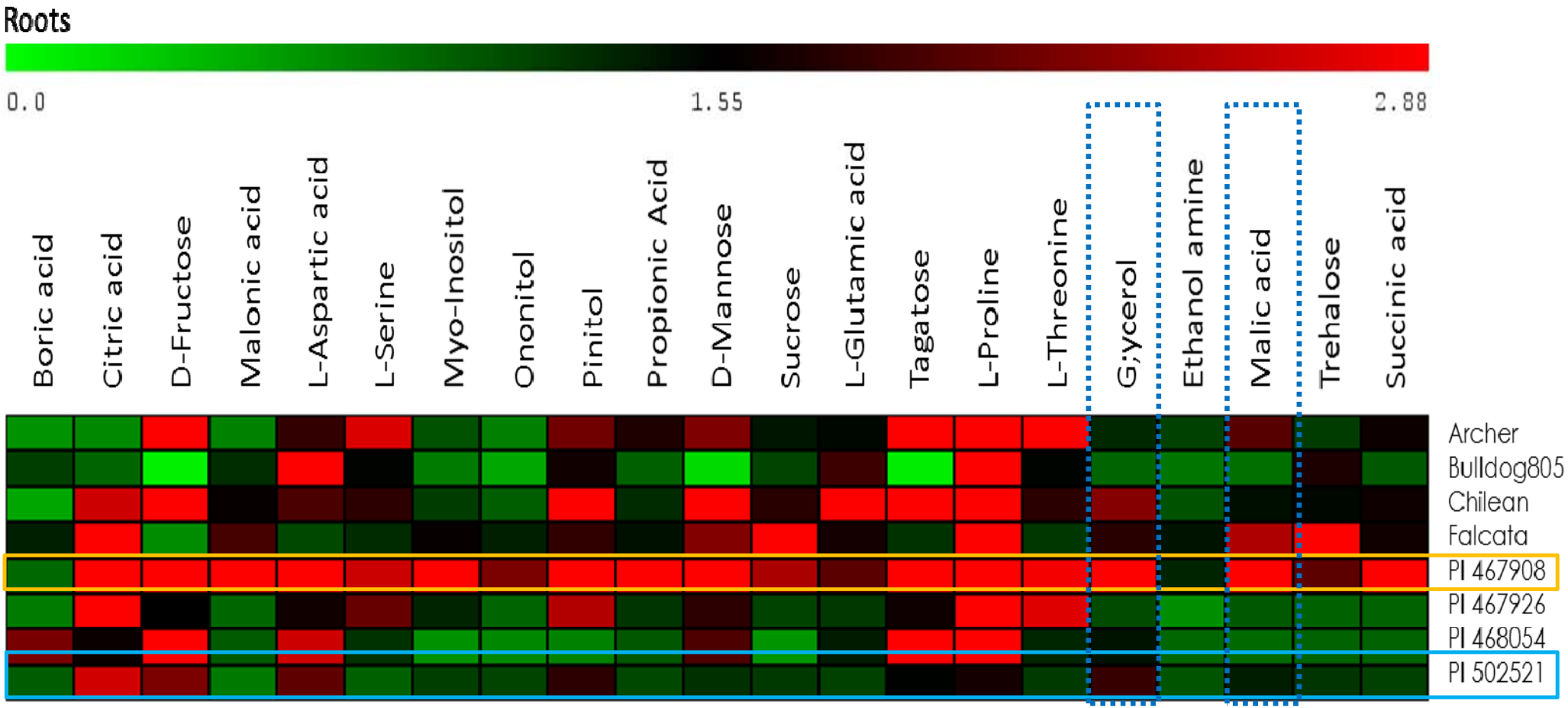


Drought Tolerant Lines Accumulate Sugars and Amino Acids

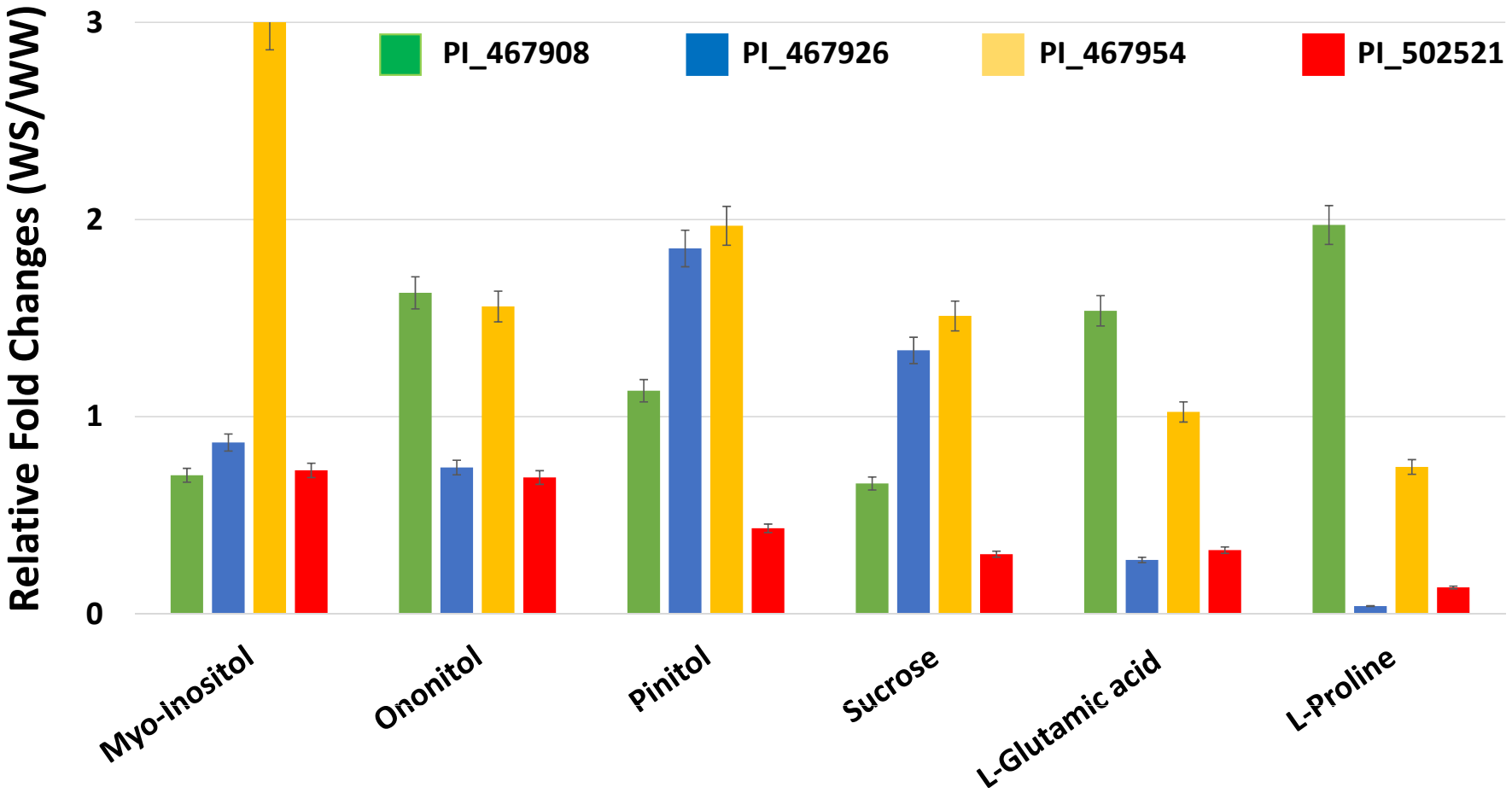
Leaves



Drought Tolerant Lines Accumulate Organic Acids



Differences in Accumulation of Osmoprotectants



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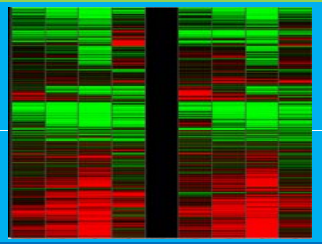
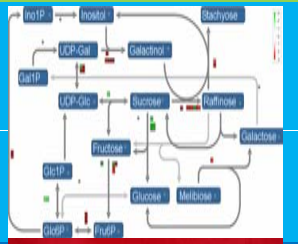
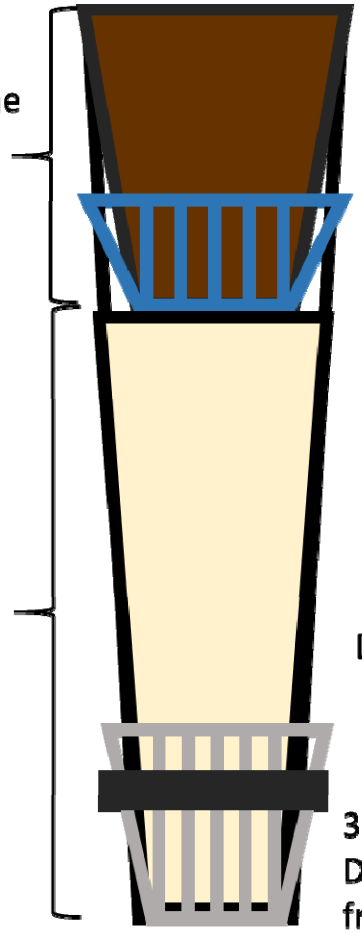




D. Develop populations

Crossing Block for Population Development

Design for Screening Drought Tolerance of F1 Populations



300 ml volume
MM360,
autoclaved

D40L inner deepot cut to 10 cm

Blue blotting paper disk, 13.5 cm Anchor Paper Co., moistened in water to become flexible and moldable to the cut deepot. The D40L and blot paper fit tightly inside the D60L

D60L outer deepot

3 coffee filters secured around outside of D60L to hold in sand. Cut a small band from D40L to secure filters in place.

