BISC 367 - Plant Physiology Lab
Spring 2009

Notices:

- **Due date for the 2nd report is March 12**

- **Reading material:**

  1. **Negi et al., (2008) Ethylene regulates lateral root formation and auxin transport in Arabidopsis thaliana. Plant J. 55: 175-187.**

Root Data - observations

- *etr1* mutant
  - Avg root length control WT = 36.7 mm
  - Avg root length salt WT = 27.4 mm
    - Salt reduced growth by 25%
  - Avg root length control *etr1* = 34.3 mm (1 plate had short roots – deleted)
  - Avg root length salt *etr1* = 25.8 mm
    - Salt reduced growth by 25%

- *ein2* mutant
  - Avg root length control WT = 37.1 mm
  - Avg root length salt WT = 34.7 mm
    - Salt reduced growth by 6%
  - Avg root length control *ein2* = 36.5 mm
  - Avg root length salt *ein2* = 32.4 mm
    - Salt reduced growth by 11%
  - **Looks like WT – don't include in analyses**

Root Data

- *aba1* mutant
  - Avg root length control WT = 35 mm
  - Avg root length salt WT = 35 mm
    - Salt had not effect on growth
  - Avg root length control *aba1* = 14.7 mm
  - Avg root length salt *aba1* = 16 mm
    - Salt had no effect on growth

- *abi1* mutant
  - Avg root length control WT = 33.61 mm
  - Avg root length salt WT = 33.1 mm
    - Salt had no effect on root growth
  - Avg root length control *abi1* = 31.2 mm
  - Avg root length salt *abi1* = 21.2 mm
    - Salt reduced growth by 32%

Root Data

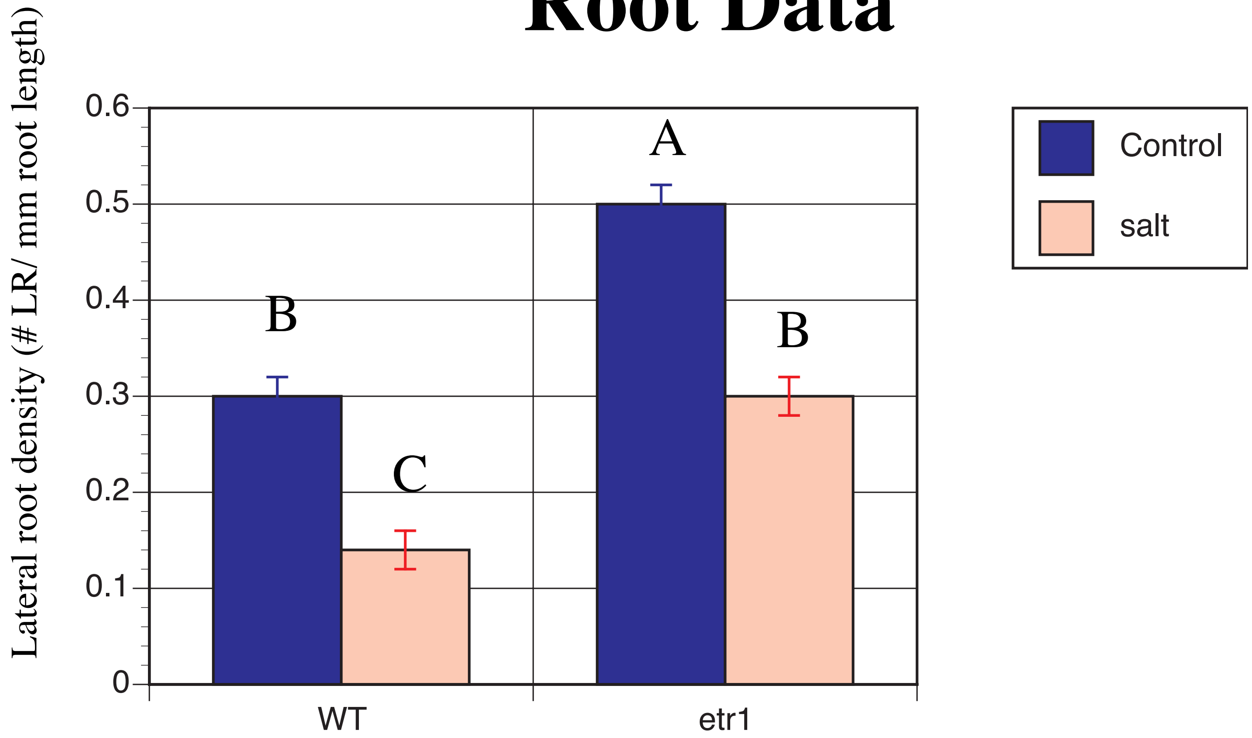
- Data crunching……..
  - On excel spreadsheet delete seedlings with roots that are < 50% of the mean length
    - When you delete a seedling, delete all data for that seedling (i.e. LR data too)
  - Create spreadsheet for all ABA mutants and another for all ET mutants
    - Combine all WT data and include in both spreadsheets
  - Calculate average root growth and LRD for all WT seedlings
  - Calculate average for root growth and LRD for mutant genotypes
    - Do this on a per plate (replicate) basis as each plate should give similar values
    - Then calculate a mean for all seedlings for both replicate plates
  - Normalize root length and LRD data for mutant genotypes by control
    - Put normalized data in a separate column
  - Organize excel worksheet so that all data are in **separate rows**
  - Save data as .csv

