

2026-06-10 Progress Report + Meeting with Professor Kyle

☒ Buy required materials for the experiment

1. 13 cm x 13 cm square petri dish
2. Square petri dish rack
3. Place the square petri dish (50 mL medium per dish) and keep the lid open at approximately a 45° angle and dry the agar for about 10 minutes.



4. ½ Murashige and Skoog (MS) without sucrose and agar
5. MES sodium salt (0.5g/1L)
6. Plastic sheet

☒ Adjust ½ Murashige and Skoog (MS) recipe

1. Adjust the sucrose contain (original 3% to 1%)
2. Adjust the pH to 5.7-5.8 (Use KOH and NEVER use NaOH!)

☒ Identifying developmental phases in the Arabidopsis

1. Reference paper

Boyces, D. C., Zayed, A. M., Ascenzi, R., McCaskill, A. J., Hoffman, N. E., Davis, K. R., & Görlach, J.

(2001). Growth Stage–Based Phenotypic Analysis of Arabidopsis. *The Plant Cell*, 13(7),

1499–1510. <https://doi.org/10.1105/tpc.010011>

☒ Point out some problems

- ☒ Arabidopsis not healthy (Increasing the light intensity)
- ☐ Strong airflow in growth chamber (cover with plastic sheet to maintain humidity)
- ☒ Planting soil suggestion (Using 泥炭土 or Jiffy-7 peat pellets: 無肥另給花寶2號)-花園城堡can buy everything gardening materials



泥炭土

Jiffy-7 peat pellets

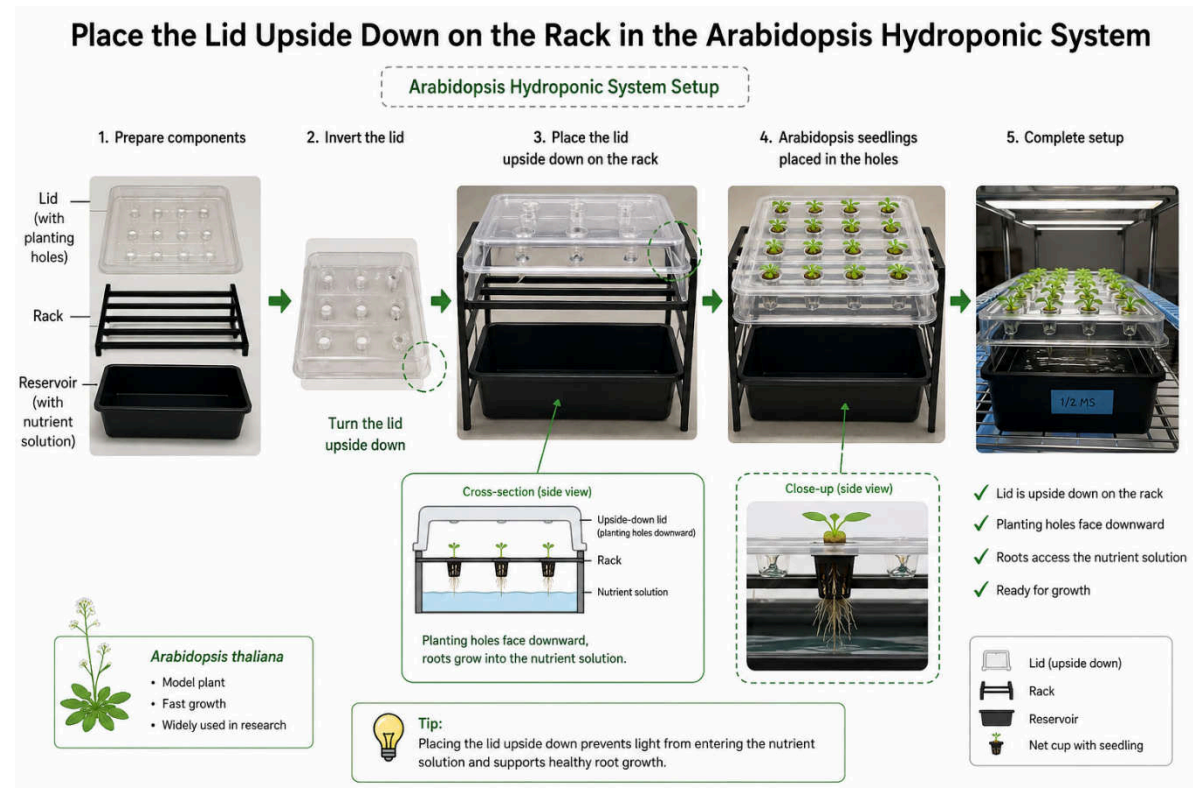
☐ 澆水乾溼分明

☒ **About our hydroponic system provide some suggestion**

Our original ideas

1. Use a tube rack to germinate seeds.
2. Use seedlings instead of direct seed germination in our hydroponic system. (About 4-7 days)

New ideas:



☒ **Provide experimental guidance**

1. Pouring agar plate
2. Swon seeding on vertical agar plate
3. Seedling transfer methods
4. Measurement fresh weight
5. Chlorophyll Extraction
6. Take pictures of agar plates

☐ **Check the experimental protocols**

Toniutti, L., Breitler, J., Guittin, C., Doulebeau, S., Etienne, H., Campa, C., Lambot, C., Pinilla, J. H., &

Bertrand, B. (2019). An Altered Circadian Clock Coupled with a Higher Photosynthesis

Efficiency Could Explain the Better Agronomic Performance of a New Coffee Clone When

Compared with a Standard Variety. *International Journal of Molecular Sciences*, 20(3), 736.

<https://doi.org/10.3390/ijms20030736>

• **Measurement Fresh weight**

1. Tare a 1.5 mL microcentrifuge tube on the precision balance.
2. Transfer 10 Arabidopsis seedlings (see different stages of plant to decide the pooling number) into the tube.
3. Measure and record the fresh weight, then label the tube accordingly.

- **Chlorophyll Extraction**

1. Add three glass beads to each 1.5ml microcentrifuge tube. (316 or 304 Stainless steel ball or handheld grinding gun)
2. Freeze plant samples at -80°C .
3. Add 1 mL of 80% acetone to each tube. (Buy Eppendorf tubes with tight-fitting lids to decrease acetone leakage)
4. Vortex thoroughly until chlorophyll is completely extracted from the leaves. The leaves will change from green to white.
5. Centrifuge the samples to pellet the plant debris.
6. Transfer the supernatant to a 96-well plate in biological and technical triplicates for absorbance measurement.
7. Measurement OD645 and OD663

$$(20.2 \times A_{645} + 8.02 \times A_{663}) \times V / (1000 \times \text{freshweight})$$

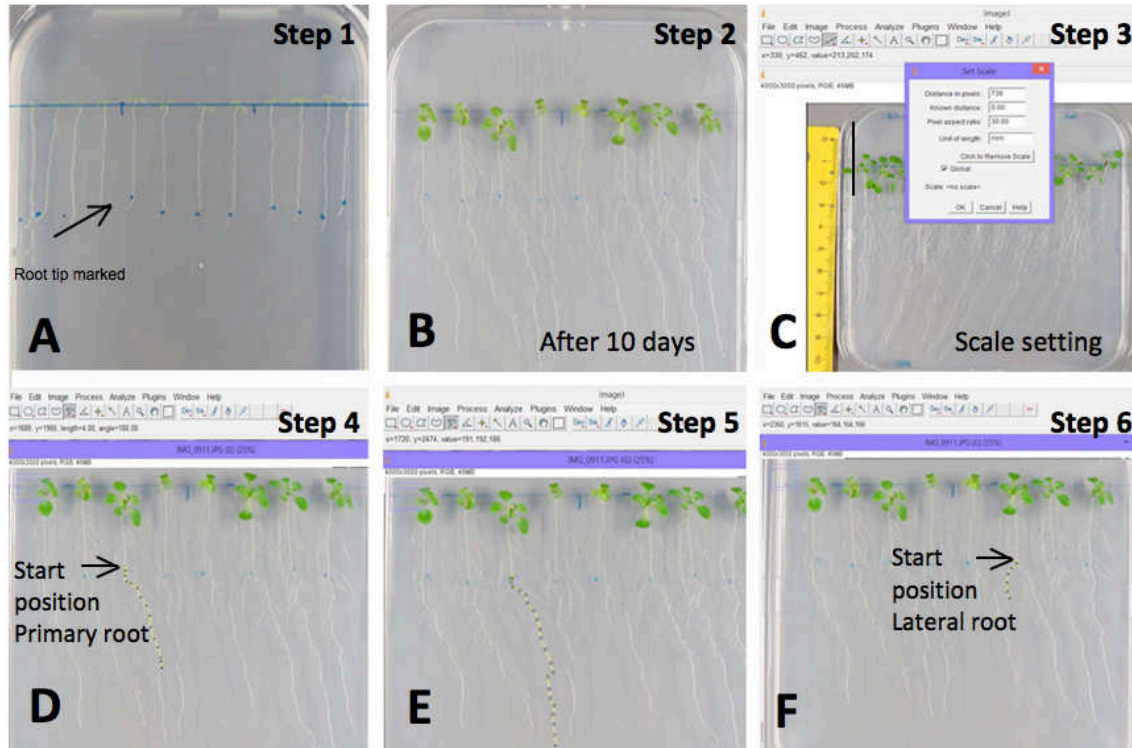
- **Future works might need the help**

1. Measurement root length or lateral root (measure total number or length)
2. or using Smartroot

Lobet, G. (n.d.). *SmartRoot*. <https://smartroot.github.io/>

(related protocol

Corrales, A., Carrillo, L., Nebauer, S., Renau-Morata, B., Sánchez-Perales, M., Fernández-Nohales, P., Marqués, J., Granell, A., Pollmann, S., Vicente-Carbajosa, J., Molina, R., & Medina, J. (2014). Salinity assay in Arabidopsis. *BIO-PROTOCOL*, 4(16).
<https://doi.org/10.21769/bioprotoc.1216>)

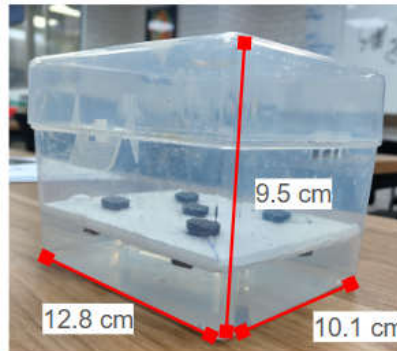


3. Start stress test experiments (salinity and heat) -> Observe the phenotypes.
4. Any questions during the experiment.

2026-06-17 Progress Report + Meeting with Professor Kyle

Plant growth

1. Hydroponic system establishment (Aim: Test our protectants)
 - About the design: We have four prototypes now.These two prototypes will be used in our following experiments.

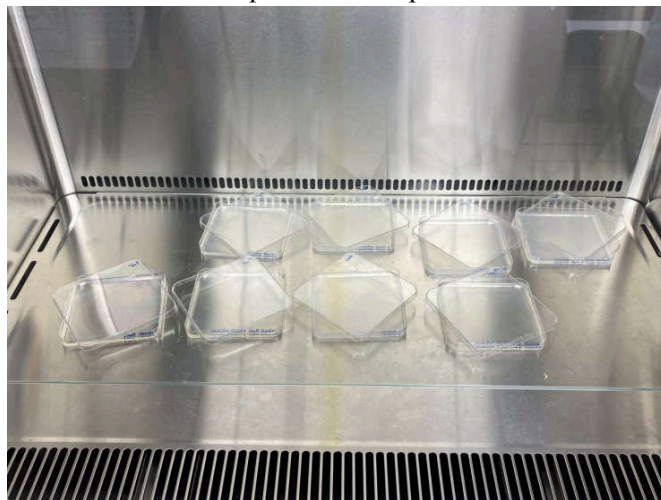


- Plant stage used in the experiment : We decide to use 4–7-day-old seedlings and transfer them directly to a hydroponic box.
 - Future work: Add protectants in the hydroponic system
2. Salinity stress condition test (Aim: Test different conditions to determine the optimal rescue concentration of trehalose)
 - Trehalose concentration: 0, 0.25, 0.5, 1mM
 - NaCl concentration: 100mM
 - Need for a time-course experiment?

No, we can keep testing the single conditions until trehalose (as our positive control) shows a rescue effect.

What are the actual experimental groups we need to include?

- (1) Use 13cm x 13 cm square vertical plate



↑ This is how we dry agar plates after pouring ↑

- (2) $\frac{1}{2}$ MS agar plate $\rightarrow$ 50ml/ (with 1% sucrose, MES buffer and 1% agar)

- (3) We decide transfer 18-20 seedling on agar plate (9-10 seedling per row)
- (4) Plant stage: 4–7-day-old seedlings
- (5) Experiment readout: Root length, Fresh weight, Chlorophyll content

4. Study how to induce heat stress on papers

Summarize information after meeting

1. Test germination rate under salinity stress
 - ☐ Should be in round plate: Sow at least 50 seeds in one plates
 - ☐ Stratification seeds directly on condition plates (Standardize stratification at 2 days)

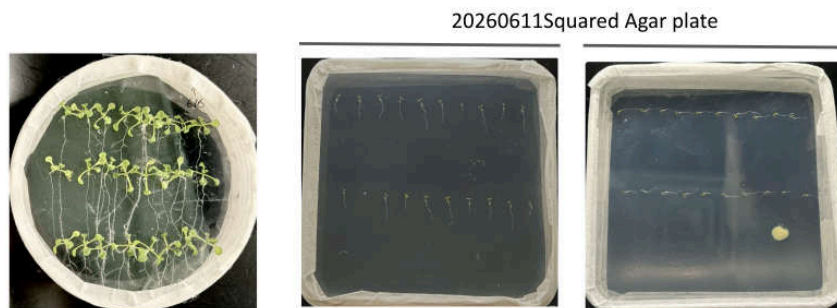
Suggestions on arabidopsis planting

- don't use 1% of MS medium for horizontal planting, it will be too thick → around 0.7-0.8% (reminder)

Observation

- if we are to see germination rate → we should plant more seeds per plates such as 50 seedlings, more seedlings more easier to organize
- if we are to see the trehalose rescue effectiveness → after germinating, look at physical quantitative measures like plant/root length or seed growth size

Day 4 (6/17) - Vertical planting

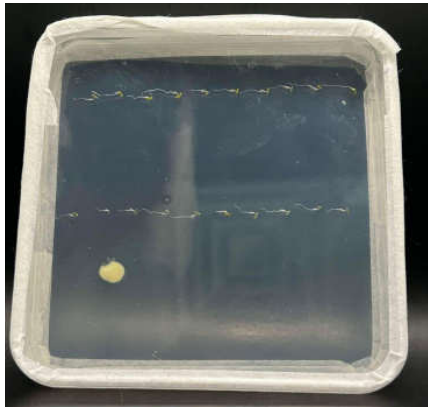


- 20260611 stratified 0614 to growth chamber Vertical Squared and Round Agar plate: the plant lacks light - the root is too thin - the tape causes some shading → needs to add light tube



