Hormonal Regulation of Root Growth

Plants produce extensive root systems, which are dynamic and designed to maximize water and nutrient uptake from the soil environment. The dynamic nature of root systems is necessary because roots experience fluctuating and unpredictable environments due to the heterogeneous nature of the soil environment in which they grow. Frequent changes in root system architecture maintain an optimal root to soil interaction and are accomplished via alterations in the initiation and growth of lateral roots and changes in the rate of root elongation. Root systems typically balance the extent of branching and elongation growth so as to optimize water and mineral uptake and promote growth into new soil territories.

Root elongation and the initiation of lateral roots and their growth are governed by hormones including auxin, ethylene (ET), abscisic acid (ABA), and cytokinins. In addition, interactions between these hormones influence root growth and development. For example, ABA is a general inhibitor of root growth and this is mediated by the ET signaling pathway. As a result, ET-insensitive mutants have reduced sensitivity to ABA-induced root growth inhibition (Beaudoin et al., 2000). ET also inhibits root growth and root bending (gravitropic growth) via the accumulation of auxin and the inhibition of auxin transport (references in Beaudoin et al., 2000). As a result, auxin accumulates and root growth is inhibited. Furthermore, cytokinin is a negative regulator of root growth and inhibits root growth by stimulating ET synthesis (references in Beaudoin et al., 2000).

Root growth and root system architecture are affected by soil conditions that impose an osmotic stress on roots. Reduced water potential (Ψ_w) of the root growing medium decreases elongation growth and the number and length of lateral roots (Deak and Malamy, 2005). However, under these circumstances root growth is maintained relative to growth of the shoot (which is reduced), which enhances the plants ability to seek out new water resources. Maintenance of root growth at low Ψ_w is achieved by ABA that accumulates in the root tip of water-deficit-stressed roots. The essential role that ABA plays is to suppress ET accumulation and / or action and so avoid ET-mediated growth inhibition (Sharp and LeNoble, 2002). As a result, the roots of ABA-deficient plants exhibit greater growth inhibition relative to wild-type at low Ψ_w and produce higher levels of ET and reactive oxygen species, both of which contribute to growth inhibition. ABA also inhibits lateral root formation resulting in a reduced number of lateral roots on roots of water-deficit-stressed seedlings (Deak and Malamy, 2005). The overall effect of these interactions is to change the architecture of the root system in water-deficit-stressed plants whereby roots are thinner with resources dedicated to maintaining root elongation via the restriction of lateral cell expansion and lateral root production.

During this lab you will examine the influence of ET and ABA on the regulation of root system architecture during seedling growth under normal and salt-stressed conditions. To achieve this you will:

a) Use *Arabidopsis thaliana* mutants that are affected in their ability to synthesize or respond to either ET or ABA

b) Measure the growth of hormone mutant and wild-type seedling roots growing under normal and salt-stressed conditions

c) Count the number of lateral roots produced on hormone mutant and wild-type seedling roots in the presence and absence of salt stress.

This experiment will allow you to assess the involvement of hormones in orchestrating changes in root system architecture not only in response to abiotic stress but under optimal growing conditions.

METHODOLOGY:

Material:

1. Arabidopsis mutant (*aba1-3*, *abi1*, *ein2*, *etr1-1*) and wild-type germinated seeds. The seeds were surface-sterilized, plated onto MS agar plates, and moist-chilled (stratified) for four days before germination.
2. MS plates with 1.5% sucrose and 0.8% agar.
3. MS plates with 1.5% sucrose, 0.8% agar, and 75 mM NaCl
4. Computer running NIH Image (freeware program)
5. Forceps, 70% ethanol, autoclaved toothpicks.
6. Light bank with racks that can accommodate Petri plates
7. Laminar flow hood
8. Surgical tape
9. Fine marker pens
10. Digital camera
11. Plastic rulers marked with 1 mm divisions

Plating surface sterilized seeds onto MS agar plates

1. Working as part of a group select one set of Arabidopsis mutant and wild-type plates with germinated seeds for your experiment.
2. Take 4 MS plates and 4 MS + NaCl plates. Label 2 of the MS plates WT (wild-type) and the other 2 with the name of your mutant genotype. Do the same for the MS + NaCL plates. You will plate wild-type germinated seeds onto your WT control plates (MS) and salt stress plates (MS + NaCl) and mutant seeds onto the control and salt-stress plates labelled with the name of your mutant genotypes.
3. Draw a horizontal line near the top of each agar plate (draw on the underside). This line is a guide for placing the seeds when you plate.
4. Working in a clean laminar flow hood, wet the tip of a toothpick by pressing it to the corner of the agar media and use it to remove some Arabidopsis seeds. Place the seeds in a row on the agar media (10 seeds per plate, evenly spaced, use your black line as a guide). Use a new toothpick for each plate to reduce the chance of

contamination. By the time you are done you should have two plates per treatment per genotype.