  - Open data in JMP (JMP can be downloaded for free http://www.microstore.sfu.ca/downloads.htm

Root Data

- Analysing data in JMP (statistical analyses package)
  1. On the data sheet everything except the data columns are “nominal”
     1. Data columns are “continuous”
  2. Select menu “Analyse”
     1. Select “Fit Model”
  3. In “Fit Model” window select “Root length” from under “Select Columns” and hit button “Y”
  4. Select “Treatment” and “Genotype” under “Select Columns” and then the “Macros” button under “Construct model Effects”
     1. When the menu drops select “Factorial to Degree”. Ensure that “2” is entered next to degree
  5. Hit “Run Model”
  6. In analyses window “Genotype*Treatment” use “least sq means” and “std error” to construct data graphs
  7. Hit the down arrow to the left of “Genotype*Treatment” and select “student’s t-test” to note the statistical classes

Root Data



- Complete data analyses for your normalized data too
  - Select “normalized by control” as “Y” (step 3 on previous slide)

Root Data

- Data for paper:
  - Plot 1: absolute root length data for ET mutants vs WT
  - Plot 2: normalized root length data for ET mutants vs WT
  - Plot 3: absolute root length data for ABA mutants vs WT
  - Plot 4: normalized root length data for ABA mutants vs WT

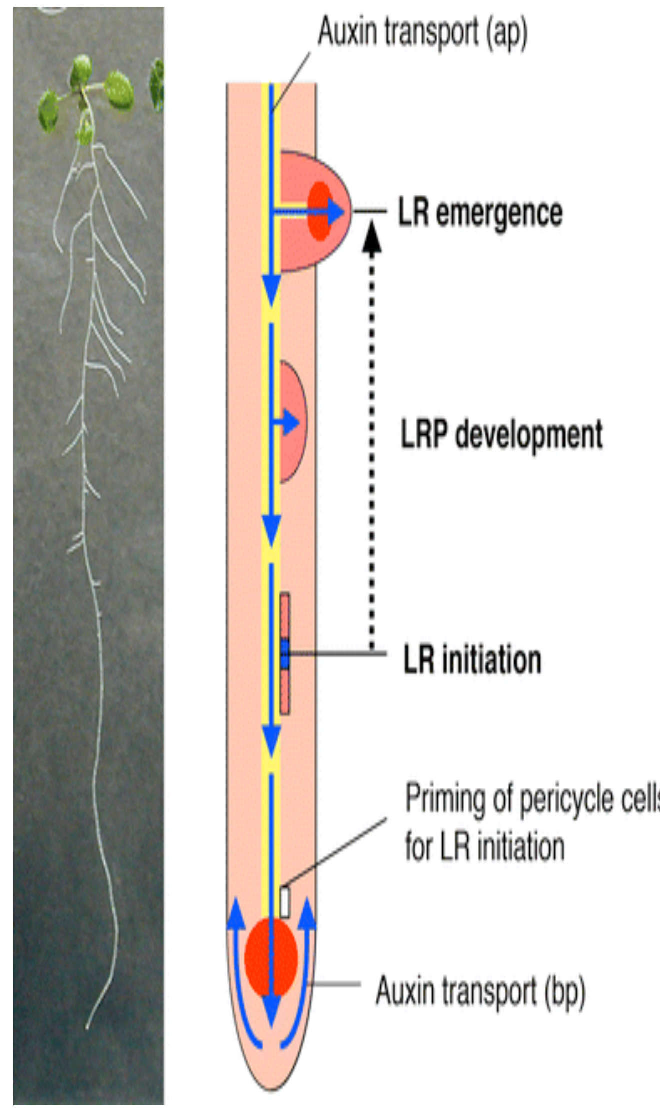
  - Plot 5: absolute LRD for ET mutants vs WT
  - Plot 6: normalized LRD for ET mutants vs WT
  - Plot 7: absolute LRD for ABA mutants vs WT
  - Plot 8: normalized LRD for ABA mutants vs WT