-
- At a slight angle at around 10°C
- use 0.5mM as the trehalose concentration and test on different stresses
- Bleached seeds will stick together
- for bacterial contamination: might caused by original seeds contamination → can use chlorine disinfection

Incident (wrong direction of root growth):



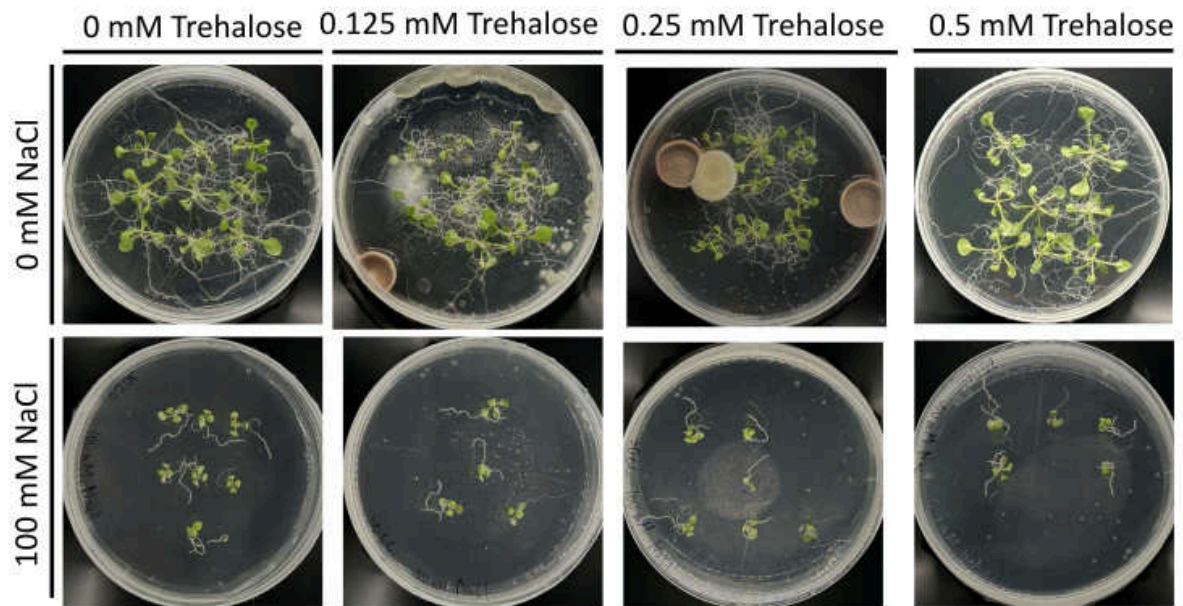
Improvement:

1. Double check before placing plates back on the rack in the growth chamber: Plates need to be placed vertically in the correct direction where roots grow down

plate contamination

- every plates need to be labeled → prevent putting sideways
- fungi and bacteria contamination

Day 14 (6/12) - Black background



- 20260530 growth chamber: the second plate has contamination over the plate edges - Dr. Kyle suggested that it might be water that bring bacteria / when taking out the agar plates we put our hands into the plastic bag, therefore causing contamination
- future improvement: when taking out agar plates, push through the plastic bag don't direct contact, and always seal the plastic bag when not using (take it out one by one)
- When there's contamination on agar plates during growth observation period, don't open the lid again because spores would fly out and diffuse to everywhere
- future improvement: label clearly or tape the lid to prevent any bacteria flying out
- seed

Box B can be implied and keep on using
auto-filling

- if medium evaporates, don't refill the medium → would make the concentration too high because only water evaporates, and the salt/sugar content don't
- Soil needs to be moist before transferring plant to soil

Hydroponics box contamination

- Box A: top part contaminated - dried medium(salt)

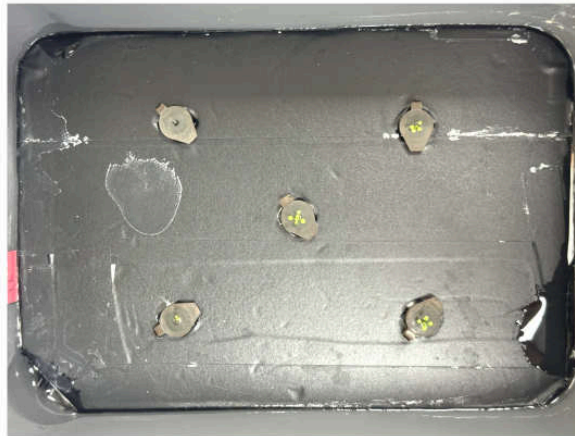
prototype 1 (A-1): 6/14

Observed Germination: 4

Any problems: yes

Observed problems:

- contamination



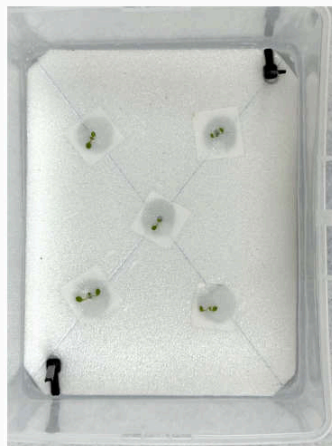
- Box B: not contaminated

prototype 4 (B-1) : 6/14

Day 0: 2026/6/7

Observed Germination: 5

Any Problems?: No (changed water)



- add water: use spray application(mist form)

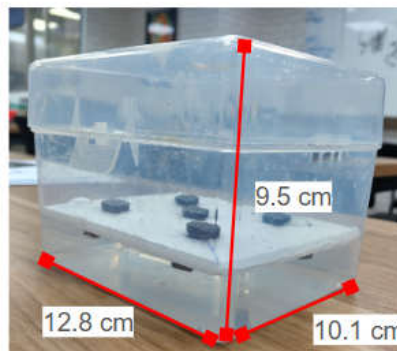
2026-06-24 Progress Report + Meeting with Professor Kyle

Overall Progress:

1. Conducting experimental set 4 - vertical plantings(6/21 transferred)
 - a. Salinity test trial: 0, 100mM NaCl and 0.25, 0.5, 1.0 mM Trehalose
 - b. Tested root length(endpoints of roots start from the same base line)
2. 20260611 square plate stratification, 0613 in growth chamber
3. 20260615 stratified 0617 in growth chamber squared agar plates

Planned trials:

1. Different NaCl concentrations (50mM, 100mM, 150mM) on 0.5mM trehalose
 2. Different NaCl concentrations on different trehalose concentrations for math modeling purposes(connected with dry lab)-> concentrations still discussing
-
1. Plant growth stage observation (Aim: Establish more stable plant cultivation techniques)
 - Current Status: (1) Record each stage of plant growth
(2) Try our best to avoid contamination on agar plates
(3) Practicing seedling transfer techniques
(4) Soil-grown Arabidopsis
 2. Hydroponic system establishment (Aim: Test our protectants)
 - About the design: We have four prototypes now.
These two prototypes will be used in our following experiments.

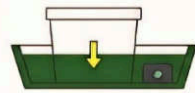


After meeting with Prof. Chen, Wen-Liang (June 20)

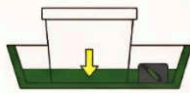
He recommend developing Recirculating(closed loop) Hydroponic System

Autopot system (patent)

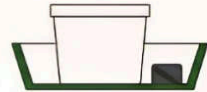
自主潮汐：Smart Valve 的智慧核心



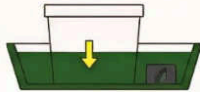
1. 智能閥 (Smart Valve) 將淺盤注水至約 3 cm 的淺水位。



2. 閥門關閉，等待植物將水分完全吸收或蒸散。



3. 當水位降至 0 cm (完全用盡) 時，閥門才會再次打開。



4. 自動補充營養液，使水位重回 3 cm。



The Result

這個過程創造了一個自然的 乾→濕→乾→濕 循環，完美模擬植物水份吸收的自然“潮汐機制”，為根部提供絕佳的空氣與養分交換條件。

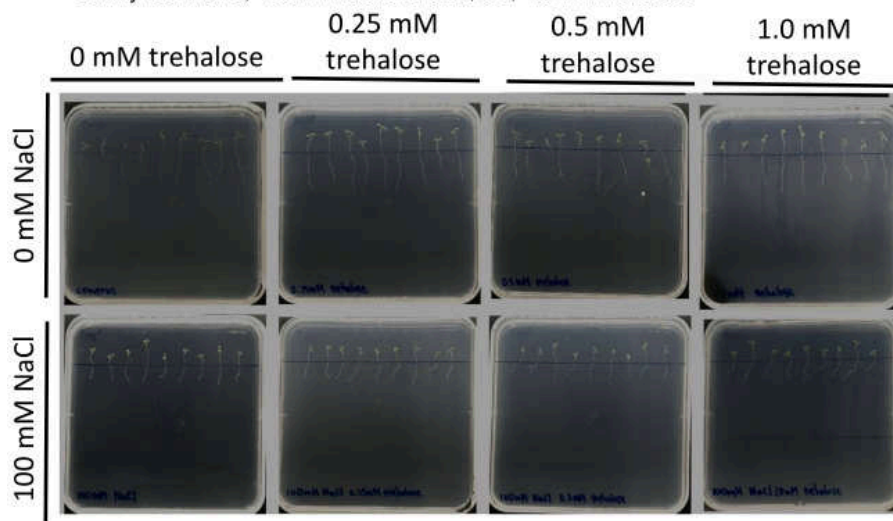
星芝國際農業科技有限公司. (n.d.). <https://www.cosmosis.com.tw/>

- Plant stage used in the experiment : We decide to use 4–7-day-old seedlings and transfer them directly to a hydroponic box.
 - Future work: Add protectants in the hydroponic system
3. Salinity stress condition test (Aim: Test different conditions to determine the optimal rescue concentration of trehalose)
- **Trial 1** Trehalose concentration: 0, 0.25, 0.5, 1mM
NaCl concentration: 100mM
June 17 Placed in growth chamber
June 21 4 days old seedling transferred onto vertical condition agar plates

experimental set 4 - vertical plantings

6/21 transferred (6/24 record)

Salinity test trial: 0, 100mM NaCl and 0.25, 0.5, 1.0 mM Trehalose



Observations for now

- (1) salinity inhibition is shown, but no obvious trehalose rescue effects.

- (2) plants within 100NaCl plates show root growths toward the left while the control groups show normal downward growth.

Possible reasons for no obvious trehalose rescue effects:

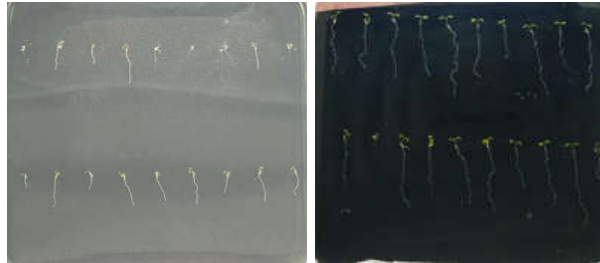
1. Concentration threshold too low: The tested concentrations of trehalose 0.25mM, 0.5mM, and 1.0mM may be insufficient to counteract the severe osmotic and ionic stress exerted by 100mM NaCl increase trehalose concentrations in the next trials?

Possible reasons for skewed growth:

1. Halotropic Response or Uneven Gradients: Salt stress is known to interfere with a plant's normal gravitropic response (growing straight down). The medium might not be mixed well with the NaCl, evaporation created a localized concentration gradient.

Current design

1. Use 13cm x 13 cm square vertical plate
2. $\frac{1}{2}$ MS agar plate-> 50ml/ (with 1% sucrose, MES buffer and 1% agar)
3. We decide transfer 9-10 seedling per row on agar plate
4. Plant stage: 4-day-old seedlings
5. Experiment readout: Root length, Fresh weight, Chlorophyll content
6. Observation days: 10 days



4 days old seedling

7 days old seedling

- 20260611 square plate stratification, 0613 in growth chamber

Day 6 (6/19) - Vertical planting

20260611 Squared Agar plate



Observations for now:

- (1) The root lengths are somewhat inconsistent, which suggests there may be some variation in germination timing.

Suggestions and improvements:

- (1) To have better consistency when doing stressed trials, increase the n value(sample size) of the neutral plates to have enough plants to select to transfer that have similar growth conditions

4. Study how to induce heat stress on papers

- **Test on agar plate**

Plant stage used in the experiment : 7-day-old seedlings

Heat stress condition: Submerge the agar plate containing 7-day-old seedlings in a water bath set at 37°C-> Recover at 22°C for 2hr

Readout: Western blot (Hsp100)

1. What is the recommended number of seedlings required for Western blot analysis?
2. How to do sample pretreatment?

- **Test in hydroponic system**

Plant stage used in the experiment : 7-day-old seedlings

Heat stress condition (short term of heat shock): heat shock at 45 °C for 14min and recover for 4 days

Readout: Chlorophyll content

1. Transplant Arabidopsis into new pot (original soil not enough)
2. Capture Arabidopsis by Camera for higher quality

2026-07-01 Progress Report + Meeting with Professor Kyle

1. Plant growth stage observation (Aim: Establish more stable plant cultivation techniques)
 - Current Status: (1) Record each stage of plant growth
 - (2) Try our best to avoid contamination on agar plates
 - (3) Practicing seedling transfer techniques
 - (4) Soil-grown Arabidopsis

4-week- old of Arabidopsis (0627 record)

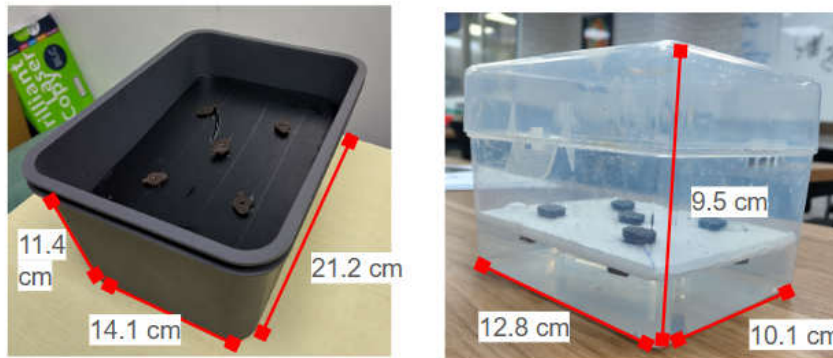


(A) and (B) Transfer to a larger pot



2. Hydroponic system establishment (Aim: Test our protectants)
 - About the design: We have four prototypes now.

2-1 These two prototypes will be used in our following experiments.



