Overview of Talk

- 'Omics approaches to uncover molecular responses to drought stress in a single genotype of Medicago truncatula
- Comparative approaches to identify key differences between drought tolerant and sensitive genotypes of alfalfa (M. sativa)
- Genome-Wide Association Studies (GWAS) of drought-related traits in M. truncatula

Medicago truncatula drought stress regime and physiological impact

Plants grown for 24 d (watered daily) before water withholding for 14 d (D14D) and re-watering for 1 d.

Effects of drought on leaf water potential ($\Psi_w$) and relative water content (RWC)

Zhang et al. (2014) Plant Cell Environ. 37, 2553–2576

Global transcriptional responses to drought in shoots and roots

Zhang et al. (2014) Plant Cell Environ. 37, 2553–2576
Massive, progressive changes in gene expression in shoots and roots in response to drought

Drought responsive transcription factor genes in Medicago truncatula

Shoots and Roots

Key: Red = induced, green = repressed, relative to watered control (D2W)

Zhang et al. (2014) Plant Cell Environ. 37, 2553–2576

Drought leads to accumulation of specific solutes in shoots and roots

Proline and pinitol increase in Medicago roots and shoots in response to drought

Zhang et al. (2014) Plant Cell Environ. 37, 2553–2576

Summary

- Transcriptome analysis of roots and shoots from control, mildly, moderately and severely stressed, and rewated plants, identified many thousands of genes, including hundreds of transcription factor genes, which were altered in expression in response to drought.
  - Metabolite profiling of drought-stressed plants revealed the presence of 135 polar and 165 non-polar compounds in roots and shoots. Combining Medicago metabolomic data with transcriptomic data yielded insight into the regulation of metabolic pathways operating under drought stress.
  - Among the metabolites detected in drought-stressed Medicago plants, proline, pinitol, and myo-inositol had striking regulatory profiles indicating involvement in Medicago drought tolerance.
- Given the complexity of the transcriptional responses to drought, it is difficult to identify key genes/alleles that could be targeted for translational approaches to enhance drought tolerance in legume species.

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Alfalfa: a global forage legume and major US crop

2008 data

Of the nine historically recognized alfalfa germplasm resources in North America, Wisfal (M. sativa ssp. falcata var. Wisfal) has the highest WUE and Chilean (M. sativa ssp. sativa var. Chilean) amongst the lowest WUE.

Wisfal has faster root growth following drought stress

Wisfal is better able to “stay green” under drought stress

Wisfal and Chilean cultivars have contrasting water use efficiency (WUE)

Wisfal has lower stomatal density and lower leaf conductance under well-watered conditions

Transcriptome responses to drought are qualitatively similar in Chilean and Wisfal

HCA analysis of gene expression changes (total 10984)
Wisfal exhibited higher expression of genes for flavonoid (and other) metabolism in shoots after 11 days drought

Concentrations of (iso)flavonoids are higher in Wisfal than Chilean shoots and roots after drought

Summary of comparative physiology/genomics in alfalfa

• Lower leaf gas conductance (lower stomatal density) but similar photosynthesis rate under control and drought conditions probably contribute to higher WUE in Wisfal compared to Chilean
• Wisfal leaves maintain chlorophyll better than Chilean under drought stress
• Chilean and Wisfal transcriptome responses to drought are qualitatively similar, with some interesting quantitative differences
• Chilean and Wisfal metabolome responses to drought are qualitatively similar. However, RSOs and salicylic acid are higher and may play more important roles in Wisfal than in Chilean. Additionally, Wisfal shoots accumulate more (iso)flavonoids than Chilean during drought, pointing to stronger anti-oxidative capacity in the former.

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Introduction to the Medicago HapMap project

• HapMap = HaplotypeMap
• Genetic variants are often inherited together in segments of DNA called haplotypes.
• 384 inbred lines of Medicago truncatula are being sequenced (262 available) to at least 5X coverage. 30 lines deep sequenced with 20X coverage.
• SNP information for lines are available to public (http://www.medicagohapmap.org/)
Genome-wide association study (GWAS) of drought stress related traits in *M. truncatula*

- Screen for drought stress related and biomass traits of *Medicago truncatula* ecotype collections.
- Map phenotypic data to the HapMap (genetic map of SNPs), identify those SNPs or regions of SNPs that are correlated with each trait.
- Identify and validate key genes in these regions.

**Drought-related and biomass traits characterized**

- Shoot dry weight (DW)
- Maximum leaf size
- Leaf specific weight (dry weight/area)
- Stomatal density
- Trichome density
- In vitro dehydration stress - PEG treatment

**Phenotyping:**

Plants were well-watered

Samples were taken when plants were 21-day old

Three replicates, 6 plants each replicate. Randomized complete block design.

**Manhattan plots of all drought-related traits**

**Selected genes identified in the drought GWAS study**

- TASSEL software used for analysis
- Mixed linear model (MLM) was used to predict phenotype/genotype correlation
- Total # of SNPs: > 2 million; SNP Density: one SNP/120-150 nucleotides
Summary

• Eight drought related and biomass traits were characterized on 220 or 84 M. truncatula inbred lines.
• For each trait, multiple significant marker-trait association chromosome regions in high linkage disequilibrium were identified.
• Potential causative genes associated with each trait were selected and are under further investigation, using a Tnt1-insertion mutant population of M. truncatula for reverse genetics.

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