Hormone regulation of root system architecture

- Lateral root (LR) formation is also influenced by hormones:
  - Auxin
  - Ethylene
  - ABA
  - Cytokinins
    - Interactions among hormones (synthesis, signaling, transport etc) complicate analyses

LRs arise from pericycle founder cells adjacent to xylem poles

- Auxin moving acropetally stimulates pericycle founder cell division and LR primordium initiation
- LR site position is determined in a region between the meristem and elongation zone



Fukaki and Tasaka (2009) Plant Mol. Biol. 69: 437-449

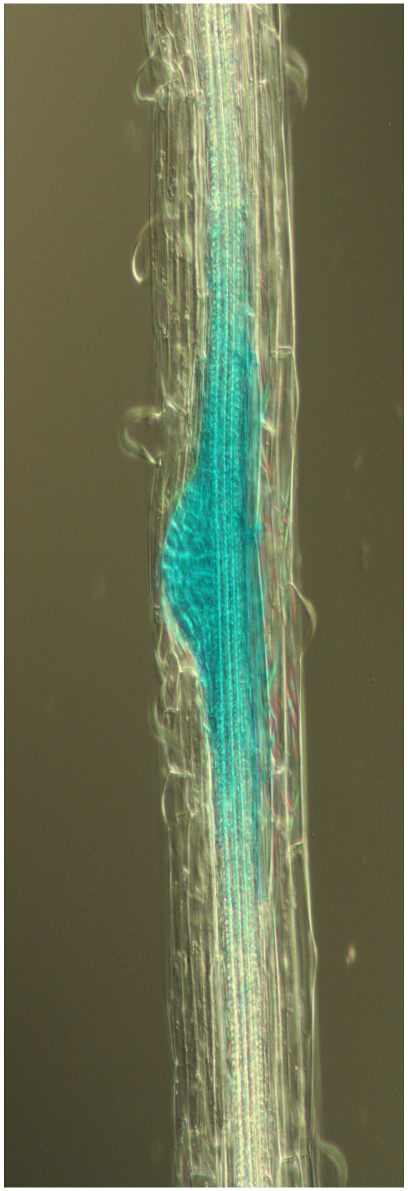
Hormonal regulation of LR formation

- Auxin is an important regulator of LR formation
  - Auxin transport, both acropetal and basipetal, is important for LR formation
  - Auxin affects LR initiation, primordium development and LR emergence

- Cytokinins are negative regulators of LR formation
  - Cytokinis affect the pericycle cells and block the developmental program of LR formation

- ABA is a negative regulator of LR formation
  - Exogenous ABA inhibits LR emergence prior to activation of the LR meristem
  - Auxin and ABA are believed to interact during LR development
  - ABA mediates the ability of nitrate to inhibit LR development

- ET stimulates adventitious root formation but represses LR formation
  - Increased ET synthesis and enhanced ET signaling reduce LR formation
  - Decreased ET signaling increases LR formation
  - ET affects auxin synthesis, transport and signaling and may influence different aspects of LR formation