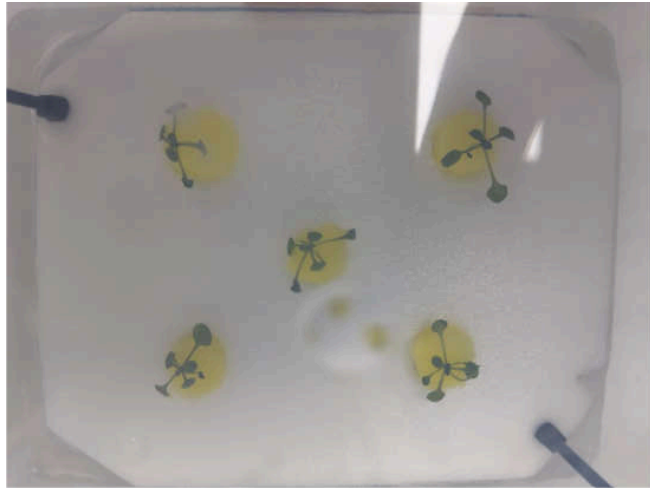
After meeting with Prof. Chen, Wen-Liang (June 20)
He recommend developing Recirculating(closed loop) Hydroponic System
Autopot system (patent)



星芝國際農業科技有限公司. (n.d.). <https://www.cosmosis.com.tw/>

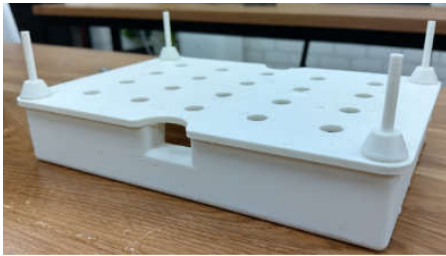
2-2 Plant stage used in the experiment : We decide to use 4–7-day-old seedlings and transfer them directly to a hydroponic box.

- ❖ We tested placing 2-week-old Arabidopsis seedlings with a sponge into the tip box.
- ❖ Difficult to put in because the root length is longer and the static electricity of the foam board makes it more difficult.

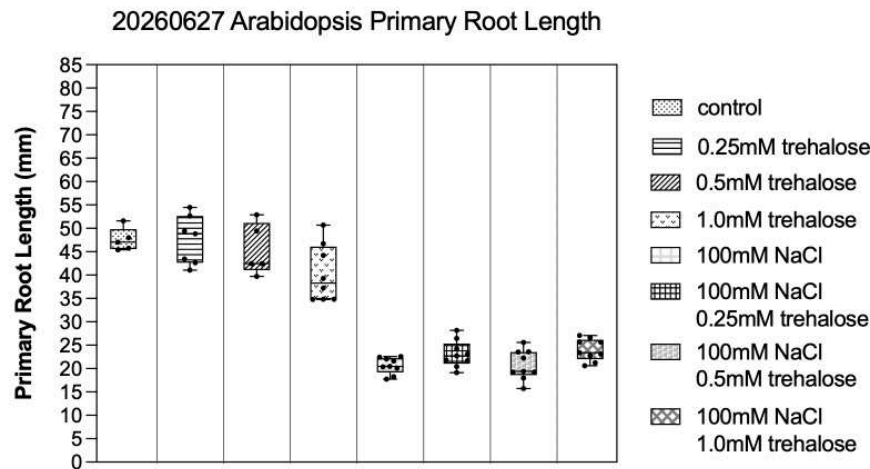


2-3 3D print of our floating board (still updating our initial design)

- (1) Adjust the thickness
- (2) Print an extra one for the tip box



3. Salinity stress condition test (Aim: Test different conditions to determine the optimal rescue concentration of trehalose)
 - ❖ Use 13cm x 13 cm square vertical plate
 - ❖ ½ MS agar plate-> 50ml/ (with 1% sucrose, MES buffer and 1% agar)
 - ❖ We decide transfer 9-10 seedling per row on agar plate
 - ❖ Plant stage: 4-day-old seedlings
 - ❖ Experiment readout: Root length, Fresh weight, Chlorophyll content
 - ❖ Observation days: 10 days
- **Trial 1** Trehalose concentration: 0, 0.25, 0.5, 1mM
 NaCl concentration: 0, 100mM
 June 17 Placed in growth chamber
 June 21: 4-day old seedling transferred onto vertical condition agar plates
 June 27: 6-day observation and ImageJ quantification.



Comments and Feedback:

As the number of days increased, the differences in root length among the various trehalose concentrations (under 100 mM NaCl stress) indeed became more pronounced. However, there is no consistent trend between trehalose concentration and root length. Currently, 0.5 mM trehalose shows no significant effect, whereas 1 mM and 0.25 mM show more notable results. This suggests that, under salt stress, the primary function of trehalose may not be the restoration of root growth.

Existing research on how trehalose enhances salt tolerance in plants primarily focuses on the size of the above-ground parts. Therefore, we should also analyze the fresh weight of the plants to see if any differences emerge.

Suggestions for next time: Measure fresh weight as well!

- **Trial 2**_Trehalose concentration: 0, 1,5,10 mM
NaCl concentration: 0, 100,150, 200mM

4. Study how to induce heat stress on papers

- **Test on agar plate**
 - ❖ Plant stage used in the experiment : 7-day-old seedlings
 - ❖ Heat stress condition: Submerge the agar plate containing 7-day-old seedlings in a water bath set at 37°C-> Recover at 22°C for 2hr
 - ❖ Readout: Western blot (Hsp100)
- Hsp101 antibody is available Western blot analysis?
1. How to do sample pretreatment

- **Test in hydroponic system**

Plant stage used in the experiment : 7-day-old seedlings

Heat stress condition (short term of heat shock): heat shock at 45 °C for 14min and recover for 4 days

Readout: Chlorophyll content

2026-07-08 Progress Report + Meeting with Professor Kyle

Overall Progress:

1. Doing experimental set 5-vertical plantings (20260629 stratified 0703 to growth chamber)
-> transferred on 7/7
 - a. Salinity testing trial: 0, 1, 5, 10mM trehalose vs. 0, 150mM and 250mM NaCl
 - i. Total of 12 groups
 - ii. Purpose
 1. Change the stratification date: 2 -> 4 days
 - a. Better consistency in the seedlings' growth conditions/in sync
 2. Increase the trehalose/NaCl concentration due to limited rescue effectiveness of previous concentrations
 3. More diverse data for dry lab modeling
 - iii. Current status: 1 day after transferring to the salinity plates
2. Finished and concluded experiment set 4: 0, 100mM NaCl and 0.25, 0.5, 1.0 mM Trehalose
 - Did image J analysis + box plot from 6/27 to 6/30
 - Purpose
 - Practice using image j to measure root lengths and organizing quantification data
 - Outcome
 - No obvious rescue effect in 0.5mM trehalose but 1.0 and 0.25mM more obvious
 - The relationship between trehalose concentration and the plants' root lengths is not linear (can't make definite conclusion)
 - Problems faced
 - Molds blocking growth of plants(inaccurate measurement and insufficient growth)-> not reliable(test trial)
3. Arabidopsis picture background setting update

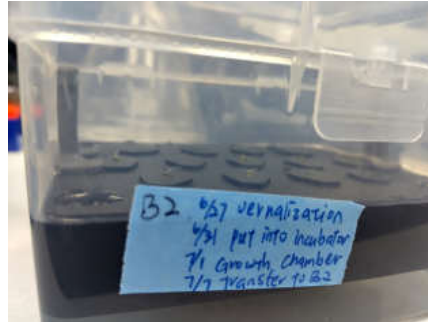
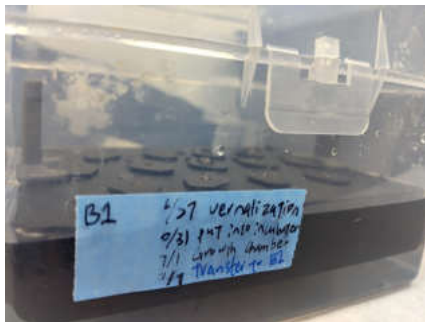


- Purpose
 - To solve the problem of plants hanging in the air or moving places due to horizontal placement while taking photos
 - Higher quality images and consistent placement of plates
- Equipment
 - Camera
 - 3D printed vertical plate holder
 - Slide the plates inside to take photos

- Black background with lights on top
4. Installed light tubes

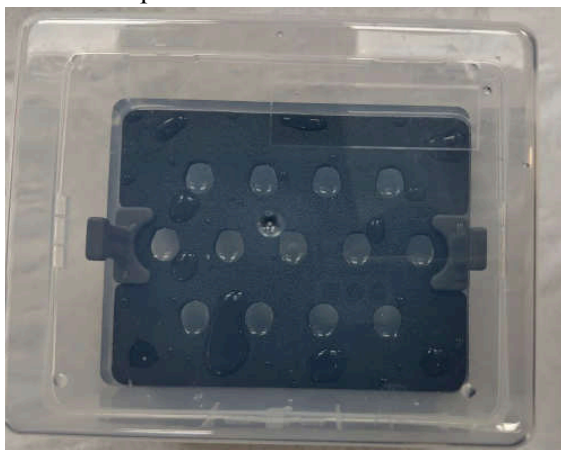


Hydroponics:



- We transferred arabidopsis from Box C into these 2 boxes (Box B).
- The floating board we used is the new 3D printed boards.
- The germination rate of Box C is quite low, and the plants are not growing very well.

-> New 3D printed board



Soil:



- Growth condition well
- No need to cut out main stems

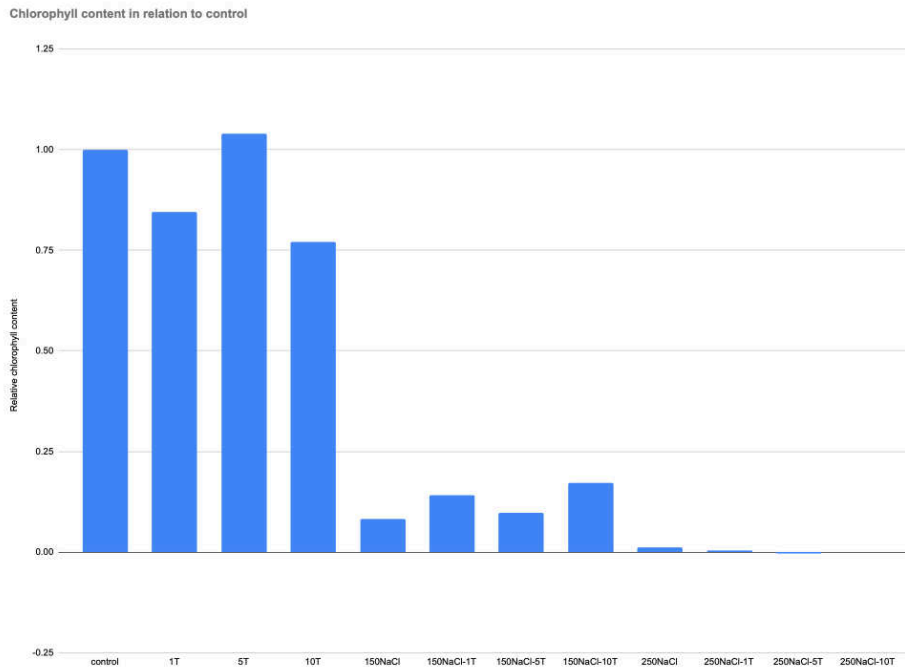
2026-07-15 Progress Report + Meeting with Professor Kyle

Overall Progress:

1. Redoing experimental set 5 - vertical planting (0, 1, 5, 10mM trehalose vs. 0, 150mM and 250mM NaCl)
 - a. Bleaching in the leaves appeared early into the experiment
 - b. Mold and bacterial colonies appearing on the agar plate after 4 days of experiment
 - i. Aseptic technique issues during transferring the plants
2. Exp set #5 Analysis / Summary
 - a. 5mM had the most consistent and best rescue effectiveness in terms of root length growth
 - b. Chlorophyll content in the 150 mM NaCl and 250 mM NaCl was extremely low even though the experiment was only conducted for a few days
 - i. Because the experiment stopped earlier the samples and data collected might not be as accurate

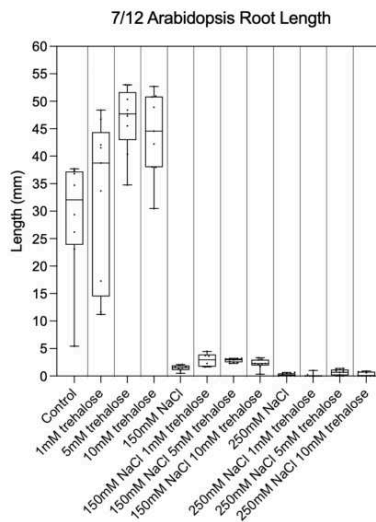
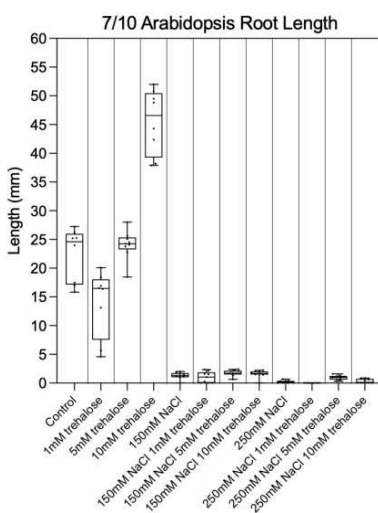


- **Chlorosis and bleaching** started to appear in the third day of experiment
- Mostly from 250 NaCl groups → considering tune/lower the concentration to 200 NaCl in the next set
- Almost every plant in 250 NaCl, 1 mM trehalose group had the symptoms of chlorosis or bleaching around the fifth day



Conclusions:

- 250mM NaCl ones had a negative chlorophyll content in relation to the control agar plate after subtracting blank OD
 - Most of them had already showed symptoms of bleaching and chlorosis-> wouldn't have accurate data
- All of the 150mM and 250mM NaCl plates had extremely low chlorophyll in relation to the controls
- Problem: No definite linear relationship of chlorophyll content between the groups with different trehalose concentrations



Conclusions:

- 250 NaCl and 1 mM trehalose group had almost no growth (average of 0.621mm growth from

7/10 to 7/12)

- After 7/10, 250 mM NaCl groups root lengths barely grow longer
 - Too high of salinity stress-> may slowly kill the plants in the early stages of experiment
- 5mM and 10mM trehalose in control showed dramatic increase in root length compared to standard control
- 5mM trehalose had the most consistent and best rescue effectiveness in both 150 mM and 250 mM NaCl conditions, followed by 10mM trehalose; while on 7/12, in 150 NaCl condition, 1 mM grew slightly longer over 5mM trehalose group

Suggestions and solutions:

1. Lower salinity concentration from 250 mM NaCl as the immediate bleaching and chlorosis of plants do not allow linear observation of protectant's effectiveness

Equipment update



- LABGIC handheld grinder for better efficiency in extracting chlorophyll

2026-07-22 Progress Report + Meeting with Professor Kyle

Overall Progress:

1. Redoing experimental set #5 (0,1,5,10mM trehalose vs. 0,150,250mM NaCl)
 - a. transferred arabidopsis seedlings to salinity plates on 7/21
 - i. changed to 8 seeds per plate so that there is space for roots to grow
2. Stratified around 8 plates (216 seeds in total) with seeds provided by Dr. Kyle
 - a. Plan to transfer into the growth chamber-> 0723

Planned trials:

1. Future experimental designs
 - b. Experimental set #6: fine tuned lower concentrations, and smaller NaCl intervals (0,5 mM trehalose vs. 0,75,100,150mM NaCl) to find optimal observation range
 - c. Experimental set #7: testing with CH bio peptide protectants by 500x dilution rate (0.1ml CH CH bio peptide vs. 0,75,100,150 mM NaCl)
 - i. Another commercial protectant for baseline tuning-> see if better effectiveness than trehalose
 - d. Remain same formula for agar
 - i. 50ml total volume per vertical square agar plate ($\frac{1}{2}$ MS agar, 1% sucrose, MES buffer, 1% agar plate)

Purpose of experiment set 6 (0,5 mM trehalose vs. 0,75,100,150mM NaCl)

- Previous experimental set #5 has revealed the excessively saline environment by 250 mM NaCl caused plants to bleach in the early phases of salinity test (~Day 3), indicating the excessive salt stress kills the plants earlier before the effectiveness of trehalose was able to show. Results from the bleached seedlings would not be accessible and comprehensible.
- Aside from the bleaching/chlorosis phenomenon, data from chlorophyll content and root length growth concludes that 5mM trehalose presents the best and most consistent of rescue effectiveness compared to 1 and 10 mM trehalose.
- Therefore, experimental set #6 fine tunes lower both trehalose and NaCl concentration, only applying one specific trehalose concentration (5mM), and shortening the NaCl concentration intervals: 0, 5 mM trehalose vs. 0, 75,100,150 mM NaCl
- Progression:
 - 7/19 stratification
 - 7/23 place as vertical condition into growth chamber
 - 7/27 transfer to salinity plates (start salinity test experiment)

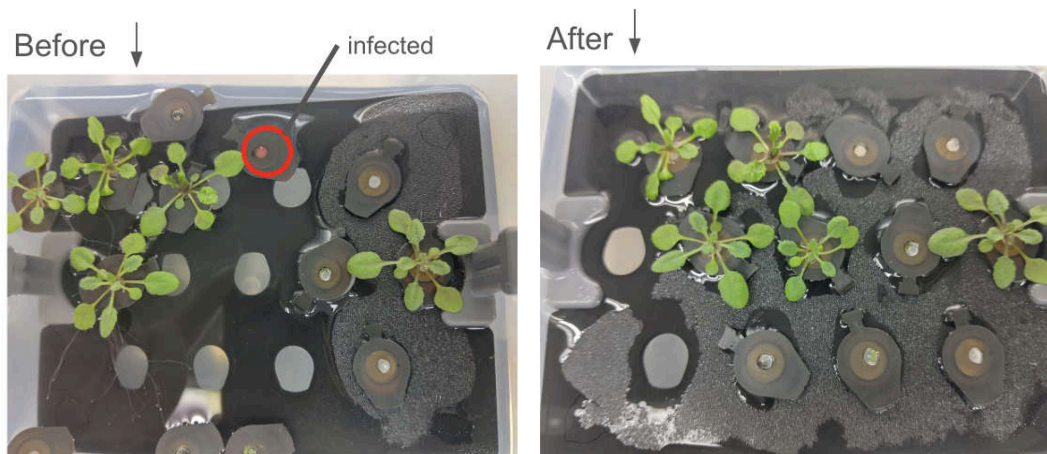
Experiment set #7



- After a deep session of discussion with the CH biotech protectant company, our teammates brought back the CH bio peptides that we can use to test on salinity plates. Based on the recipe they provided: dilution rate 1/500, the concentration of CH protectants in each agar plate would be 100 μ l/50ml of agar.
- Ultimately, the concentrations of experimental set #7: **0, 100 μ l of CH bio peptide vs. 0, 75,100,150 mM NaCl**
- Progression
 - 7/19 stratification
 - 7/23 place as vertical condition into growth chamber
 - 7/27 transfer to salinity plates (start salinity test experiment)

Hydroponics:

- observed problems



1. Observed problems of Box B1
 - 3D printed boards sink into the medium
 - lids will float on the medium and the roots will stick on top of the board
 - Many leaves covered by liquid and block stomata
 - The agar in 2 lids had been infected

- red
- medium is contaminated-> cloudy looking

-> Solutions: changed the medium, changed a new 3D board, and took away the plants that are not growing well with tweezers



2. Observed problems of Box B2

- all plants are infected by bacteria-> red coloring

- progress

7/20 Transferred plants from tube rack

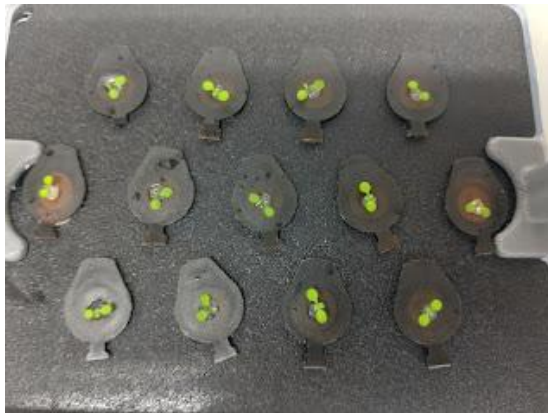
- germination rate : $41/43 = \text{about } 95\%$



- Box 1



- Box 2



- Box 3

- New set of arabidopsis seeds
- 1. 7/19 stratify
- 2. 7/23 put into growth chamber

2026-07-29 Progress Report + Meeting with Professor Kyle

Overall Progress:

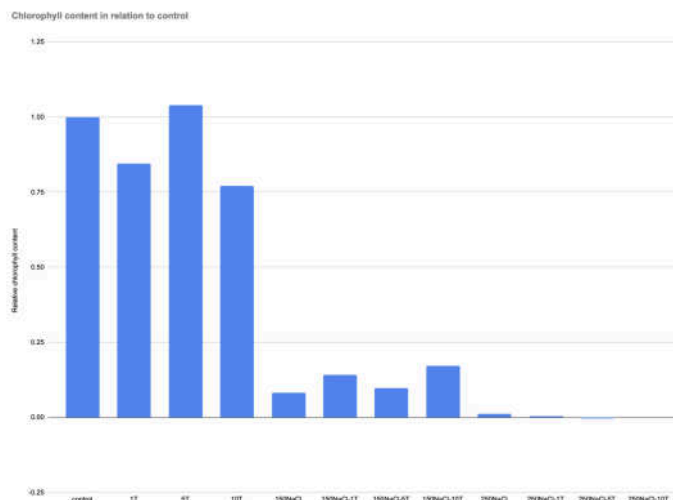
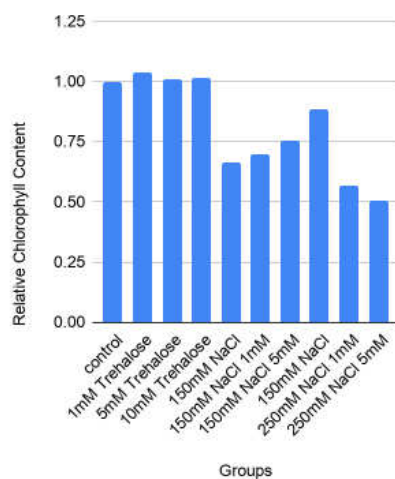
1. Ended Exp set #5 (0,150, 250 mM NaCl vs. 0, 1, 5, 10 mM trehalose)
 - a. root length analysis
 - b. chlorophyll content
2. Conducting Experimental set #6 (0,75,100,150mM NaCl vs. 0, 5 mM trehalose)
 - a. 7/28 transferred
3. Stratified 25 seeds/plate in 8 circular plates in neutral $\frac{1}{2}$ MS agar(for 4 days)
 - a. 7/31 move to growth chamber
4. Arabidopsis soil planting pretest
 - a. Added water/humidity checker (2 each pot)

Chlorophyll extraction data for Exp set #5 (0,150, 250 mM NaCl vs. 0, 1, 5, 10 mM trehalose)

	total chlorophyll content (mg/g) V in this case = 2mL				
Group	Replicate 1	Replicate 2	Replicate 3	avg	freshweight
control	0.2480808874	0.1210116041	0.1135421502	0.1608782139	0.1172
1mM Trehalose	0.2707756881	0.1378848624	0.1200477064	0.1762360856	0.0872
5mM Trehalose	0.2522221402	0.121049631	0.1152728782	0.1628482165	0.1084
10mM Trehalose	0.2503750687	0.1236709441	0.1175609533	0.1638689887	0.1091
150mM NaCl	0.2727721739	0.1339321739	0.1339321739	0.1802121739	0.0115
150mM NaCl 1mM Trehalose	0.2698740157	0.1371590551	0.1327149606	0.1799160105	0.0127
150mM NaCl 5mM Trehalose	0.3312301075	0.1747182796	0.173855914	0.2266014337	0.0093
150mM NaCl 10mM Trehalose	0.3995363636	0.1924431818	0.19565	0.2625431818	0.0088
250mM NaCl 1mM Trehalose	0.5834736842	0.2896263158	0.2747736842	0.3826245614	0.0038
250mM NaCl 5mM Trehalose	0.4640622222	0.2320311111	0.2383022222	0.3114651852	0.0045

- Due to contamination, 250mM NaCl control group does not have data
- 5mM trehalose in both 150 and 250mM NaCl, has the heaviest freshweight
- 1mM Trehalose in 250mM NaCl has the most chlorophyll content ; while 10mM Trehalose in 150mM NaCl has the most chlorophyll content

Exp set #5 trial 2: Chlorophyll Content In relation to control

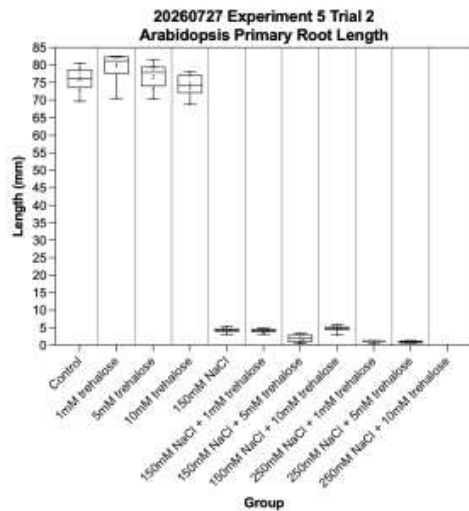


Left graph: experiment set 5 trial 2

Right graph: experiment set 5 trial 1(for comparison)

- Overall, 1mM trehalose in 250mM NaCl has the most relative chlorophyll content, followed by 250NaCl-5T
- In 150mM NaCl, 10mM Trehalose has the most chlorophyll content, followed by 5T (opposite with 250NaCl group)
- All trehalose concentrations had descent rescue effectiveness across NaCl groups in this trial

Rootlength data for Exp set #5 (0,150, 250 mM NaCl vs. 0, 1, 5, 10 mM trehalose)



Predicted timeline: Experiment set 7

- Testing with CH bio peptide protectants by 500x dilution rate (0.1ml CH CH bio peptide vs. 0,75,100,150 mM NaCl)
 - 7/27 placed seeds for vernalization in 8 neutral ½ MS circular plates
 - 7/31 move to growth chamber
 - 8/4 add CH bio peptide protectant to pretreatment group(0, CH bio peptide and 100mM NaCl, CH bio peptide)
 - 8/5 add 100mM NaCl to pretreatment and posttreatment group
 - 8/6 add CH bio peptide protectant to posttreatment group
 - -> 8/10 or 8/11(observe for 6-7 days before collecting data)
- Initial idea
 - soak seeds during vernalization with CH bio peptide for pretreatment group and sterilized water for control groups
 - 12 seeds per circular plate group
- Suggestions and changes
 - To better compare pretreatment and posttreatment of protectants, the seeds should be grown on neutral agar plates for a few days then apply liquid NaCl as stress and CH bio peptide protectant depending on different timing
 - If stratify seeds in different solutions(sterilized water and CH biopeptide), the consistencies of plants will differ significantly-> different starting points and growth conditions
 - Timing of the liquid salinity and protectant?
 - Start with doing pretreatment a day before salinity stress application
 - Reason: peptide easily degrades. If the protectant degrades too quickly, it

will be difficult to determine its tolerance and effectiveness. One day should allow the plants to absorb/utilize the peptide and have observable phenotypical changes.

- Future direction: can also move the pretreatment to 3 days prior to the stress if 1 day demonstrates consistent effectiveness
- Circular plates can hold up to 30-40 plants per plate and distribute sample amounts for different measuring aspects

Soil Planting



Tray 1
(4 arabidopsis/pot)

testing soil moisture
detector's accuracy
• added on (7/29)

Arabidopsis location indicator
• because the plant is
still too small, we put
micropipette tips beside
the plant to mark the
specific location and
track growth

Arabidopsis Soil Planting Protocol(1st version)

Phase 1: Seed Sterilization & Germination

1. Sterilization & Sowing: Sterilize Arabidopsis seeds with ethanol and sow them under sterile conditions on 1/2 MS square agar plates.
2. Stratification & Growth: Stratify the plates at 4°C in the dark for 3 days (cold treatment). Transfer them to white light and culture vertically for 7 days.

Phase 2: Soil & Pot Preparation

3. (Optional) Sanitization: Pre-soak pots and water trays in 1:100 diluted bleach for 10 minutes to sanitize. Rinse thoroughly with clean water multiple times until all bleach odor is completely gone. This can be done inside a hood to avoid strong chemical odors.
4. Soil Preparation: Mix peat soil and autoclave it. Fill 3-inch pots with soil, lightly press down until 80% full, and thoroughly water from the surface to ensure the entire soil body is fully moistened before starting seedling transplantation.

Phase 3: Transplantation

5. Seedling Transfer:
 - a. Make a small hole (about one knuckle deep) in the center of the soil using a finger (if planting four seedlings per pot, space four holes evenly across the soil surface).
 - b. Using tweezers, gently lift one or four 7-day-old seedlings (select only uniform, healthy seedlings).
 - c. Place the root against the sidewall of the hole.

- d. Use a wash bottle to rinse soil over the roots so they are naturally covered. Avoid touching the root system directly with tweezers or fingers.
- e. Note: During this process, use tweezers only to support the seedling from beneath the cotyledons (never clamp the seedling directly to prevent tissue damage).