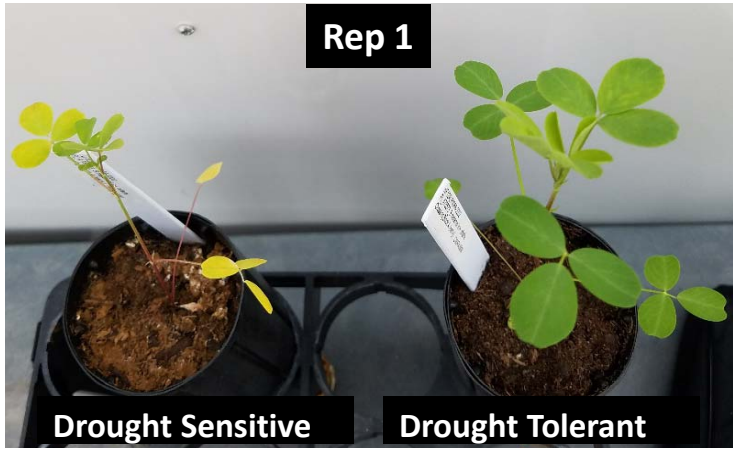
Growth containers

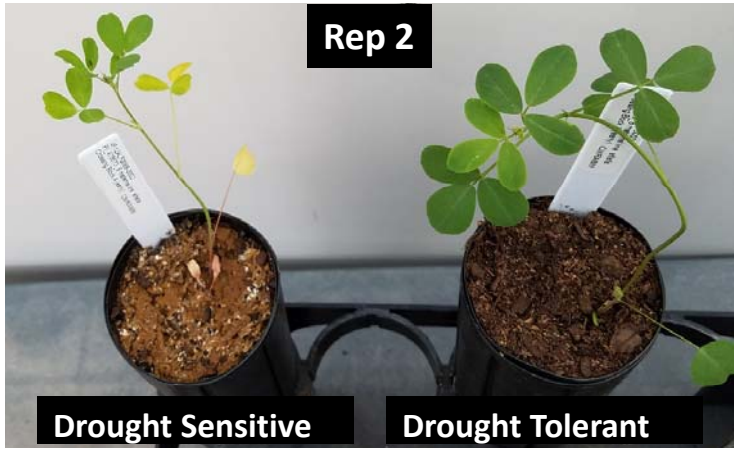
Ref: Christy Motes

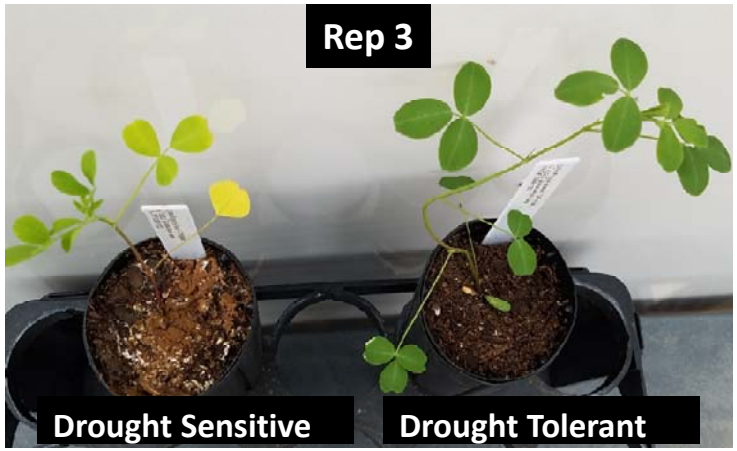
Deepot Screening Drought Tolerance of F1 Populations

