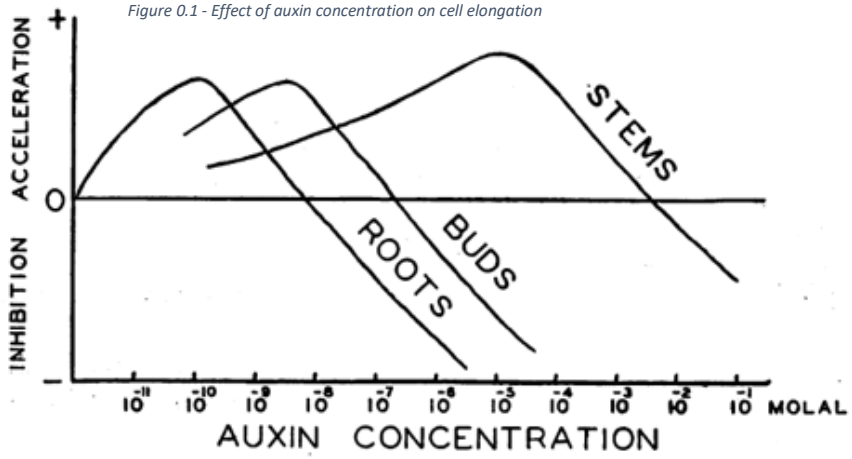
Practical 2 – The role of auxin in root development

In this practical we will test the effects of auxin on root growth.

We will use the seeds of the DR5::GUS line to monitor the effects of auxin application on auxin signalling and correlate it to the root phenotypes. We will use two concentrations of NAA, Naphthalene 1-acetic acid, a synthetic auxin. Depending its concentration, auxin may have opposite effects: inhibiting cell elongation at high concentration, favoring cell elongation at low concentration (Fig. 6.1). In this practical we will germinate seeds on 0.1 uM and 10 pM NAA.

Commented [HRB1]: We need to test the concentrations

Figure 0.1 - Effect of auxin concentration on cell elongation



1.1. Materials

1.1.1. Seeds

Seeds were sterilized for you by chlorine gas method
DR5::GUS

1.1.2. Material

Sterile 50 ml tube
Petri dishes
Micropore tape
Aluminum foil
Stereomicroscope
Toothpicks
Marker pen
Tubes for the seeds
Well plates
Gloves
Pipettes (1000, 200, 10, 2ul)

37°C incubator
scanner
slides and cover-slides
microscope

1.1.3. Solutions

1/2 MS
10 mM NAA stock solution
GUS staining (solutions are prepared)
GUS wash buffer
50 mM $NaPO_4$ pH7 (stock of 500 mM)
5 mM Ferro-cyanide ($K_4Fe(CN)_6$) (stock of 50 mM)
GUS staining buffer
50 mM $NaPO_4$ pH7 (stock of 500 mM)
5 mM Ferro-cyanide ($K_4Fe(CN)_6$) (stock of 50 mM)
0.05% Triton X-100
X-Gluc added in the last moment in the required volume at 0.5 mg/ml final (stock 20 mg/ml in DMSO)
20% lactic acid in 20% glycerol (in PBS)

1.2. Experiment

1.2.1. Timetable

This experiment is designed to take ±fifteen days. We will start the experiment on Monday 9th, November and then move the petri dishes from cold treatment on Thursday 12th. The seeds will grow for 11 days. Phenotype will be scored at Day After Germination (DAG) 4, 6, 8 and 11. Seedlings of each treatment will be collected and stained for GUS on November 23rd. Microscopic observations will be on Monday 30th November and analysis of the results on Monday 7th December.

1.2.2. Sowing

All this work is done in sterile conditions under flow benches in room 1S26. You will have to calculate the volume of NAA to add for one plate for the given final concentrations. You will be provided with 200 mL of 1/2MS and will have to prepare 3 plates: control (no NAA added), high NAA (100nM), low NAA (100pM). One plate contains 50mL of medium.

- Calculate the volume of NAA to add for 50mL MS from a stock solution of 10mM for a final concentration of 100nM and 100pM. Propose how to have an accurate pipetting with the given stock solution.
- Label your plates with the treatment (control, 100pM NAA, 100nM NAA), the name of the seeds that will be sown (DR5::GUS), your name and the date
- You may draw one line with the marker pen at the back of the plate
- Work under sterile flow, using the 50mL-tube, pour MS into the control plate, then the low concentration NAA plate (by adding the proper volume of NAA into 50 mL MS in the falcon tube), and then the high concentration NAA plate
- Leave the plate open under the flow for ± 30 minutes to solidify
- Take a toothpick and wet its tip by tapping it in the corner of the plate.

- Using the wet tip of the toothpick pick up one seed at a time from the tube
- Place 20 seeds for each genotype on the Petri dishes, following the line you draw. Separate nicely the seeds along the line.
- Place the lid on the Petri dishes and wrap the edges of each Petri dish with Micropore tape completely sealing them shut.
- Wrap their stack completely with aluminum foil so that no light reaches the seeds.
- Label the wrapped Petri dishes using marker tape, with your name and date.
- Put the wrapped Petri dishes in the fridge (room 1S16) at 4°C-6°C. This process is called stratification (or vernalization). Stratification is important for breaking seed dormancy and synchronizing the germination of the seeds. Be extremely careful when preparing, transferring or working on Petri dishes with seeds. **Rough handling can cause seeds to shift from their intended position and ruin the experiment.**
- All Petri dishes should be kept in cold treatment until Saturday 9th, November.

1.2.3. **Data collection**

- Scan the plates to follow the growth of the root at DAG = 4, 6 , 8 and 11.
- The scans can be used to analyze the length of the roots, and roughly quantify emergence of the lateral roots.

1.2.4. **GUS staining**

- Prepare a 12-well plate marked with your name, and in the lid, the name of the treatment in the corresponding well
- Fill each well (=3) with 2 ml GUS wash buffer
- Collect ±10 seedlings per treatment per well
- Apply vacuum for 5 minutes
- Remove GUS wash buffer and replace by 1 mL of GUS staining buffer.
- Apply vacuum for 5 minutes
- Close the lid and wrap it in aluminum
- Incubate at 37°C for 30 minutes. Monitor if the staining is enough. You may incubate longer if necessary
- Remove the GUS staining buffer and replace by 2 ml of lactic acid solution
- Leave on the table to clear
- Mount on slides (1 slide per line per treatment)
- We will be looking at the microscope and take pictures. The manipulation of the microscope will be performed under supervision following the instructions of the teacher.