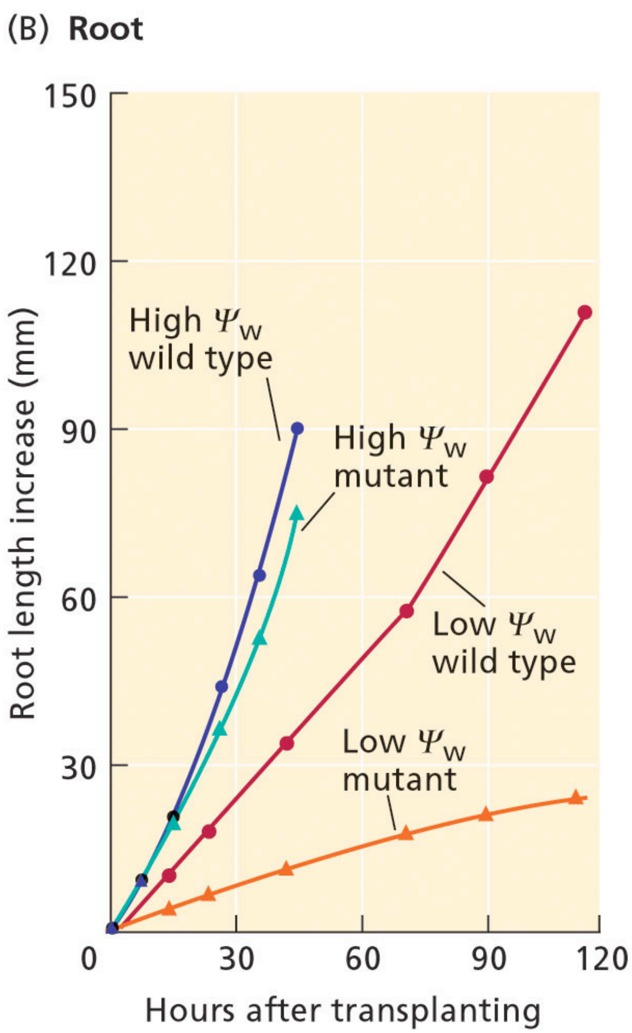
DR5-GUS expression in developing LR

Root system architecture is plastic and influenced by the environment

- In response to drought stress root elongation is maintained & LR formation is suppressed
  - This favours growth towards new soil environments
  - When roots are in better soil environment root proliferation (branching) occurs
    - LR primordia are present but their emergence has been suppressed

- ABA accumulates in the roots of drought-stressed plants and plays a role in maintaining growth.

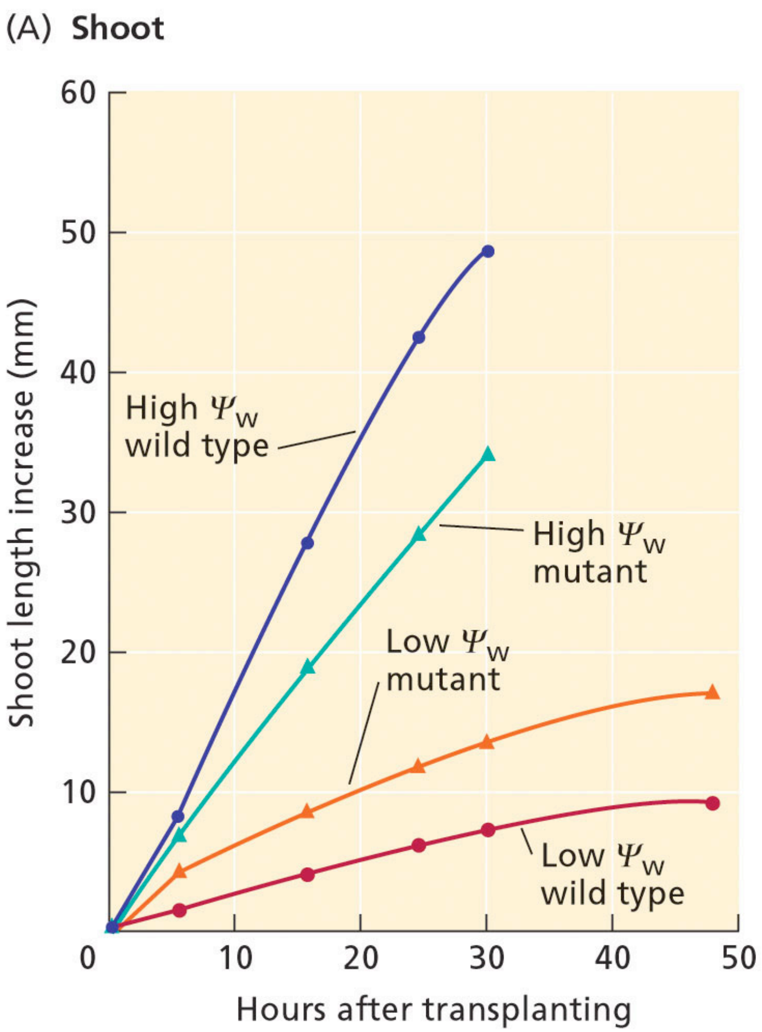
ABA maintains root growth in water deficit stressed seedlings