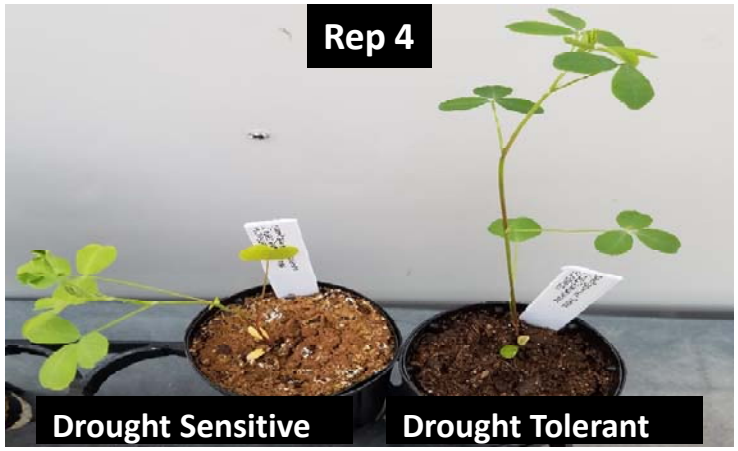
Individuals F1 population screening

Drought Sensitive vs Tolerant Progenies – Divergent Selection



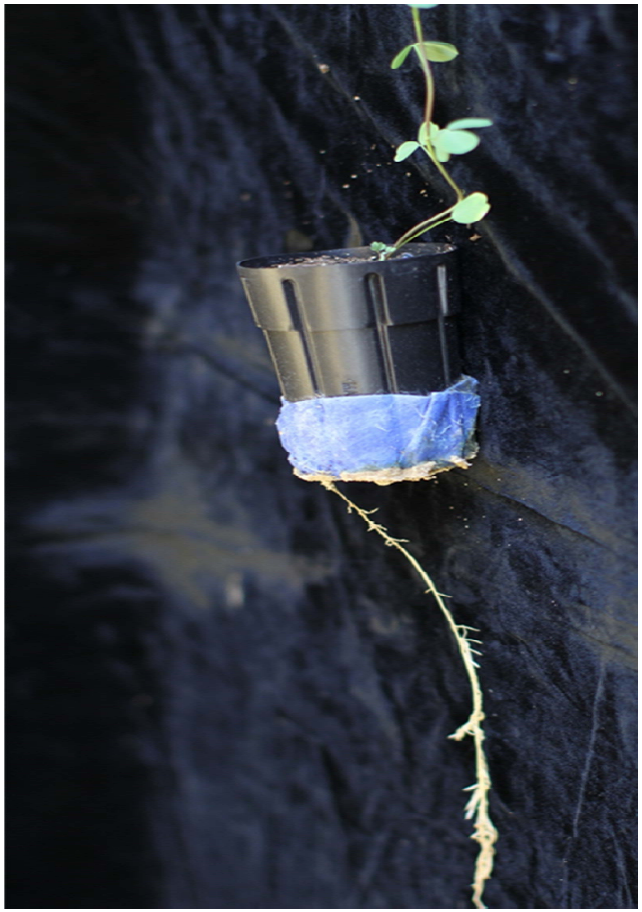




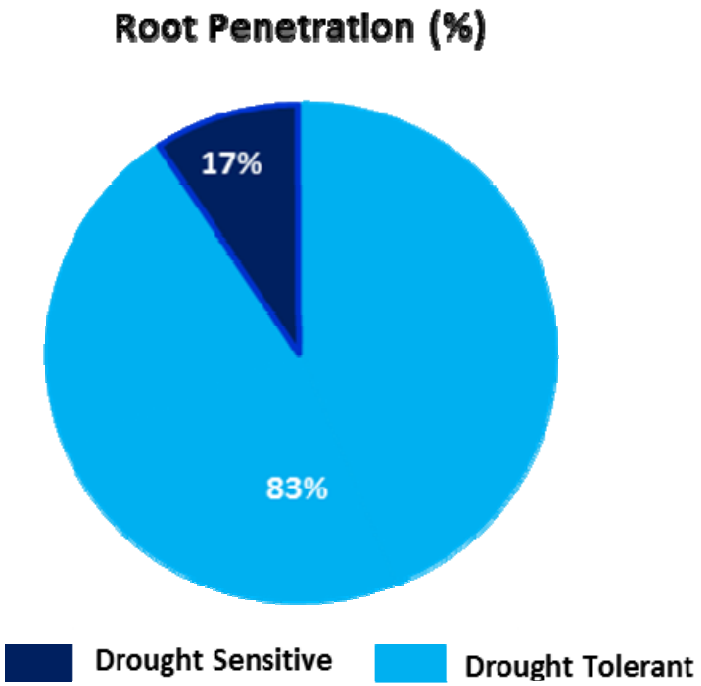


Root Penetration Ability

