BISC 367 - Plant Physiology Lab
Spring 2009

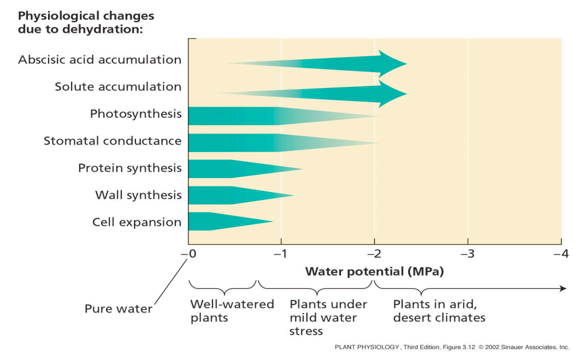
Notices:

- qPCR calculation due Thursday (March 19th)
- Lecture quiz next week (Tuesday March 24th)

- **Reading material:**
  - Cuming AC et al., (2007) New Phytologist 176:275-287
  - Wong et al., (2006) Plant Physiology 140:1437-1450
  - Bray EA (2004) Journal of Experimental Botany 55:2331-2341

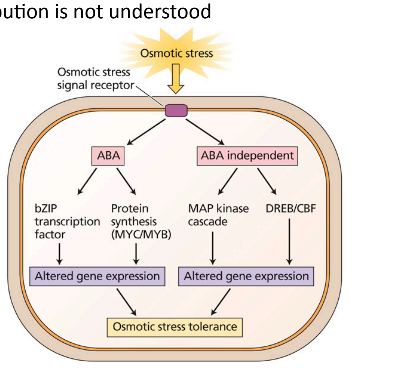
Water Deficit Stress

- Responses to water deficit stress



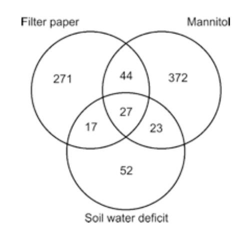
Water Deficit Stress

- Most responses to water deficit stress are underpinned by global changes in gene expression
  - ABA is the most important signal that coordinates the expression of many, but not all, genes
    - ABA-dependent and ABA-independent pathways are proposed to regulate gene expression in water-defict-stressed plants
    - How drought is perceived and translated into a change in ABA level/ distribution is not understood



Water Deficit Stress

- Microarray analyses of global changes in gene expression in drought-stressed plants has:
  - Expanded the list of known stress-responsive genes
  - Illustrated the extent of the overlap in gene expression between different environmental stresses
    - Reflects shared responses
      - Oxidative stress
      - Cell / tissue damage / repair
    - BUT response is tailored to specific stress
      - Large number of genes are specific to a given stress
  - Allowed for the identification of co-regulated genes
    - Increases understanding of regulation of stress responses



Venn diagram showing the number of genes induced by 3 abiotic stresses. 27 genes were induced by all 3 stresses. From Bray (2004) J. Exp Bot. 55:2331-2341.

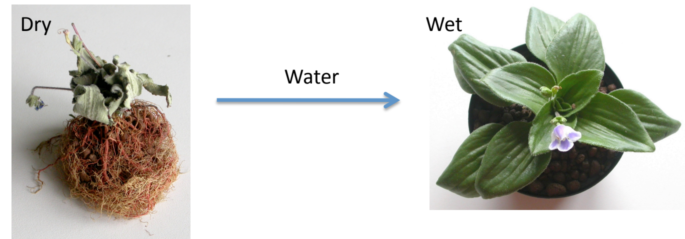
Water Deficit Stress

- The plant kingdom is derived from a single terrestrial colonization event ~ 480 million yrs ago
  - First plants had to survive in an environment with sporadic water supply and lacked:
    - Complex root system
    - Vascular tissues
    - Stomates
    - Cuticle
    - Lignin
  - These plants had physiological and biochemical adaptations to cope with drought
  - The Bryophytes are living plants that most resemble the first land plants and many are desiccation tolerance
    - Also known as anhydrobiotic survival


Drought-tolerant *Tortula ruralis* (moss)

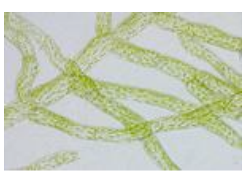
Water Deficit Stress

- Very, very few higher plants are *desiccation tolerant*
  - Most of these plants are desiccation avoiders
  - Maintain tissue hydration and "avoid" exposure to stress
  - Anhydrobiotic capability appears to have been "repressed"
  - Desiccation tolerance is restricted to reproductive propagules
    - Seeds
    - Pollen
    - Spores
- A few higher plants are desiccation tolerant
  - Desiccation tolerance has been sporadically regained during plant evolutionary history



Craterostigma plantagineum is an example of a desiccation tolerant angiosperm
Image from: http://ds9.botanik.uni-bonn.de/bartels/arbeitsgebiet.html

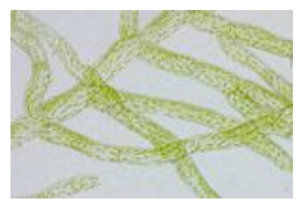
Drought stress response in *Physcomitrella patens*

Physcomitrella patens gametophytes (above) and protonema (below)

- In desiccation tolerant *Tortula ruralis* anhydrobiosis:
  - depends on repair-associated genes expressed during rehydration
    - Therefore, desiccation tolerance may be constitutive
- Desiccation tolerance is not universal among Bryophytes
- *Physcomitrella patens* is a genetic model for the Bryophytes
  - Genomic resources & microarray technologies exist
- *P. Patens* was used in a study to ask: "how has anhydrobiotic ability been suppressed in higher plant lineages"?