Phase 4: Acclimatization & Post-Transplant Care

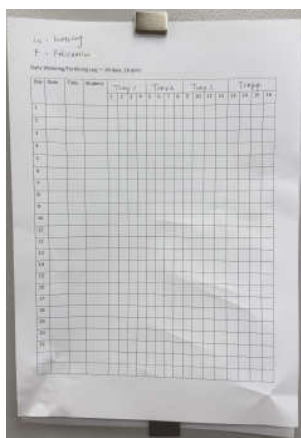
6. Humidity Management:
 - a. Place the pots onto a water tray and add water until the bottom of the pots is submerged (approx. 0.5 cm deep).
 - b. Cover with a transparent lid to maintain humidity for 2 days.
 - i. Day 1: Keep the cover fully sealed.
 - ii. Day 2: Slightly open the cover to lower humidity.
 - iii. Day 3: Remove the cover completely to ensure good air circulation and prevent fungal growth.
7. Watering: Adjust watering frequency based on growth chamber humidity. The soil surface should undergo dry-wet cycles. Because growth chambers can be very dry, keep the soil surface dry for no more than 1 day to prevent dehydration.
8. Fertilization: Fertilize with HYPONeX No. 2 diluted 1:1000. Apply by alternating between water and fertilizer with each watering cycle. Both watering and fertilization should be applied via sub-irrigation (from the tray bottom). Avoid direct watering on the soil surface unless the soil is too dry to absorb water from the bottom.

Phase 5: Experimental Treatment & Harvest

9. Experiment Timing: Protectant or stress treatments can begin approximately 3 weeks post-transplantation. Depending on plant growth, the timeframe can be adjusted between 2 to 4 weeks. Take photos regularly to track progress and discuss the optimal starting point. Treatments should begin no later than 4 weeks post-transplant to avoid bolting and growth arrest.
10. Support for Propagation: Plants not used for experiments can be kept for seed propagation. Once the inflorescence reaches 20 cm, insert a bamboo stake into the soil.
 - a. Tip: Cover the sharp end of the stake with a 200 μ L tip before inserting to prevent direct soil contact and mold growth.
 - b. Secure the inflorescence to the stake using orchid clips to prevent lodging. Take care not to damage the stem with the clips.
11. Seed Harvesting (only for the plants without any stress treatment): Siliques will begin to dry about 1.5 months post-transplant; reduce watering frequency at this stage. Once 80% of all siliques on the plant are dry, stop watering entirely to allow full drying before seed collection.

Equipment update:

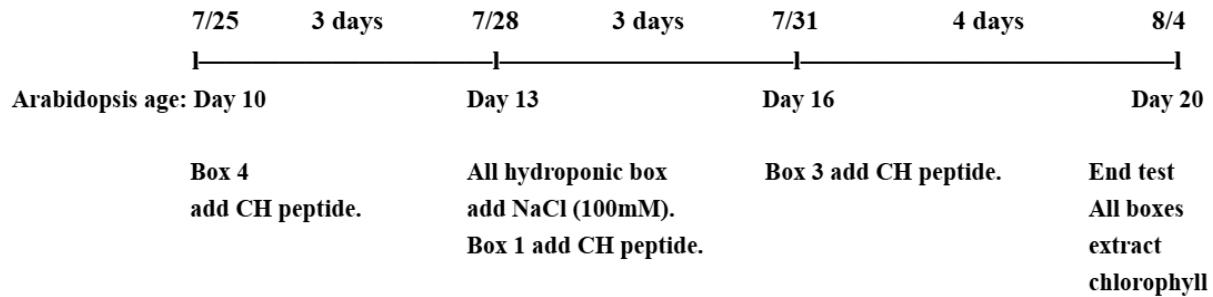
Soil daily log for watering, fertilization, and tracking of plants' conditions



Hydroponics:

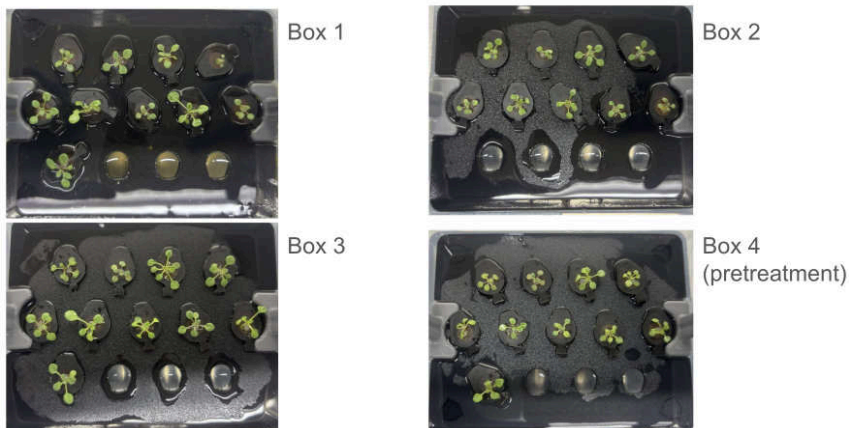
- **Hydroponic salinity testing (concentration : 100mM)**

- Timeline



- NaCl concentration in $\frac{1}{2}$ MS medium: 100mM
- Control (only add NaCl): Box 2
- Pre-treatment (add CH bio peptide protectant 3 days before NaCl): Box 4
- Add NaCl & CH bio peptide protectant at the same time(cotreatment): Box 1
- Add CH bio peptide protectant after 3 days of NaCl: Box 3

Salinity test pictures (7/29, 1 day of NaCl)



comparison of with or without Oxygen (both boxes are contaminated)

with O₂



without O₂



30 days old



-> the box without oxygen starting bolting, which means that the stress is very significant

- **Transfer arabidopsis to hydroponic boxes**

- a. 7/27 transfer
- b. germination rate: $46/48 = 96\%$
- c. transferred to 2 tip boxes and 1 black box
- d. currently no observed problems

A1:



A2:



A3:

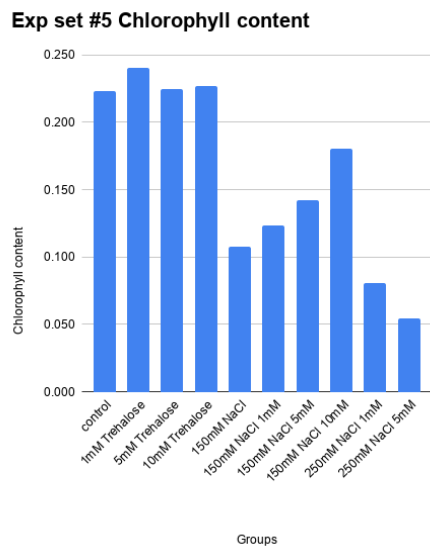


- **New set of arabidopsis (set B)**



- 7/25 stratified
- 7/29 take it out from refrigerator and put into growth chamber
- If this set is growing pretty well-> plan to do heat stress on this set

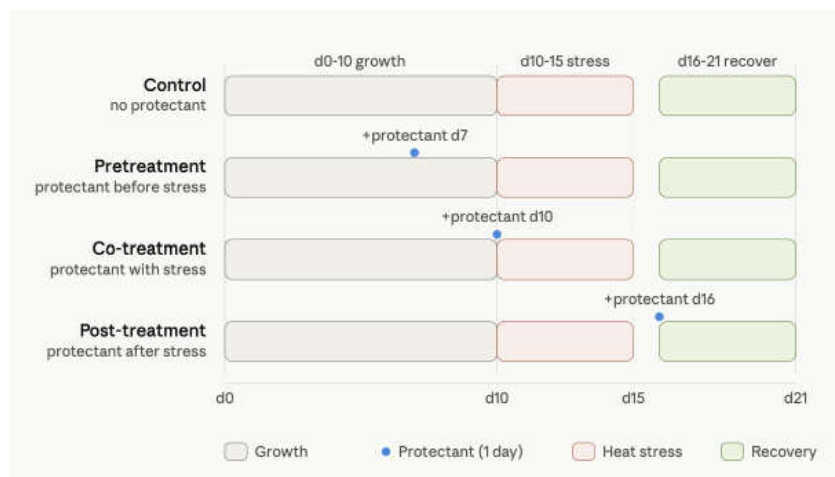
Chlorophyll data analysis of experiment set #5 (0,150, 250 mM NaCl vs. 0, 1, 5, 10 mM trehalose)



total chlorophyll content (mg/g) V in this case = 2mL						
Group	Replicate 1	Replicate 2	Replicate 3	avg	freshweight	
control	0.232	0.226	0.211	0.223	0.1172	
1mM Trehalose	0.249	0.254	0.219	0.241	0.0872	
5mM Trehalose	0.235	0.225	0.213	0.224	0.1084	
10mM Trehalose	0.233	0.230	0.218	0.227	0.1091	
150mM NaCl	0.111	0.106	0.106	0.108	0.0115	
150mM NaCl 1mM Trehalose	0.123	0.128	0.119	0.123	0.0127	
150mM NaCl 5mM Trehalose	0.131	0.149	0.147	0.143	0.0093	
150mM NaCl 10mM Trehalose	0.188	0.173	0.180	0.180	0.0088	
250mM NaCl 1mM Trehalose	0.093	0.089	0.059	0.081	0.0038	
250mM NaCl 5mM Trehalose	0.050	0.050	0.063	0.054	0.0045	

- Conclusions: Without stress, 1mM trehalose has the best rescue effectiveness. However, in 150 mM NaCl stress, 10 mM trehalose has the best rescue effectiveness
 - Need to confirm if the same results can be concluded with repeated experiments

Heat stress treatment planning for different time periods discussed:



Soil Progress



- Training for soil transfer

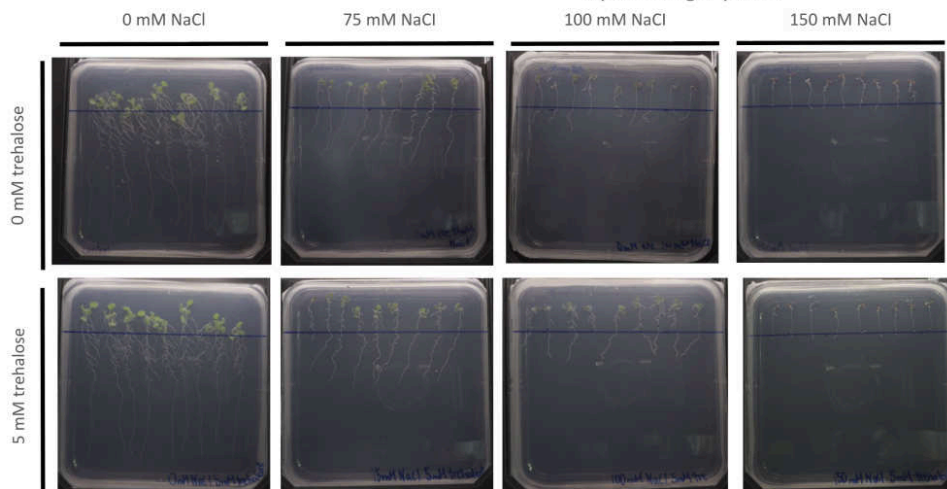
2026-08-05 Progress Report + Meeting with Professor Kyle

Overall Progress:

1. Ended Exp set #6 (0,5 mM trehalose vs. 0,75,100,150mM NaCl)
 - a. root length analysis-8/4
 - b. chlorophyll content-8/5
 - c. fresh weight-8/4
2. Exp set #7 500x dilution rate (0.1ml CH CH bio peptide vs. 0,75,100,150 mM NaCl)
 - a. added protectants to the pretreatment group - 8/4
 - b. added NaCl to both pretreatment and posttreatment group - 8/5
 - c. added protectant to day 0 - 8/5
3. Arabidopsis soil planting
 - a. 4 Tray
 - b. stratified new vertical plates(x4) for next trial+practice
4. Chlorophyll extraction
 - a. Wrong Elisa reader OD wavelength used 633 instead of 663

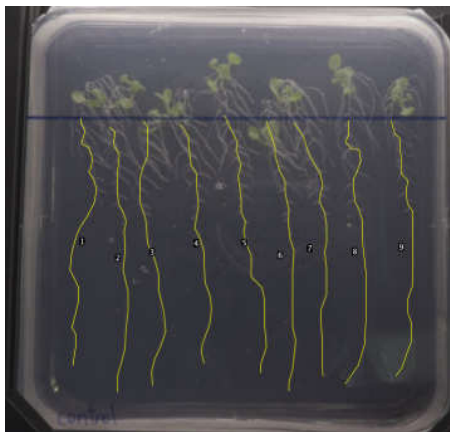
Day 6 (8/3) - Vertical planting

20260719 stratified 0723 to growth chamber
Squared Agar plates



-> 5mM Trehalose in 100mM NaCl has very obvious rescue effectiveness (relatively longer root length compared to 0 mM NaCl)

Exp set #6 (0,5 mM trehalose vs. 0,75,100,150mM NaCl) Root length analysis:

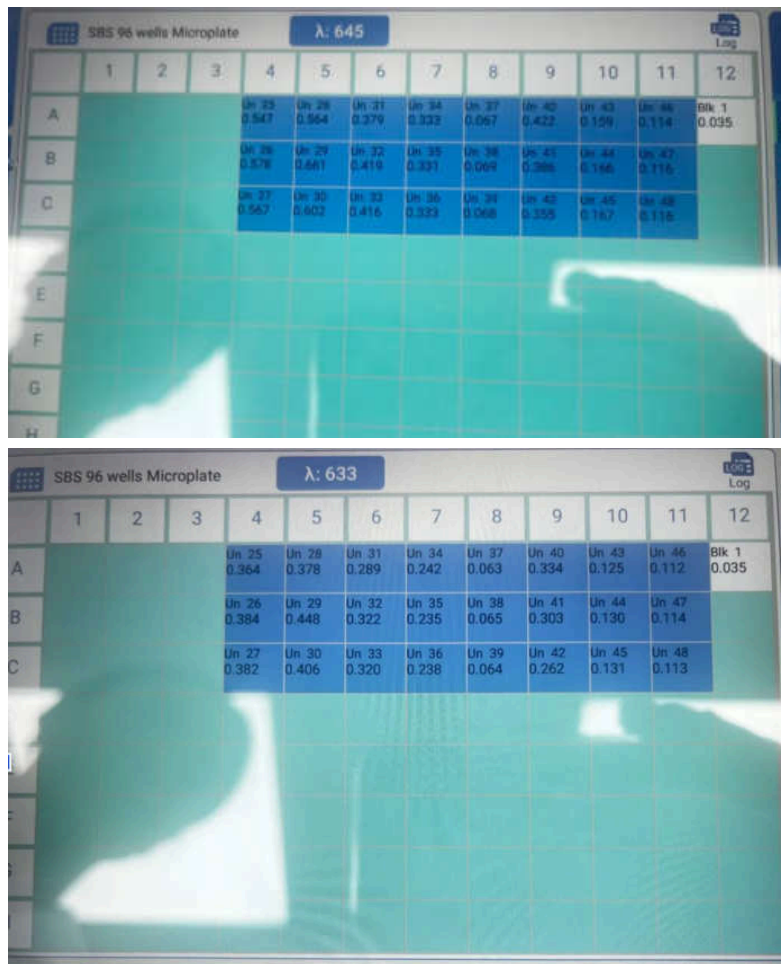
control		<table><tr><th></th><th>Area</th><th>Mean</th><th>Min</th><th>Max</th><th>Length</th></tr><tr><td>1</td><td>9.758</td><td>67.237</td><td>53.535</td><td>104.610</td><td>73.898</td></tr><tr><td>2</td><td>10.072</td><td>66.343</td><td>53.520</td><td>101.923</td><td>76.236</td></tr><tr><td>3</td><td>10.055</td><td>69.679</td><td>52.333</td><td>109.320</td><td>76.181</td></tr><tr><td>4</td><td>9.114</td><td>70.484</td><td>51.395</td><td>110.509</td><td>68.984</td></tr><tr><td>5</td><td>9.950</td><td>70.607</td><td>51.333</td><td>112.241</td><td>75.299</td></tr><tr><td>6</td><td>10.159</td><td>71.388</td><td>51.949</td><td>127.673</td><td>76.839</td></tr><tr><td>7</td><td>9.706</td><td>70.895</td><td>48.894</td><td>108.255</td><td>73.466</td></tr><tr><td>8</td><td>10.421</td><td>68.579</td><td>50.791</td><td>105.364</td><td>78.882</td></tr><tr><td>9</td><td>9.863</td><td>66.526</td><td>50.333</td><td>94.000</td><td>74.773</td></tr></table>		Area	Mean	Min	Max	Length	1	9.758	67.237	53.535	104.610	73.898	2	10.072	66.343	53.520	101.923	76.236	3	10.055	69.679	52.333	109.320	76.181	4	9.114	70.484	51.395	110.509	68.984	5	9.950	70.607	51.333	112.241	75.299	6	10.159	71.388	51.949	127.673	76.839	7	9.706	70.895	48.894	108.255	73.466	8	10.421	68.579	50.791	105.364	78.882	9	9.863	66.526	50.333	94.000	74.773	74.951
	Area	Mean	Min	Max	Length																																																										
1	9.758	67.237	53.535	104.610	73.898																																																										
2	10.072	66.343	53.520	101.923	76.236																																																										
3	10.055	69.679	52.333	109.320	76.181																																																										
4	9.114	70.484	51.395	110.509	68.984																																																										
5	9.950	70.607	51.333	112.241	75.299																																																										
6	10.159	71.388	51.949	127.673	76.839																																																										
7	9.706	70.895	48.894	108.255	73.466																																																										
8	10.421	68.579	50.791	105.364	78.882																																																										
9	9.863	66.526	50.333	94.000	74.773																																																										
5mM T		<table><tr><th></th><th>Area</th><th>Mean</th><th>Min</th><th>Max</th><th>Length</th></tr><tr><td>1</td><td>9.037</td><td>70.741</td><td>51.154</td><td>113.289</td><td>72.059</td></tr><tr><td>2</td><td>9.415</td><td>74.775</td><td>53.503</td><td>123.498</td><td>75.018</td></tr><tr><td>3</td><td>9.195</td><td>72.166</td><td>51.533</td><td>113.401</td><td>73.200</td></tr><tr><td>4</td><td>9.667</td><td>76.671</td><td>54.367</td><td>126.097</td><td>77.030</td></tr><tr><td>5</td><td>9.226</td><td>76.795</td><td>51.664</td><td>121.667</td><td>73.561</td></tr><tr><td>6</td><td>8.754</td><td>75.589</td><td>57.407</td><td>120.795</td><td>69.776</td></tr><tr><td>7</td><td>8.911</td><td>72.698</td><td>53.333</td><td>118.566</td><td>71.072</td></tr><tr><td>8</td><td>8.282</td><td>71.481</td><td>53.136</td><td>130.575</td><td>65.958</td></tr><tr><td>9</td><td>8.943</td><td>68.722</td><td>49.333</td><td>119.141</td><td>71.286</td></tr></table>		Area	Mean	Min	Max	Length	1	9.037	70.741	51.154	113.289	72.059	2	9.415	74.775	53.503	123.498	75.018	3	9.195	72.166	51.533	113.401	73.200	4	9.667	76.671	54.367	126.097	77.030	5	9.226	76.795	51.664	121.667	73.561	6	8.754	75.589	57.407	120.795	69.776	7	8.911	72.698	53.333	118.566	71.072	8	8.282	71.481	53.136	130.575	65.958	9	8.943	68.722	49.333	119.141	71.286	72.107
	Area	Mean	Min	Max	Length																																																										
1	9.037	70.741	51.154	113.289	72.059																																																										
2	9.415	74.775	53.503	123.498	75.018																																																										
3	9.195	72.166	51.533	113.401	73.200																																																										
4	9.667	76.671	54.367	126.097	77.030																																																										
5	9.226	76.795	51.664	121.667	73.561																																																										
6	8.754	75.589	57.407	120.795	69.776																																																										
7	8.911	72.698	53.333	118.566	71.072																																																										
8	8.282	71.481	53.136	130.575	65.958																																																										
9	8.943	68.722	49.333	119.141	71.286																																																										
75mM NaCl		<table><tr><th></th><th>Area</th><th>Mean</th><th>Min</th><th>Max</th><th>Length</th></tr><tr><td>1</td><td>4.024</td><td>81.510</td><td>70.666</td><td>97.872</td><td>30.682</td></tr><tr><td>2</td><td>3.627</td><td>87.266</td><td>77.754</td><td>103.473</td><td>27.588</td></tr><tr><td>3</td><td>4.697</td><td>86.493</td><td>73.333</td><td>99.334</td><td>35.686</td></tr><tr><td>4</td><td>4.438</td><td>86.711</td><td>74.234</td><td>110.386</td><td>33.735</td></tr><tr><td>5</td><td>4.007</td><td>87.376</td><td>74.257</td><td>115.507</td><td>30.490</td></tr><tr><td>6</td><td>5.077</td><td>84.143</td><td>71.975</td><td>106.356</td><td>38.696</td></tr><tr><td>7</td><td>5.267</td><td>85.638</td><td>63.667</td><td>110.821</td><td>40.118</td></tr><tr><td>8</td><td>5.129</td><td>82.386</td><td>64.367</td><td>125.289</td><td>39.115</td></tr><tr><td>9</td><td>4.836</td><td>75.807</td><td>59.000</td><td>96.233</td><td>36.797</td></tr></table>		Area	Mean	Min	Max	Length	1	4.024	81.510	70.666	97.872	30.682	2	3.627	87.266	77.754	103.473	27.588	3	4.697	86.493	73.333	99.334	35.686	4	4.438	86.711	74.234	110.386	33.735	5	4.007	87.376	74.257	115.507	30.490	6	5.077	84.143	71.975	106.356	38.696	7	5.267	85.638	63.667	110.821	40.118	8	5.129	82.386	64.367	125.289	39.115	9	4.836	75.807	59.000	96.233	36.797	34.767
	Area	Mean	Min	Max	Length																																																										
1	4.024	81.510	70.666	97.872	30.682																																																										
2	3.627	87.266	77.754	103.473	27.588																																																										
3	4.697	86.493	73.333	99.334	35.686																																																										
4	4.438	86.711	74.234	110.386	33.735																																																										
5	4.007	87.376	74.257	115.507	30.490																																																										
6	5.077	84.143	71.975	106.356	38.696																																																										
7	5.267	85.638	63.667	110.821	40.118																																																										
8	5.129	82.386	64.367	125.289	39.115																																																										
9	4.836	75.807	59.000	96.233	36.797																																																										

75mM NaCl 5T		<table><tr><th></th><th>Area</th><th>Mean</th><th>Min</th><th>Max</th><th>Length</th></tr><tr><td>1</td><td>0.833</td><td>85.698</td><td>48.133</td><td>126.427</td><td>6.599</td></tr><tr><td>2</td><td>3.589</td><td>77.866</td><td>51.782</td><td>123.198</td><td>28.341</td></tr><tr><td>3</td><td>4.150</td><td>82.005</td><td>60.120</td><td>124.794</td><td>32.751</td></tr><tr><td>4</td><td>4.438</td><td>86.825</td><td>61.753</td><td>134.333</td><td>35.172</td></tr><tr><td>5</td><td>3.605</td><td>87.945</td><td>68.480</td><td>137.060</td><td>28.476</td></tr><tr><td>6</td><td>4.310</td><td>92.860</td><td>64.000</td><td>127.019</td><td>34.056</td></tr><tr><td>7</td><td>4.566</td><td>90.528</td><td>68.290</td><td>137.910</td><td>36.187</td></tr><tr><td>8</td><td>3.669</td><td>92.772</td><td>69.570</td><td>131.186</td><td>28.967</td></tr><tr><td>9</td><td>4.054</td><td>91.066</td><td>66.562</td><td>139.448</td><td>32.033</td></tr></table>		Area	Mean	Min	Max	Length	1	0.833	85.698	48.133	126.427	6.599	2	3.589	77.866	51.782	123.198	28.341	3	4.150	82.005	60.120	124.794	32.751	4	4.438	86.825	61.753	134.333	35.172	5	3.605	87.945	68.480	137.060	28.476	6	4.310	92.860	64.000	127.019	34.056	7	4.566	90.528	68.290	137.910	36.187	8	3.669	92.772	69.570	131.186	28.967	9	4.054	91.066	66.562	139.448	32.033	29.176
	Area	Mean	Min	Max	Length																																																										
1	0.833	85.698	48.133	126.427	6.599																																																										
2	3.589	77.866	51.782	123.198	28.341																																																										
3	4.150	82.005	60.120	124.794	32.751																																																										
4	4.438	86.825	61.753	134.333	35.172																																																										
5	3.605	87.945	68.480	137.060	28.476																																																										
6	4.310	92.860	64.000	127.019	34.056																																																										
7	4.566	90.528	68.290	137.910	36.187																																																										
8	3.669	92.772	69.570	131.186	28.967																																																										
9	4.054	91.066	66.562	139.448	32.033																																																										
100mM NaCl		<table><tr><th></th><th>Area</th><th>Mean</th><th>Min</th><th>Max</th><th>Length</th></tr><tr><td>1</td><td>1.742</td><td>85.472</td><td>65.560</td><td>118.794</td><td>12.650</td></tr><tr><td>2</td><td>1.591</td><td>90.779</td><td>67.510</td><td>139.533</td><td>11.526</td></tr><tr><td>3</td><td>2.405</td><td>95.699</td><td>73.243</td><td>142.180</td><td>17.512</td></tr><tr><td>4</td><td>0.644</td><td>106.344</td><td>96.313</td><td>120.476</td><td>4.649</td></tr><tr><td>5</td><td>1.761</td><td>99.771</td><td>59.107</td><td>144.624</td><td>12.821</td></tr><tr><td>6</td><td>1.629</td><td>96.329</td><td>80.000</td><td>118.395</td><td>11.756</td></tr><tr><td>7</td><td>1.818</td><td>107.857</td><td>82.624</td><td>137.808</td><td>13.283</td></tr><tr><td>8</td><td>0.701</td><td>89.296</td><td>72.424</td><td>110.642</td><td>4.998</td></tr><tr><td>9</td><td>1.364</td><td>96.827</td><td>69.835</td><td>123.659</td><td>9.792</td></tr></table>		Area	Mean	Min	Max	Length	1	1.742	85.472	65.560	118.794	12.650	2	1.591	90.779	67.510	139.533	11.526	3	2.405	95.699	73.243	142.180	17.512	4	0.644	106.344	96.313	120.476	4.649	5	1.761	99.771	59.107	144.624	12.821	6	1.629	96.329	80.000	118.395	11.756	7	1.818	107.857	82.624	137.808	13.283	8	0.701	89.296	72.424	110.642	4.998	9	1.364	96.827	69.835	123.659	9.792	10.999
	Area	Mean	Min	Max	Length																																																										
1	1.742	85.472	65.560	118.794	12.650																																																										
2	1.591	90.779	67.510	139.533	11.526																																																										
3	2.405	95.699	73.243	142.180	17.512																																																										
4	0.644	106.344	96.313	120.476	4.649																																																										
5	1.761	99.771	59.107	144.624	12.821																																																										
6	1.629	96.329	80.000	118.395	11.756																																																										
7	1.818	107.857	82.624	137.808	13.283																																																										
8	0.701	89.296	72.424	110.642	4.998																																																										
9	1.364	96.827	69.835	123.659	9.792																																																										
100mM NaCl 5T		<table><tr><th></th><th>Area</th><th>Mean</th><th>Min</th><th>Max</th><th>Length</th></tr><tr><td>1</td><td>1.478</td><td>86.221</td><td>61.117</td><td>108.097</td><td>12.240</td></tr><tr><td>2</td><td>2.180</td><td>86.136</td><td>61.051</td><td>100.784</td><td>17.936</td></tr><tr><td>3</td><td>2.224</td><td>89.490</td><td>69.667</td><td>107.347</td><td>18.344</td></tr><tr><td>4</td><td>0.146</td><td>86.969</td><td>78.667</td><td>97.232</td><td>1.148</td></tr><tr><td>5</td><td>2.385</td><td>90.911</td><td>75.869</td><td>109.405</td><td>19.720</td></tr><tr><td>6</td><td>1.873</td><td>98.797</td><td>76.667</td><td>112.832</td><td>15.416</td></tr><tr><td>7</td><td>1.844</td><td>97.186</td><td>74.073</td><td>116.158</td><td>15.188</td></tr><tr><td>8</td><td>1.814</td><td>91.027</td><td>61.667</td><td>106.000</td><td>14.989</td></tr><tr><td>9</td><td>2.107</td><td>92.959</td><td>74.066</td><td>109.575</td><td>17.440</td></tr></table>		Area	Mean	Min	Max	Length	1	1.478	86.221	61.117	108.097	12.240	2	2.180	86.136	61.051	100.784	17.936	3	2.224	89.490	69.667	107.347	18.344	4	0.146	86.969	78.667	97.232	1.148	5	2.385	90.911	75.869	109.405	19.720	6	1.873	98.797	76.667	112.832	15.416	7	1.844	97.186	74.073	116.158	15.188	8	1.814	91.027	61.667	106.000	14.989	9	2.107	92.959	74.066	109.575	17.440	13.602
	Area	Mean	Min	Max	Length																																																										
1	1.478	86.221	61.117	108.097	12.240																																																										
2	2.180	86.136	61.051	100.784	17.936																																																										
3	2.224	89.490	69.667	107.347	18.344																																																										
4	0.146	86.969	78.667	97.232	1.148																																																										
5	2.385	90.911	75.869	109.405	19.720																																																										
6	1.873	98.797	76.667	112.832	15.416																																																										
7	1.844	97.186	74.073	116.158	15.188																																																										
8	1.814	91.027	61.667	106.000	14.989																																																										
9	2.107	92.959	74.066	109.575	17.440																																																										