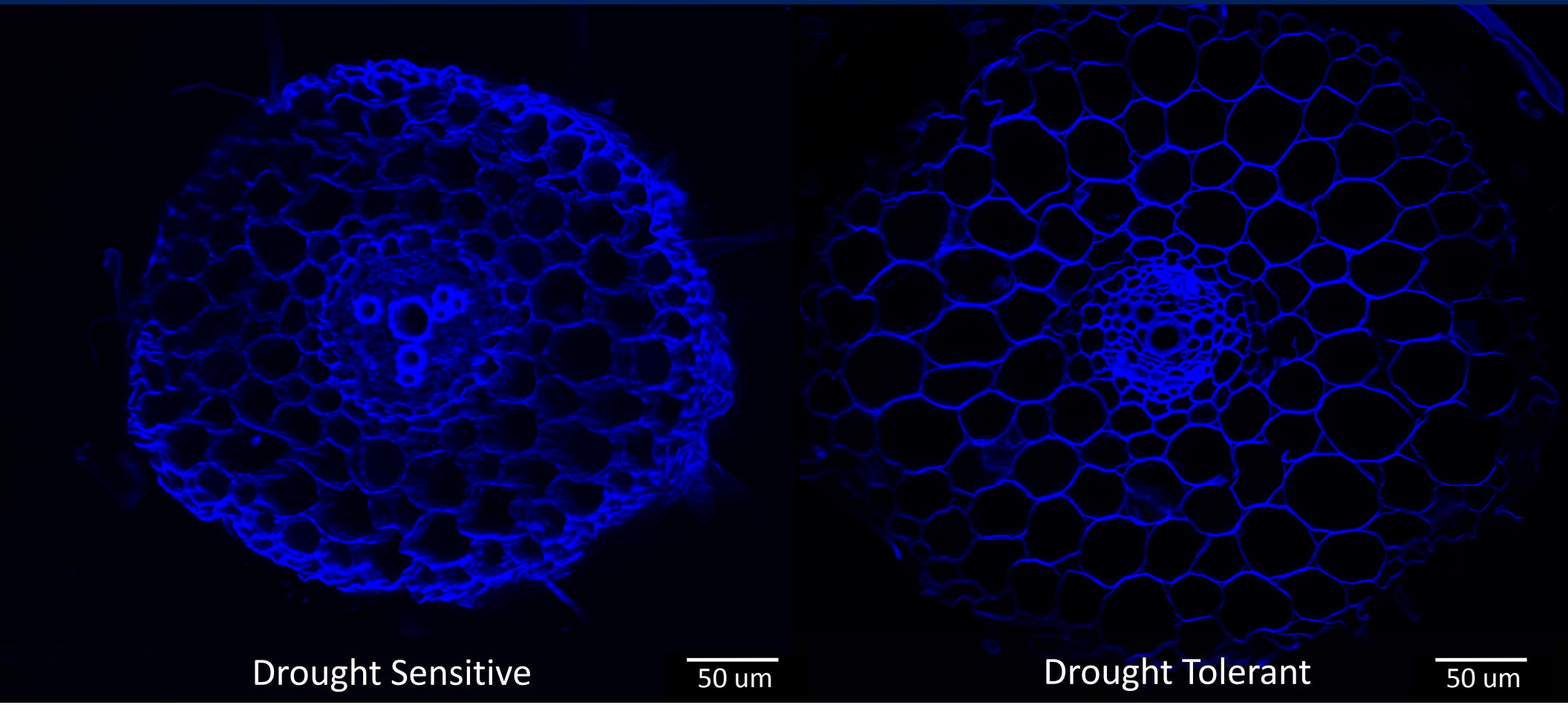
Drought Sensitive



Drought Tolerant



Root Phenes Predict Root Penetration Ability



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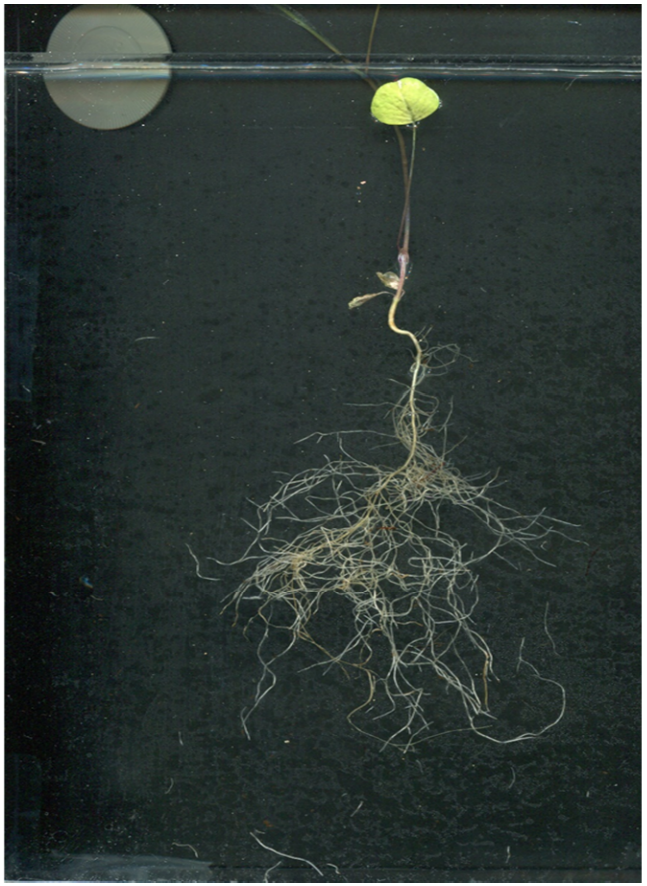
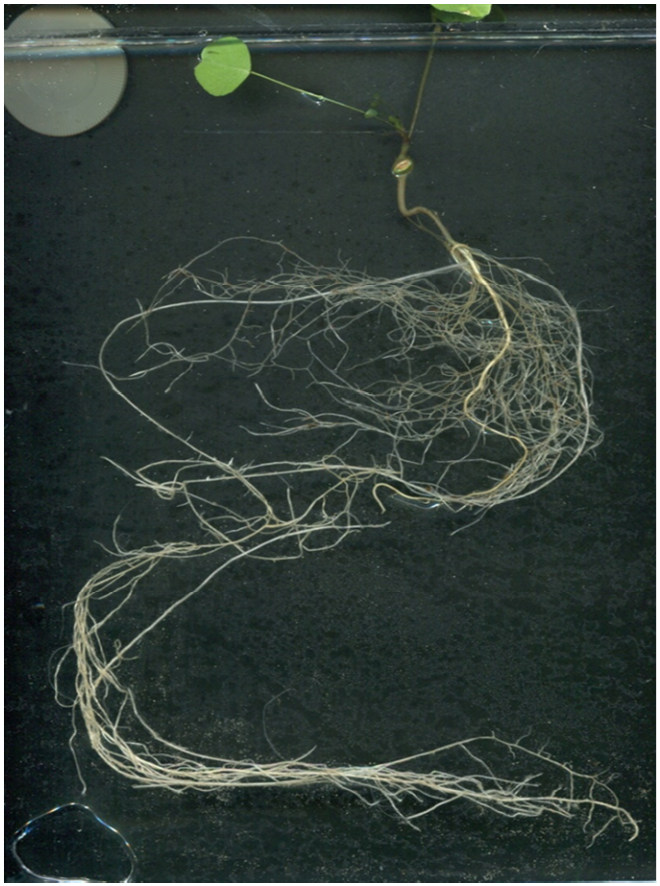
NF16LF009-0066