Drought stress response in *Physcomitrella patens*



- Experimental treatments performed on protonema
- Treatments included:
  - ABA (10^{-5} M)
  - Mannitol (10% - osmoticum)
  - NaCl (0.3 M)
  - Dehydration (air)

Drought stress response in *Physcomitrella patens*

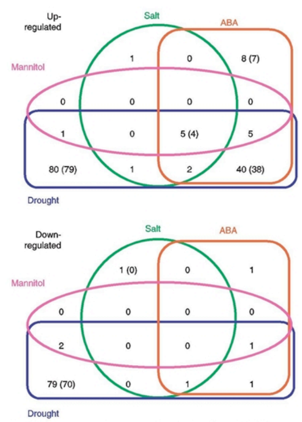
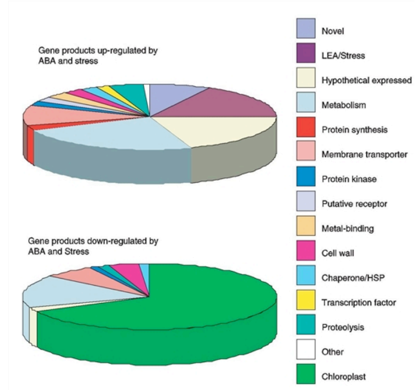


Fig. 3 Genes up- and down-regulated by abscisic acid (ABA) and stress treatments. Transcripts exhibiting significant changes (> twofold) in abundance following 2 h treatment with 10^{-5} M ABA (ABA), 2 h incubation on 10% mannitol (mannitol), 2 h incubation on 0.3 M NaCl (salt) and dehydration to 84% fresh weight loss (drought). Numbers in brackets indicate the numbers of genes, where some chip features correspond to different sequences within the same transcript.

- What did they find?
  - The expression of:
    - Many genes was responsive to drought stress
    - Many genes was responsive to ABA
    - Only a few genes was responsive to salt stress or an osmotic stress
  - Chloroplast associated genes were down-regulated
  - Many genes that were drought responsive were also responsive to ABA
    - Included genes associated with the acquisition of desiccation tolerance e.g. *LEA* (Late embryogenesis abundant) genes

Drought stress response in *Physcomitrella patens*



- The molecular response of *P. patens* to drought resembles the seed development program of higher plants for desiccation tolerance
  - *P. patens* can tolerate complete desiccation if it is pre-treated with ABA
- *P. patens* is intermediate in drought tolerance between *Tortula* and higher plants
  - *P. patens* may have retained some desiccation tolerance
  - Higher plants have lost desiccation tolerance
  - In higher plants the genes for desiccation tolerance appear to have been "developmentally sequestered" to the reproductive life cycle stages

Drought "tolerance" in higher plants

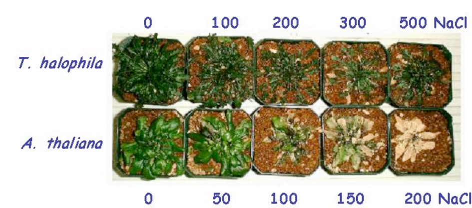
- Many studies compare drought tolerant and drought susceptible plants to understand how plants tolerate drought
- *Thellungiella salsuginea* is a model ecotype for molecular studies on abiotic stress tolerance
  - Closely related to Arabidopsis
    - Share many features e.g. short life cycle, small genome, easy to transform etc.
  - Native to harsh environments
    - 2 ecotypes have been studied
    - Shandong ecotype from high salinity coastal areas of China
    - Yukon ecotype from the Takhini salt flats nr. Whitehorse, Yukon Territories
  - Stress tolerant
    - Tolerates 500 mM NaCl
    - Desiccation to lose >40% F. wt.

Thellungiella halophila
ASPB

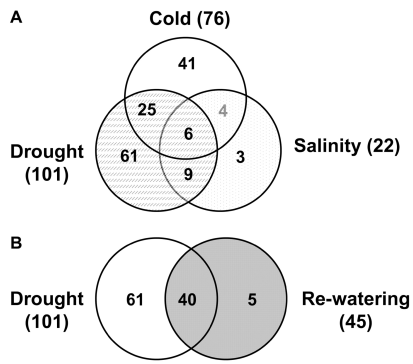
Drought "tolerance" in higher plants

- Arabidopsis cDNA microarrays have been used to study the molecular response of *Thellungiella* to drought
  - Only 6 genes were differentially expressed in salt-stressed *Thellungiella* (Shandong)
  - Attributed to a constitutive high level of stress-responsive gene expressive in *Thellungiella* compared to Arabidopsis
  - Suggests that stress tolerance occurs because stress-responsive genes are constitutively expressed in *Thellungiella*
- Work with the Yukon ecotype
  - Made a "boutique" array with 3,628 cDNAs from stressed plants
    - Enriched for stress-responsive genes

T. Halophila can tolerate 500 mM NaCl. Arabidopsis cannot survive 200 mM NaCl



Drought "tolerance" in higher plants



Wong, C. E., et al. Plant Physiol. 2006;140:1437-1450

- Used array to look at molecular response to drought, cold, salinity and re-watering

- What did they find?
  - Most genes responded to drought or cold treatments
  - Very few genes responded to salinity
    - *Thellungiella* is good at discriminating against sodium in favour of potassium
  - Very few genes were shared between stresses
    - Indicates that response is tailored to specific stress condition
  - Most down-regulated genes were P/S genes