- Wild type and ABA-deficient seedlings were transferred to a low or high Ψ_w growing medium
  - Growth was measured

- **In roots:**
- At low Ψ_w the ABA-deficient mutant roots **grew less** than WT roots
  - ABA is involved in maintaining root growth at low Ψ_w
  - ABA promotes osmotic adjustment in the root meristem
    - Maintains cell division and expansion
    - Changes in the cell wall (loosening) aid growth

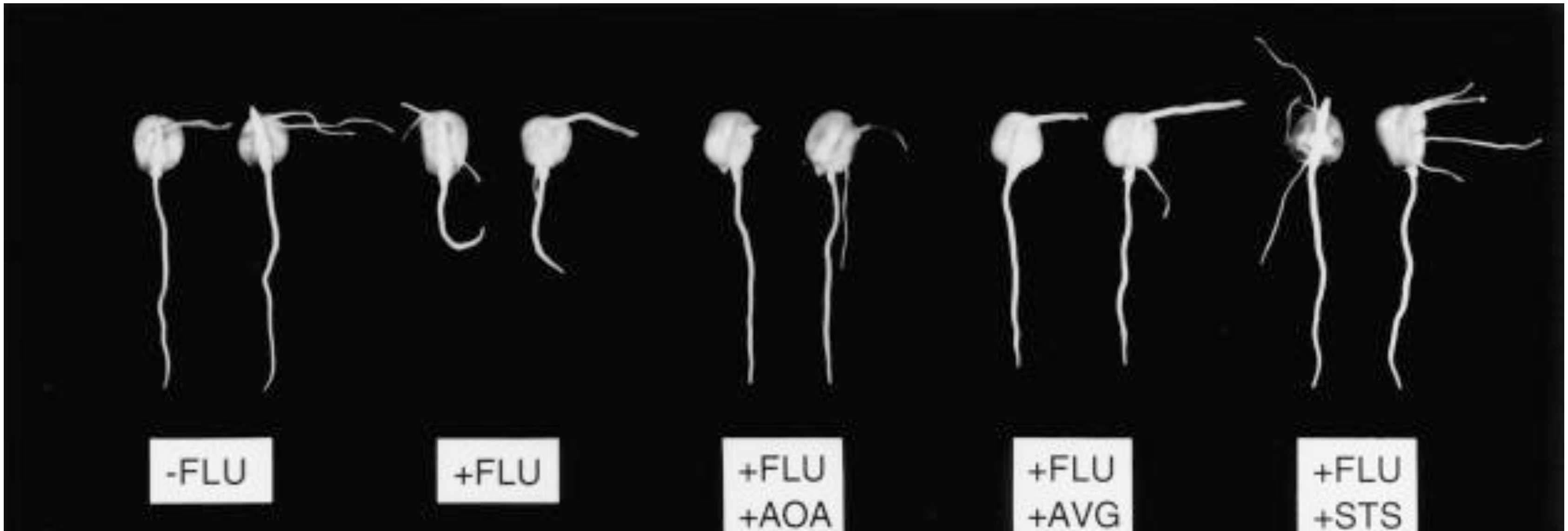
ABA reduces shoot growth in water deficit stressed seedlings



- Recall that in the shoot ABA inhibits growth:
- The shoot of ABA-deficient seedlings **grew more** under low Ψ_w than WT seedlings
  - ABA inhibits shoot growth in low Ψ_w plants
- Adaptive significance:
  - Increases root:shoot ratio in water deficit-stressed plants
  - Increases vol. of soil that can be explored for water uptake