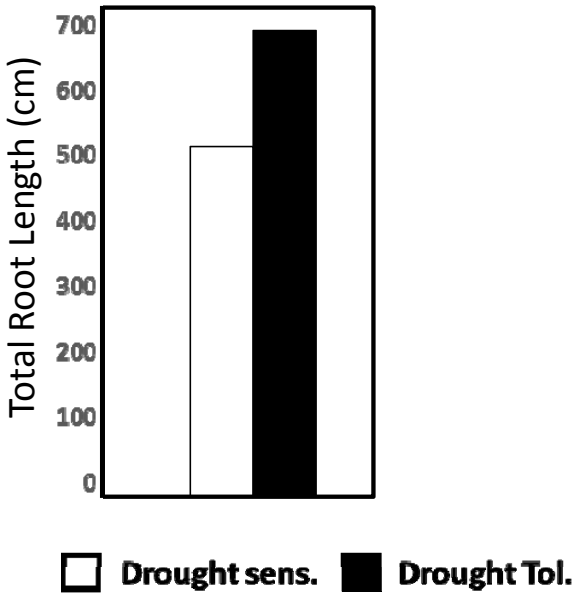
Differences in Root System in Tolerant and Sensitive Progenies



Drought Sensitive

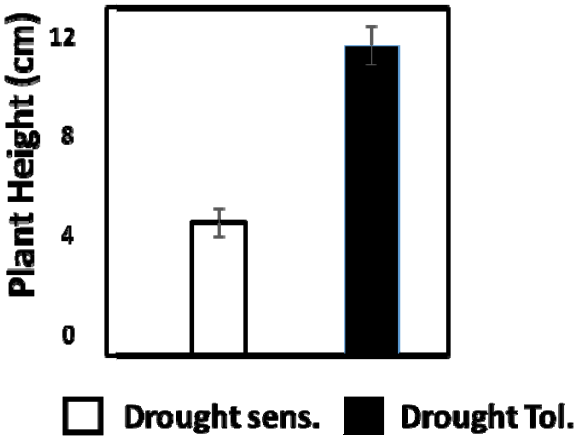


Drought Tolerant



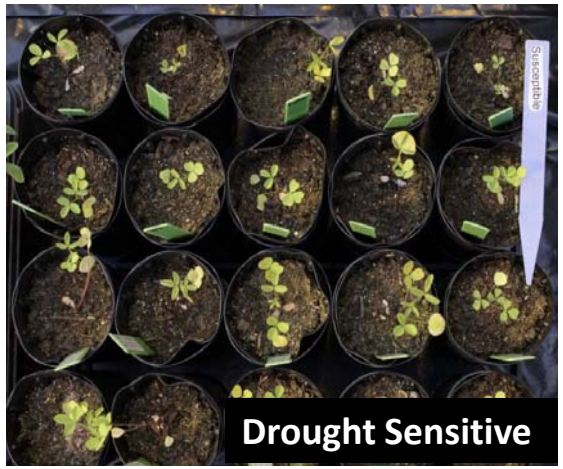
Drought Recovery of Half-Sib Progenies

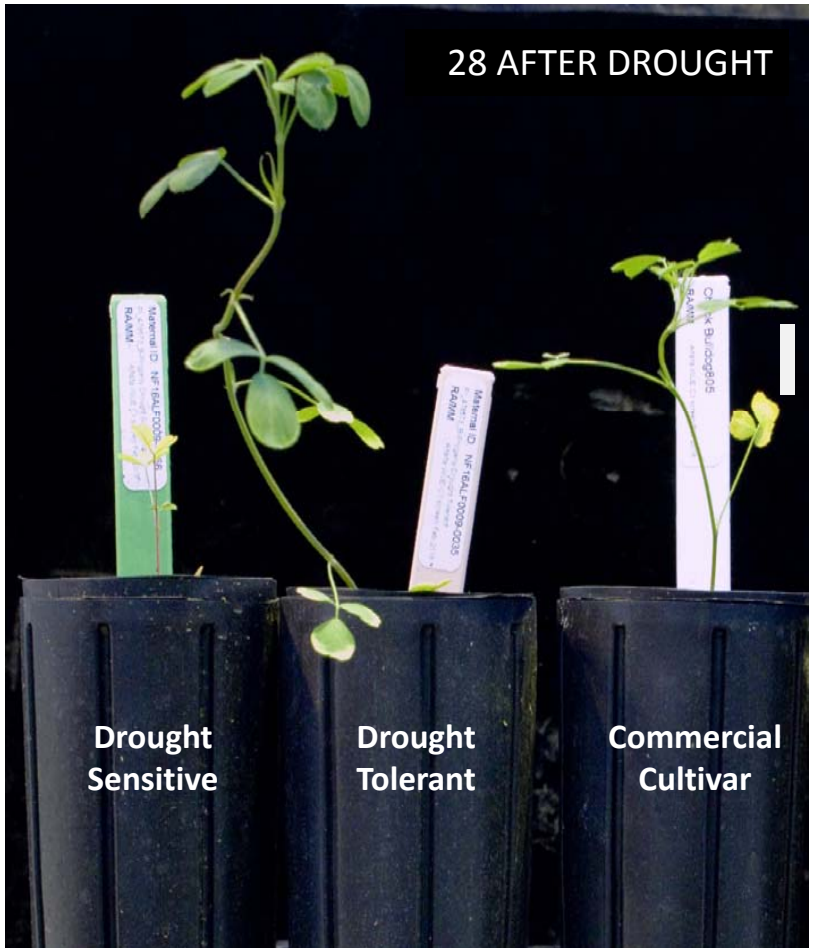
Five days after transplanting



Twelve days after transplanting

Summary of Drought Population Development



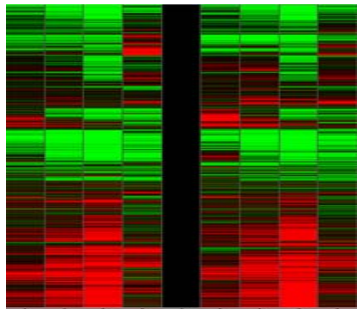


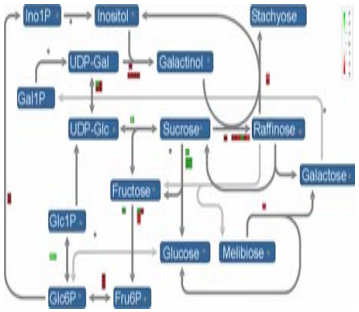
Research Summary

- Identified genetic variation under drought stress conditions in the field.
- Developed alfalfa populations from field-selected plants based on yield and persistence.
- Developed standardized protocol for drought screening under greenhouse conditions.
- Explored high root penetration mechanisms for drought tolerant lines.

Research Summary

- Identified differential accumulation of osmoprotectants under drought stress.
- Detected differential gene expression between well watered and water stressed plants.
- Observed accumulation of secondary metabolites in water stressed plants.
- Developed divergent alfalfa populations from greenhouse drought screening.