150 NaCl		<table><tr><th></th><th>Area</th><th>Mean</th><th>Min</th><th>Max</th><th>Length</th></tr><tr><td>1</td><td>0.211</td><td>89.056</td><td>82.852</td><td>100.858</td><td>1.534</td></tr><tr><td>2</td><td>0.260</td><td>92.507</td><td>74.333</td><td>109.342</td><td>2.014</td></tr><tr><td>3</td><td>0.243</td><td>84.158</td><td>76.024</td><td>90.999</td><td>1.856</td></tr><tr><td>4</td><td>0.195</td><td>98.672</td><td>78.251</td><td>112.528</td><td>1.424</td></tr><tr><td>5</td><td>0.114</td><td>83.061</td><td>76.982</td><td>100.667</td><td>0.806</td></tr><tr><td>6</td><td>0.454</td><td>100.555</td><td>87.667</td><td>107.774</td><td>3.544</td></tr><tr><td>7</td><td>0.146</td><td>92.249</td><td>77.552</td><td>105.474</td><td>1.096</td></tr></table>		Area	Mean	Min	Max	Length	1	0.211	89.056	82.852	100.858	1.534	2	0.260	92.507	74.333	109.342	2.014	3	0.243	84.158	76.024	90.999	1.856	4	0.195	98.672	78.251	112.528	1.424	5	0.114	83.061	76.982	100.667	0.806	6	0.454	100.555	87.667	107.774	3.544	7	0.146	92.249	77.552	105.474	1.096	1.753												
	Area	Mean	Min	Max	Length																																																										
1	0.211	89.056	82.852	100.858	1.534																																																										
2	0.260	92.507	74.333	109.342	2.014																																																										
3	0.243	84.158	76.024	90.999	1.856																																																										
4	0.195	98.672	78.251	112.528	1.424																																																										
5	0.114	83.061	76.982	100.667	0.806																																																										
6	0.454	100.555	87.667	107.774	3.544																																																										
7	0.146	92.249	77.552	105.474	1.096																																																										
150 NaCl 5T		<table><tr><th></th><th>Area</th><th>Mean</th><th>Min</th><th>Max</th><th>Length</th></tr><tr><td>1</td><td>0.447</td><td>69.233</td><td>64.023</td><td>92.364</td><td>3.481</td></tr><tr><td>2</td><td>0.255</td><td>74.859</td><td>64.667</td><td>85.173</td><td>1.921</td></tr><tr><td>3</td><td>0.510</td><td>84.056</td><td>69.000</td><td>100.652</td><td>4.033</td></tr><tr><td>4</td><td>0.367</td><td>95.817</td><td>76.040</td><td>122.917</td><td>2.888</td></tr><tr><td>5</td><td>0.383</td><td>94.497</td><td>62.472</td><td>126.114</td><td>2.973</td></tr><tr><td>6</td><td>0.255</td><td>97.472</td><td>80.220</td><td>114.847</td><td>1.926</td></tr><tr><td>7</td><td>0.351</td><td>80.711</td><td>68.333</td><td>121.000</td><td>2.718</td></tr><tr><td>8</td><td>0.239</td><td>80.449</td><td>55.075</td><td>112.342</td><td>1.880</td></tr><tr><td>9</td><td>0.128</td><td>65.068</td><td>62.000</td><td>66.666</td><td>0.986</td></tr></table>		Area	Mean	Min	Max	Length	1	0.447	69.233	64.023	92.364	3.481	2	0.255	74.859	64.667	85.173	1.921	3	0.510	84.056	69.000	100.652	4.033	4	0.367	95.817	76.040	122.917	2.888	5	0.383	94.497	62.472	126.114	2.973	6	0.255	97.472	80.220	114.847	1.926	7	0.351	80.711	68.333	121.000	2.718	8	0.239	80.449	55.075	112.342	1.880	9	0.128	65.068	62.000	66.666	0.986	2.534
	Area	Mean	Min	Max	Length																																																										
1	0.447	69.233	64.023	92.364	3.481																																																										
2	0.255	74.859	64.667	85.173	1.921																																																										
3	0.510	84.056	69.000	100.652	4.033																																																										
4	0.367	95.817	76.040	122.917	2.888																																																										
5	0.383	94.497	62.472	126.114	2.973																																																										
6	0.255	97.472	80.220	114.847	1.926																																																										
7	0.351	80.711	68.333	121.000	2.718																																																										
8	0.239	80.449	55.075	112.342	1.880																																																										
9	0.128	65.068	62.000	66.666	0.986																																																										

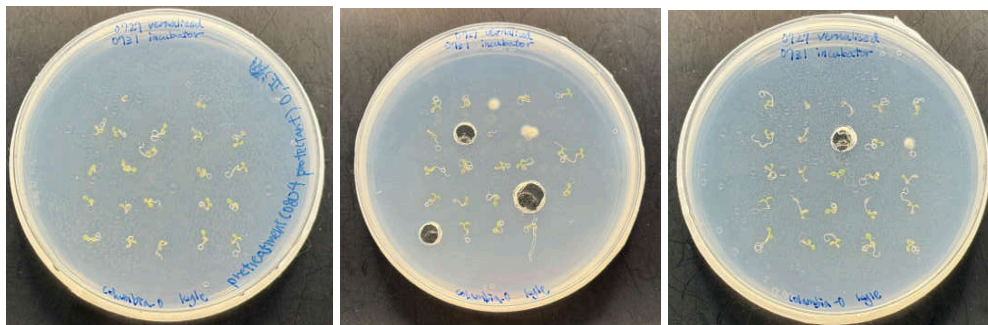
	0mM	75mM	100mM	150mM
0mM Trehalose	74.951	34.767	10.999	1.753
5mM Trehalose	72.107	29.176	13.602	2.534

Exp set #6 (0,5 mM trehalose vs. 0,75,100,150mM NaCl) Chlorophyll extraction on Elisa reader:



Exp set #7 500x dilution rate (0.1ml CH CH bio peptide vs. 0,75,100,150 mM NaCl)

Problem:



- Removed contamination with pipette tips
- Seeds may not be proper sanitized
- Too much water vapor inside the agar plates-> plants are soaked in water and during the transportation of the plates to take pictures, the water washes away the plants to different locations
- When adding the protectants, plants are easily washed away (surface tension make it harder to fill the entire surface)

Solution:

- Remove the excess water caused by vapor with pipette before adding NaCl and protectant
- While adding the protectant, spin the plate slowly to have better coverage in all areas of the plants
- Stratified new plates(0805) and 0809 move to growth for trial 2
- Will place the new plates (Exp set #7 500x dilution rate (0.1ml CH CH bio peptide vs. 0,75,100,150 mM NaCl) trial 2) in the transparent box for moisture level control-> aim to have less water in the agar

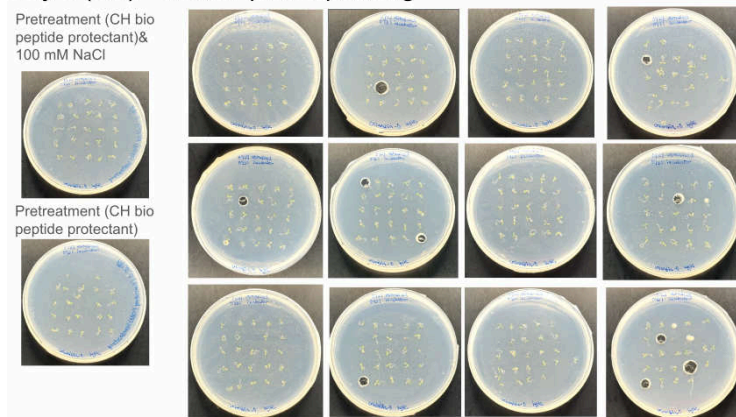
Exp #7 Progress for now:

- protectant amount: 1ml
 - shake the plate lightly left to right so the liquid covers all the plants
 - salinity(100 mM NaCl) amount: 1667 μ l of 3M NaCl liquid stock
1. changed groupings due to holes in agar(remove contamination so can't apply liquid NaCl or protectant)
 - pretreatment(add protectant day before NaCl)
 - 0 day(add protectant and NaCl on the same day)
 - 1 day(add NaCl one day before protectant)
 - no 2, 3, 4 days treatment-> do baseline treating for 1 day first

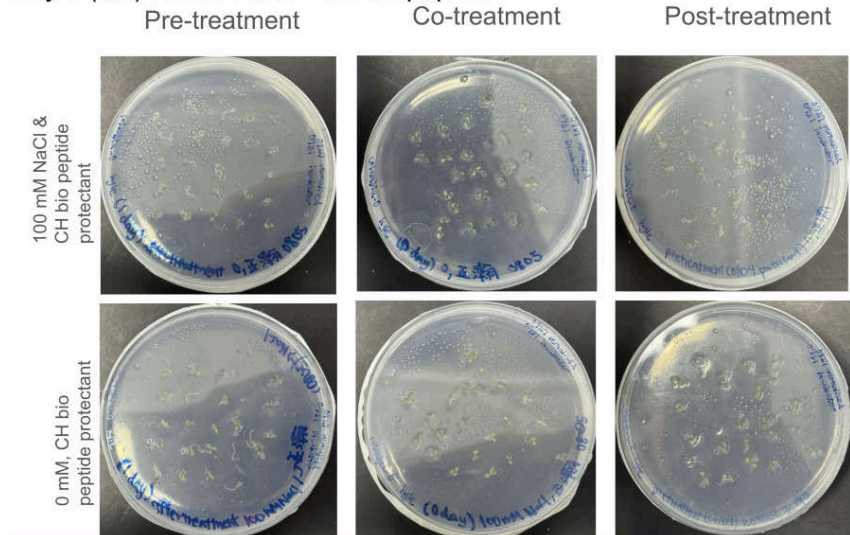
Exp #7 schedule

- 7/27 placed seeds for stratification in 8 neutral $\frac{1}{2}$ MS circular plates
- 7/31 move to growth chamber
- 8/4 add CH bio peptide protectant to pretreatment group(0, CH bio peptide and 100mM NaCl, CH bio peptide)
- 8/5 add 100mM NaCl to pretreatment and posttreatment group
- 8/6 add CH bio peptide protectant to posttreatment group
- -> 8/10 or 8/11(observe for 6-7days)

Day 5 (8/5) - circular plates planting



Day 0 (8/5) Added NaCl + CH biopeptide



Hydroponics:

- Changes made
 - After paper research reference: Changed MS medium's proportion of $\frac{1}{2}$ MS powder and MES buffer $\rightarrow$ 0.1x MS medium
 - 0.46g of $\frac{1}{2}$ MS powder per liter
 - 0.683g of MES buffer per liter (3.5 mM)
 - For salinity, still use original proportion of MS medium to keep testing conditions constant
- Salinity test results
 - 8/4 - root length & fresh weight
 - 8/5 - Chlorophyll extraction

Control:



Pretreat:



Cotreat:



Post-treat:



- Transfer arabidopsis to hydroponic box
 - 8/2 transfer
 - germination rate: $44/48 = 91.7\%$

B1:



B2:



B3:



B4:



- New set of seeds (set C&D)
 - Set C:
 - 7/30 stratify
 - 8/3 growth chamber
 - used seeds without disinfectant
 - Set D:
 - 8/3 stratify
 - 8/7 growth chamber

Soil Progress

Tray 1:



Tray 2:



Tray 3:



Tray 4:



- Feedback and changes from the soil planting protocol
 - Try to avoid watering from above the soil surface; instead, add water to the tray and let the soil absorb it from the bottom. The only exception is when the soil is too dry that the bottom watering is no longer sufficient to moisten the surface-> even so, use mist form from a distance to prevent damaging plant growth

- Most plants are growing normally and look quite healthy. Only a few are showing significantly slower growth, or have died.
 - Will improve transplanting methods and take extra care to ensure roots are not damaged
- As the plants grow larger, markers placed too close to the plants could hinder their development, so move the tips further away or remove the tips entirely-> removed on august 5th since the plants are large enough to observe now

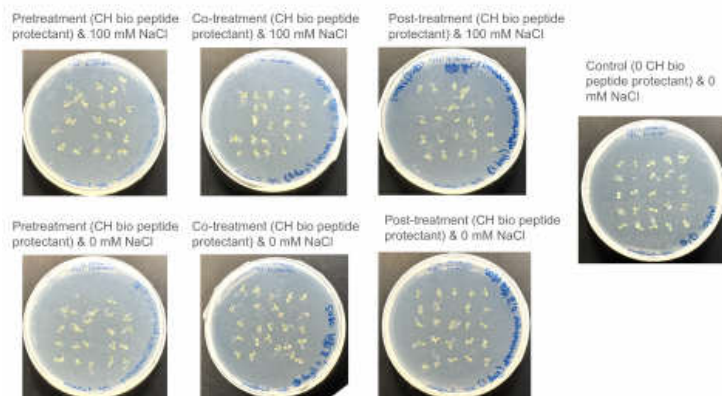
2026-08-12 Progress Report + Meeting with Professor Kyle

Overall Progress:

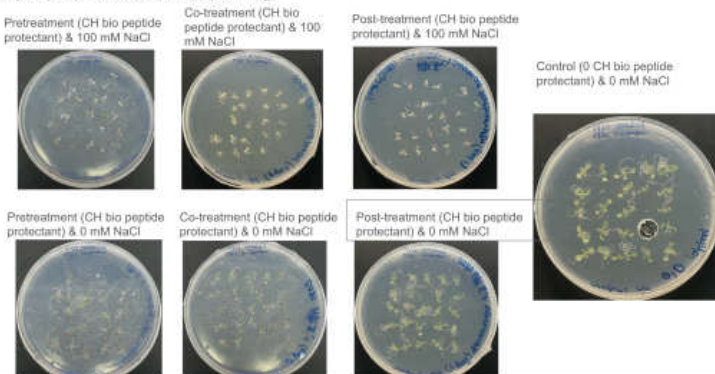
- a. Finished/Collected data for exp set #7 (pre-,co-,post-treatment of CH bio peptide vs. 100mM NaCl)
 - i. chlorophyll content
- b. Redoing exp set #7 pre-,co-,post-treatment of CH bio peptide vs. 100mM NaCl) because of the previous trial's contaminations and inability to observe clear relationship between protectant effectiveness
- c. Another experiment set with 75mM and 100mM NaCl difference but also pretreatment, cotreatment, and posttreatment (softer agar: 0.6% for roots to better penetrate)
 - i. increase sample size to 40 seeds per plate with 3D printed reference
 - ii. timeline
 1. 8/9 stratified 14 plates
 2. 8/13 move to growth chamber
 3. 8/17 add CH bio peptide to pre-treatment group
 4. 8/18 (day 0) add NaCl to all groups and CH bio peptide to the co-treatment group
 5. 8/19 add CH bio peptide to the post-treatment group