ABA maintains root growth by suppressing ethylene production

Maize seedlings growing at low Ψ_w



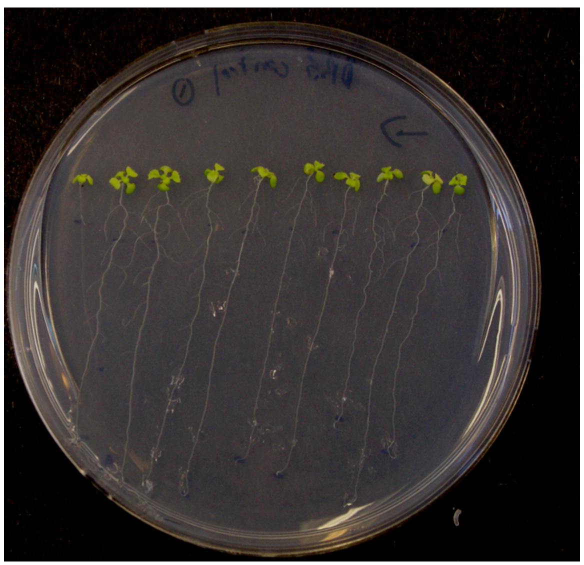
From Spollen *et al*., Plant Phys. **122**:967 (2000)

- FLU (fluridone) is an inhibitor of ABA biosynthesis
- AOA, AVG & STS are inhibitors of ethylene action or biosynthesis
- Inhibiting ethylene production or action in ABA-deficient seedlings restored growth in water deficit stressed seedlings
  - ABA suppresses ethylene production and thereby maintains root growth

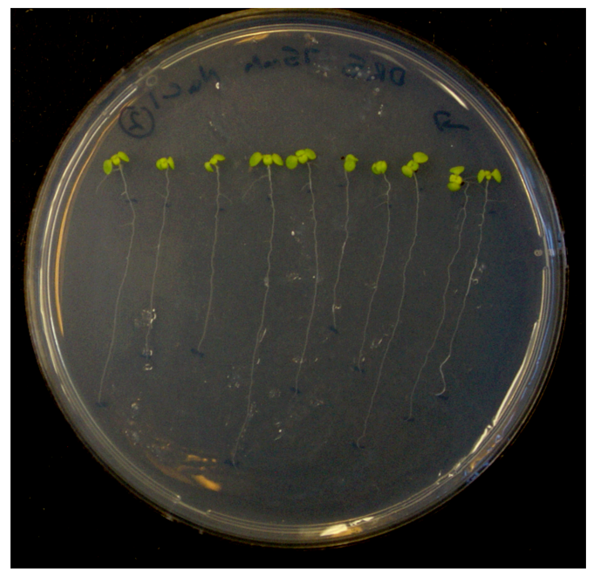
Root system architecture is plastic and influenced by the environment

LR formation is suppressed in drought stressed roots because:

- Their elongation growth is reduced
- Primordia are formed but they don’t grow out or develop into LRs
- The repression of LR formation is dependent on ABA
  - May involve ABA checkpoints that operate during LR development



Non-stressed seedlings



Salt-stressed seedlings

Root system architecture is plastic and influenced by the environment

- Root system architecture is also affected by nitrate
  - High concentrations of nitrate repress LR development
    - Arrest of LR development is relieved by lowering the nitrate level
    - Nitrate is thought to be directly responsible for the repression of LRs
  - ABA is implicated in the repression of LRs by nitrate
    - ABA insensitive mutants are less sensitive to the ability of nitrate to repress LRs
    - Some debate as to whether the repression of LRs by nitrate is an osmotic stress response

Hydrotropism

- Refers to the response of roots to a moisture gradient
  - Roots grow towards moisture (higher Ψ_w)
  - Widespread response in higher plants, relatively little is known

- Pathway interacts with gravitropism and involves the roots cap as the sensor or moisture
  - relatively little is known about hydrotropism and the interaction with gravitropism appears to vary by plant species
  - Hydrotropism is a directional growth response and auxin is definitely involved.

    - Recent review: Takahashi and Miyazawa, (2009) Plant Molecular Biology 69: 489-502.