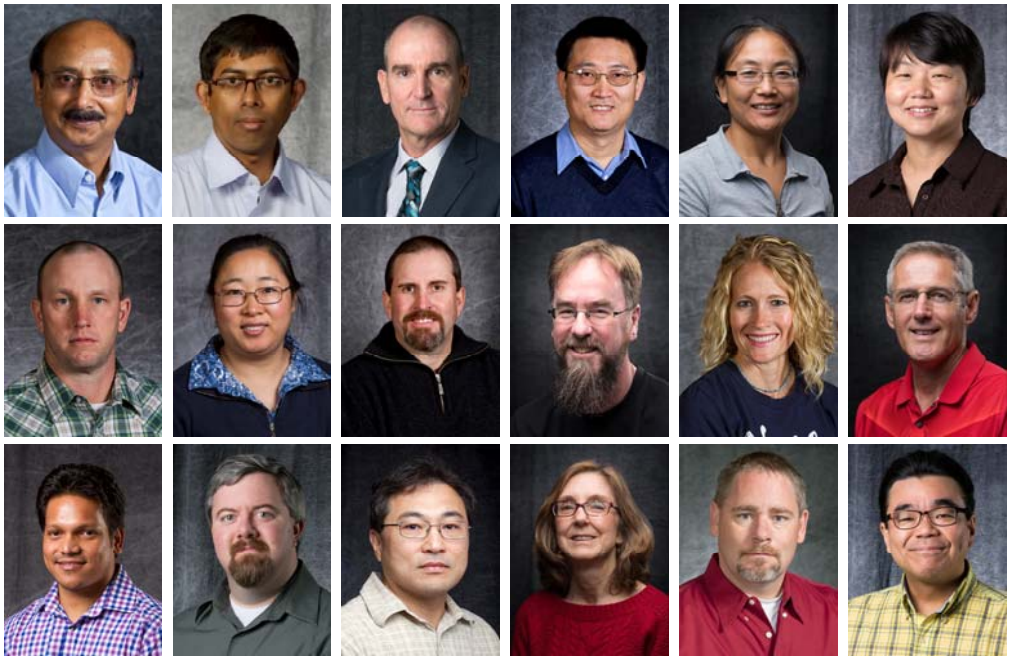




Acknowledgements

Lab Members

Noble Collaborators and Core Facilities



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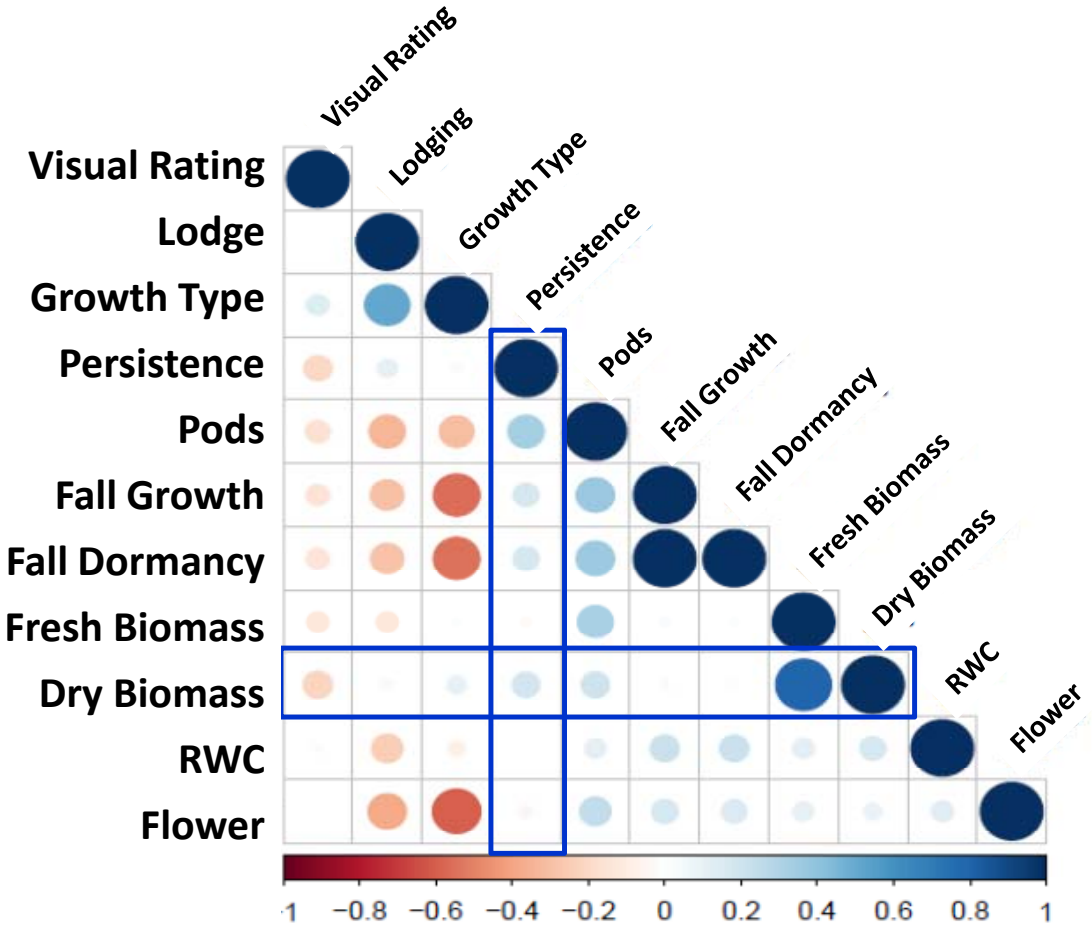
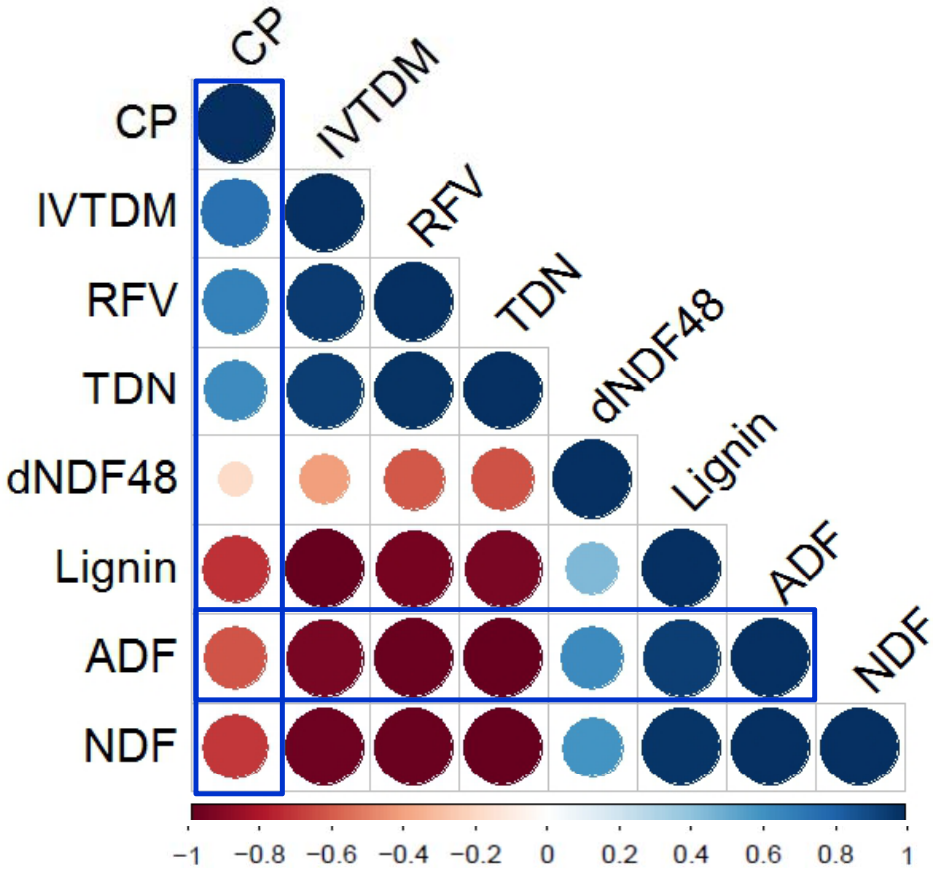
Understanding Adaptive Responses to Drought Stress in Alfalfa

Rokebul Anower
Legume Genomics Laboratory
2018 Joint Conference NAAIC
June 4-6, 2018, Logan, Utah

Correlation Between Agronomic & Forage Quality Traits





Not sure about the summary slide, so I prepared several to get some options.

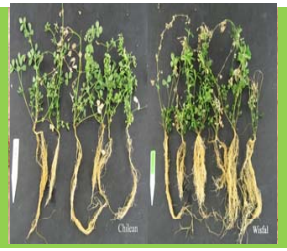
Research Summary

A. Field evaluation and selection

- Identified genetic variation under drought stress conditions in the field.
- Selection from field based on yield and persistence.
- Developed alfalfa populations from field-selected plants.