5. After plating the seeds on Petri dishes, replace the cover and seal with surgical tape to prevent desiccation. Use a fine marker to draw a short horizontal line at the level of the root tip for each seedling.
6. Place dishes vertically in the racks under the light bank in the lab.
7. **Next week** you will:
    a. measure root length using a ruler measure and record the length of the root. You will measure the distance from the horizontal line you drew to the end of the root tip.
    b. Count the number of lateral roots.
    c. Use the digital camera to obtain an image for each of your plates
    d. Import images onto the computer. We will use NIH Image to gain a more accurate measure of root length.

Analysing your root growth data

1. You have measurements for 20 seedlings (10 seedlings on 2 replicate plates) for each treatment for each genotype. Assemble your data into an Excel spreadsheet (see below). In this spreadsheet you will calculate the mean root length for the control treatments (see "Length (mean)" column below) and use this mean to normalize root growth for each seedling on the treatment plates (see "normal length by control" column below).
2. Assemble a similar spreadsheet for your lateral root data.
3. Save your excel spreadsheet as a comma delimited (.csv) file.
4. Open JMP software and then open your excel file. JMP is available to students at the SFU microcomputer store and you can download it for free so that you can complete your analyses in your own time.
5. In JMP ensure that only your data columns contain continuous data. All other columns should contain "nominal" data. You will analyse the normalized data (these are the data in "normal length by control") as well as the non-normalized data ("root length").
6. Select "Analyze" then "Fit Model". Set the "normal length by control" (or "root length") as "Y". To analyse select "Treatment" and "Genotype" and the Macro "Factorial to degree" (make sure that 2 degrees are entered in the "Degree" box). Hit "Run Model". This in one example of how these data can be analyzed, we will go through some other ways during the lab. *Can you think of some other ways to normalize these data?*
7. Plot the data +/- standard error and report those data that are statistically significant.

Date	Genotype	Treatment	Plate #	Seedling #	Root length (mm)	Length (mean)	normal length b control
19-Sep	wildtype	control	1	1	45	53.35714286	100.00
19-Sep	wildtype	control	1	2	50		100.00
19-Sep	wildtype	control	1	3	56		100.00
19-Sep	wildtype	control	1	4	48		100.00
19-Sep	wildtype	control	1	5	53		100.00
19-Sep	wildtype	control	1	6	49		100.00
19-Sep	wildtype	control	2	4	53		100.00
19-Sep	wildtype	control	2	5	62		100.00
19-Sep	wildtype	control	2	6	65		100.00
19-Sep	wildtype	control	2	7	33		100.00
19-Sep	wildtype	control	2	8	59		100.00
19-Sep	wildtype	control	2	9	59		100.00
19-Sep	wildtype	control	2	10	57		100.00
19-Sep	wildtype	control	2	11	58		100.00
19-Sep	wildtype	salt	1	1	31		58.10
19-Sep	wildtype	salt	1	2	43		80.59
19-Sep	wildtype	salt	1	3	32		59.97
19-Sep	wildtype	salt	1	4	36		67.47
19-Sep	wildtype	salt	1	5	40		74.97
19-Sep	wildtype	salt	1	6	37		69.34
19-Sep	wildtype	salt	2	8	28		52.48
19-Sep	wildtype	salt	2	9	36		67.47
19-Sep	wildtype	salt	2	10	44		82.46
19-Sep	wildtype	salt	2	11	29		54.35

Here are some things to think about:

1. Did salt reduce growth?
2. Did salt reduce growth equally for all genotypes?
3. Do the roots of the different mutants grow equally in the absence (or presence) of salt stress?
4. What predictions can you make as to the response of root growth to salt for the wild-type and mutant genotypes? Use these predictions to state your hypotheses.
5. Did salt alter the number of lateral roots?
6. Was lateral root number affected by the genotype?
7. What is the overall effect of salt on the root system of the Arabidopsis seedlings?

References:

Beaudoin N, Serizet C, Gosti F and Giraudet G (2000) Interactions between abscisic acid and ethylene signalling cascades. Plant Cell 12: 1103-1115.

Deak KI and Malamy J (2005) Osmotiv regulation of root system architecture. Plant J. 43: 17-28.

Sharp RE and LeNoble ME (2002) ABA, ethylene and the control of shoot and root growth under water stress. J. Exp.Bot. 53: 33-37.