Research Summary

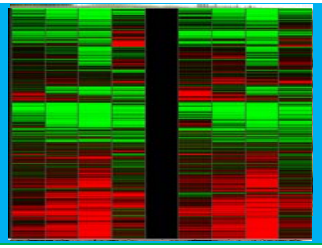
B. Greenhouse evaluations

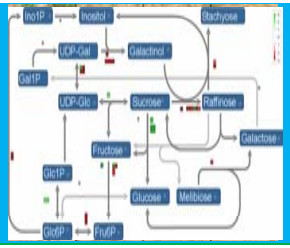


- Developed standardized protocol for drought screening under greenhouse conditions.
- Screened drought tolerant alfalfa lines selected under greenhouse conditions.
- Explored high root penetration mechanisms for drought tolerant lines

Research Summary

C. Drought tolerance mechanisms





- Identified differential accumulation of osmoprotectants under drought stress.
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Research Summary

D. Develop populations

- Developed divergent alfalfa populations from greenhouse drought screening.

Or this

Outline of Research Approaches

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Outline of Research Approaches

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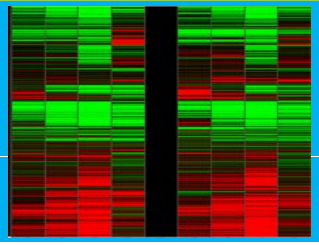

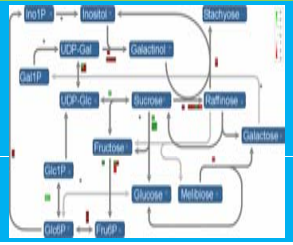

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Outline of Research Approaches

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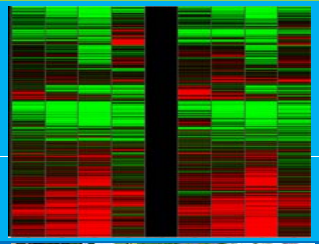

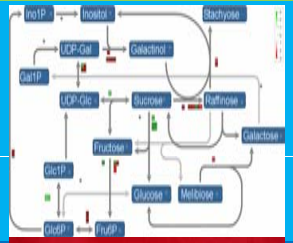

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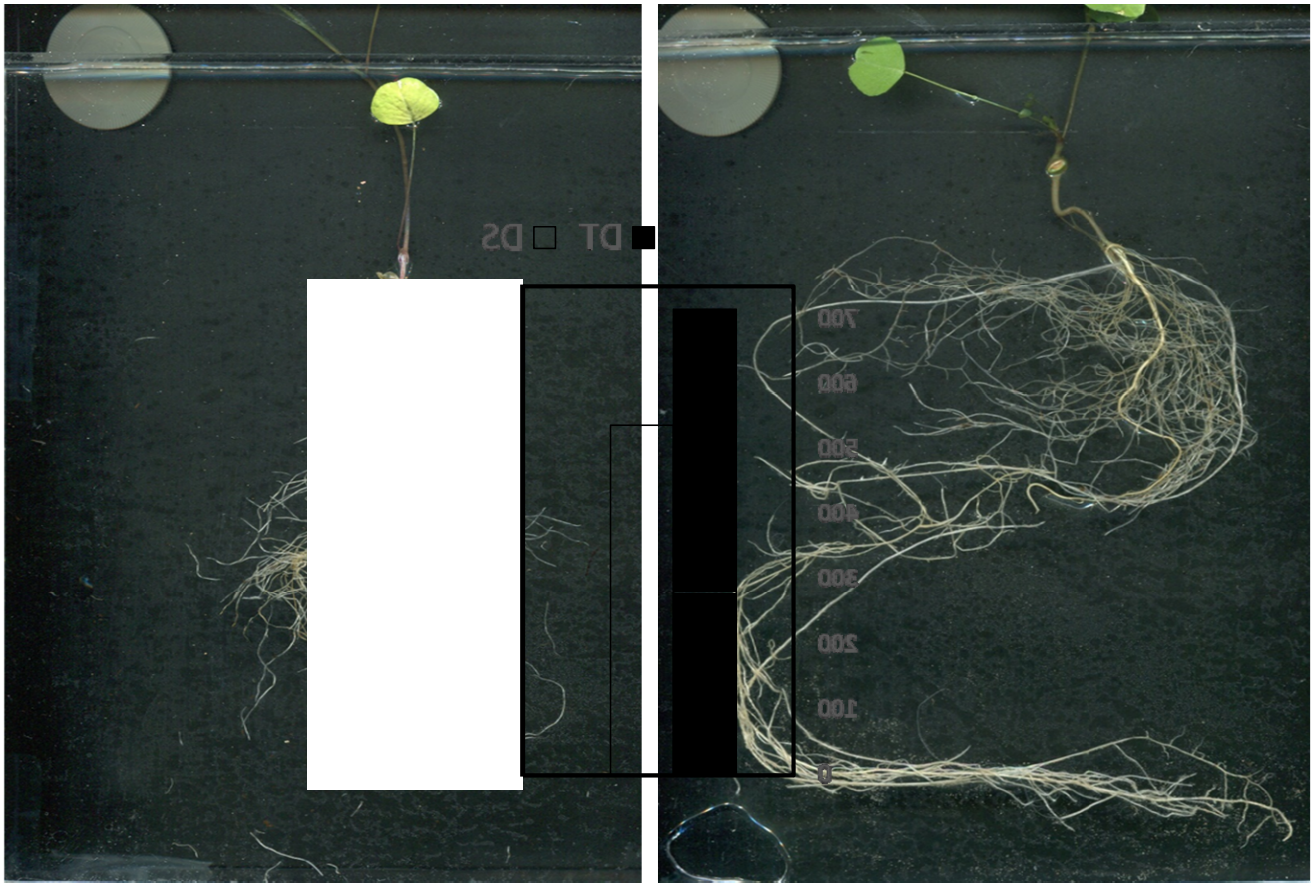
Differences in Root System in Tolerant and Sensitive Progenies



Drought Sensitive

Drought Tolerant