Exp set #7 (pre-,co-,post-treatment of CH bio peptide vs. 100mM NaCl)

Day 0 (8/5) - circular plates planting



Day 8 (8/8) - circular plates planting



- Serious bleaching of leaves in the pre, post treatment and 100 mM NaCl group
- No significant rescue effectiveness, but most leaves in the co-treatment remain green, making it relatively more effective than other two timings

Soil

- Feedback and changes from the soil planting protocol
 - Note that for the groups where seeds were sowed directly onto the soil, the soil must not be allowed to alternate between dry and wet cycle
 - If it dries out after the seeds have germinated, the seedlings will die
 - Keep soil surface consistently moist and only begin the dry-wet cycling process one week after the plants have germinated
 - Due to amount of mold buildup in some of the pots, to limit the amount of moisture and separate watering for different pots within the same plate-> use the top and bottom of the circular agar plates to cover each pot
 - Can adjust water amount for each individual pot and also ensure plants still obtain light



- Growth conditions
 - Need to keep soil moist in tray 5 and 6 (before the seeds germinate and under 7 days of germination)
 - Need to use tweezers to remove most of the mold on the soil

Tray 1:



Tray 2:



Tray 3:



Tray 4:



Tray 5:



Tray 6:



Hydroponics

1. Throw away set A (20 day old)
 - a. A1 bolted & didn't grow well



- b. A2 grow mold and didn't grow well



2. Growth status of set B (16 day old)
 - a. One board is sinking (water overflowing on the board)
 - b. Plants are very small
 - c. Didn't add O₂
 - d. throw away

B1:



B2:



B3:



B4:

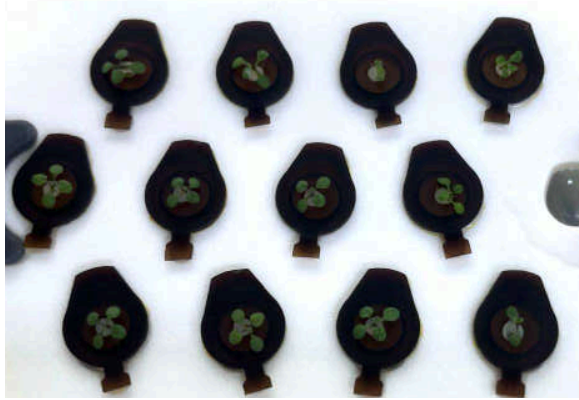


3. Growth status of set C (9 day old)
 - a. plants in C4 are smaller than plants in other boxes
 - b. germination rate: $48/48 = 100\%$
 - i. one plant grew mold (didn't put into box)
 - c. plan to do heat stress for this set

C1:



C2:



C3:



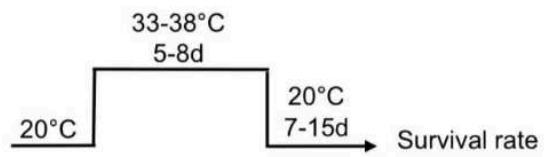
C4:



4. Set D (5 day old)
 - a. still haven't transferred to hydroponic box
 - b. also do heat stress

- Heat stress protocol

TMHT (Thermotolerance to **M**oderately **H**igh Temperature)



- 10 day old arabidopsis
- 37°C for 7 days
- recover 7 days

2026-08-13 Progress Report + Meeting with Professor Kyle

Experimental Plan

1. Conduct a larger number of horizontal plate experiments and increase the number of shelves in the growth chamber.
2. Prepare 2–3 plates for the same treatment group to increase the sample size and collect biological triplicates.
3. For the CH bio peptide protectant, three treatment application timings are currently being compared:
 - Pre-treatment
 - Co-treatment
 - Post-treatment

II. Current Experimental Issues and Improvements

Issue 1: Uneven coverage of the Protectant / NaCl solution

- Currently, some plants may not be completely immersed in the solution.
- Improvement: Add a larger volume of solution (Add 3 mL and remove 2 mL or 2.5 mL to avoid inconsistent residual volumes.) At the beginning, let it stand for 1 min, and then remove all of the liquid.
- Purpose:
 - o Ensure that every plant comes into complete contact with the solution.
 - o Avoid prolonged immersion that could cause waterlogging stress.

Issue 2: Treatment method for the Co-treatment group

- It is recommended to prepare the Protectants + NaCl as a master mix first, and then add the master mix to each agar plate.

Issue 3: Agar surface is too hard, preventing normal root penetration

- A hard agar surface may prevent roots from penetrating the agar, thereby affecting interpretation of the experimental results.
- Improvement already completed:
 1. Seal the plate immediately after pouring the agar.
 2. Open the plate lid after the agar has solidified.
 3. Place the agar upside down and allow the upper surface to dry.

III. Current Results and Follow-up Confirmation

- Based on the preliminary observations from this experiment, the Co-treatment appears to be more effective than the Pre-treatment and Post-treatment, but this result still needs to be confirmed.
- It is currently uncertain whether the CH bio peptide treatment alone causes plant growth inhibition; this needs to be continuously monitored.

Preliminary result:

Co-treatment > Pre-treatment / Post-treatment

(This is a preliminary observation and requires further validation.)

IV. Image and Data Quality

Issue 4: Insufficient resolution of plate images

- The resolution of the current plate images is insufficient and may affect subsequent phenotype assessment.
- Improvement: On the day of sample collection, open the plate and take photographs directly. (Both the front and back can be photographed.)

Issue 5: Chlorophyll data are inconsistent with the actual photographic results

- A possible reason is that the experimental groups may have been mislabeled or mismatched during sample loading.
- Improvement:
 - o Before starting sample loading, confirm and clearly label each experimental group again.

V. Recommendations for Follow-up Protectant Experiments

If the results using the CH bio peptide confirm that Co-treatment has a significant effect, the self-produced Protectants can be prioritized for Co-treatment in subsequent experiments.

However, to fully compare the effects of different treatment methods, it is still recommended to compare all three groups—Pre-treatment, Co-treatment, and Post-treatment—to determine the effect of different treatment timings on Protectant efficacy.

Hydroponic Experiment

I. Culture Conditions and Observation Parameters

1. The hydroponic system requires aeration to ensure that the plant roots receive sufficient oxygen.
2. Plants with the same growth duration can be compared to evaluate:

- o 1/2 MS medium
- o 0.1X MS medium

Determine which medium is more suitable for use as the hydroponic culture medium in subsequent experiments.

3. Observe:

- o Root system / root phenotype
- o EC value
- o Plant growth status

II. Heat Stress Conditions

- Heat stress temperature: 37°C.
- Preliminary test duration: 5–7 days.
- The purpose of the experiment is to observe plant recovery after different durations of heat stress and select the most suitable treatment duration for subsequent formal experiments.

III. Plant Grouping

Current procedure:

1. Before starting the experiment, remove plants with unhealthy growth.
2. Evenly distribute the remaining healthy plants among the treatment groups.

Total: 36 plants

Hydroponic box | Number of plants | Treatment

Box 1 | 9 | Control

Box 2 | 9 | Pre-treatment

Box 3 | 9 | Co-treatment

Box 4 | 9 | Post-treatment

IV. Heat Stress Experimental Conditions

- Temperature: 37°C
- Duration: 5 days (preliminary condition)
- Total plants: 36

- 4 hydroponic boxes
- 9 plants / box
- Treatment groups:
 - o Control
 - o Pre-treatment
 - o Co-treatment
 - o Post-treatment

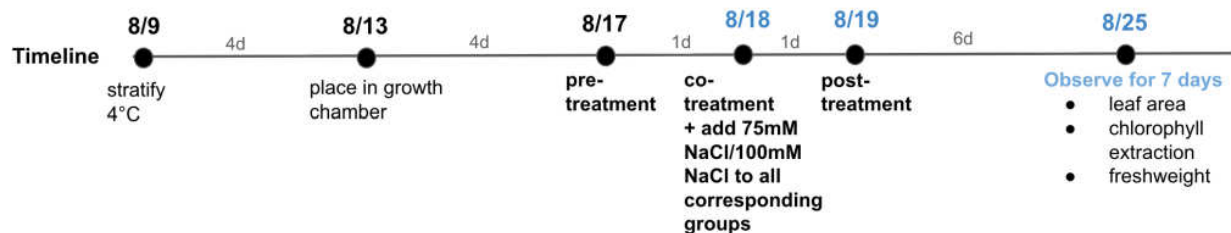
Key Points for Follow-up Confirmation

1. Confirm whether the Co-treatment with the CH bio peptide is truly superior to the Pre-treatment / Post-treatment.
2. Determine whether the peptide treatment alone causes growth inhibition.
3. Confirm whether the chlorophyll data correctly correspond to the phenotypes.
4. For heat stress, first compare 37°C with different treatment durations (5–7 days) and evaluate recovery, then determine the final experimental conditions.

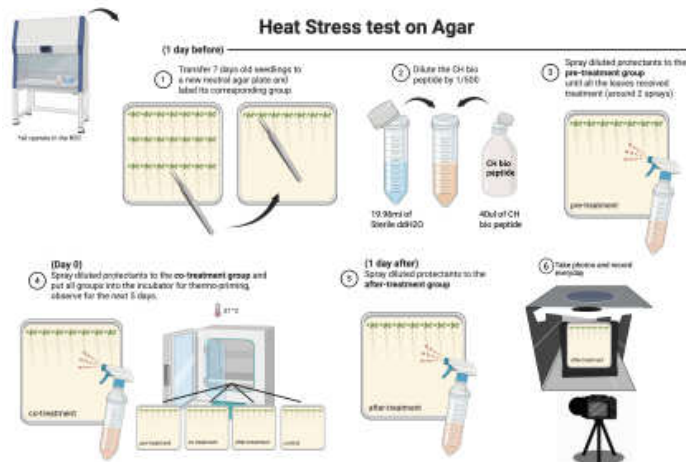
2026-08-19 Progress Report + Meeting with Professor Kyle

Overall Progress:

1. Doing Exp set #7 trial 3 (75mM, 100mM NaCl vs. CH bio peptide protectant pre, co, and post-treatment)
 - a. majority of plants penetrated the agar but a few plants in each plate were still floating around because of the water droplets caused by humidity
 - b. 12 plates in total
 - c. Timeline
 - i. 8/17 pre-treatment
 - ii. 8/18 co-treatment + apply NaCl
 1. method to prevent surface tension that causes uneven treatments: add 3ml of liquid first until the entire agar surface and plant are slightly submerged and have contact with the salt/peptide, then suck out the excessive 2ml.
 - iii. 8/19 post-treatment



2. Doing first heat stress testing on agar
 - a. Decided to do thermo priming inside the incubator instead of heat shock
 - b. vertical planting → transferred 7 days old seedlings to 4 new neutral agar plates (corresponding groups: control, pre-, co-, post-)
 - i. 12 plants/per plate

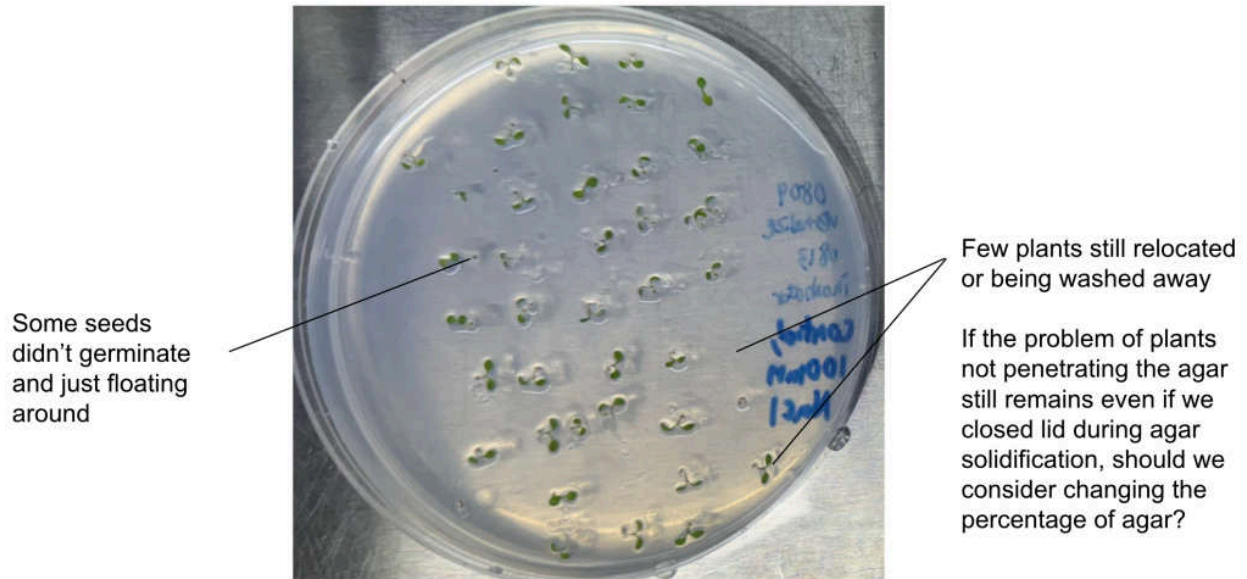


-> As it's vertical plates, we shifted our application method to spraying:

1. *Dilute the CH bio peptide into a 50ml centrifuge tube
2. apply an individual sprayer that is cleaned and sterilized
 - a. spray the exterior with alcohol evenly
 - b. clean the interior by connecting the tube to sterile ddH₂O and spray ~20 times to ensure the alcohol inside is washed out by the water
3. spray peptide with a distance ~15cm in between toward the entire plant from leaves to roots

(around 2 sprays: one on left and one on right)

Exp #7 trial 3 Problems



-> solutions and changes: suck out the excess water with pipette before taking pictures every day to prevent excess moisture and water from washing seeds away, lower agar percentage to 0.6%

Incubator setup

Because the white light itself is not long enough, we tried to adjust a position that provides light to all plants inside

Place on a rack, maintaining the slightly slanted angle



Planned Trials : ACCD testing

- 0818
 - stratified 12 plates(trial 1)
- 0819
 - stratified 12 plates(trial 2)

- refining ACCD agar testing protocol (discussing with protectant group)
- do a trial(1 and 2) first without enzyme activity assay as pretest trials

Soil

- The plants in trays 1–4 are generally growing well. However, for a few plants that have slower growth and higher accumulation of anthocyanins, won't collect their seeds (specifically, don't collect seeds from the plants in the top-right and bottom-right positions of tray 1 or the plants in the top section of tray 2)
- Noticed some small and yellowing leaves
 - likely just older leaves reaching the end of their lifespan
- The remaining plants are in good condition
 - begin inserting bamboo stakes to support the inflorescences, in preparation for future seed collection
- For tray 6, if any areas of the surface appear dry, mist them with water before replacing the cover.

Tray 1:



Tray 2:



Tray 3:



Tray 4:



Tray 5:



Tray 6:

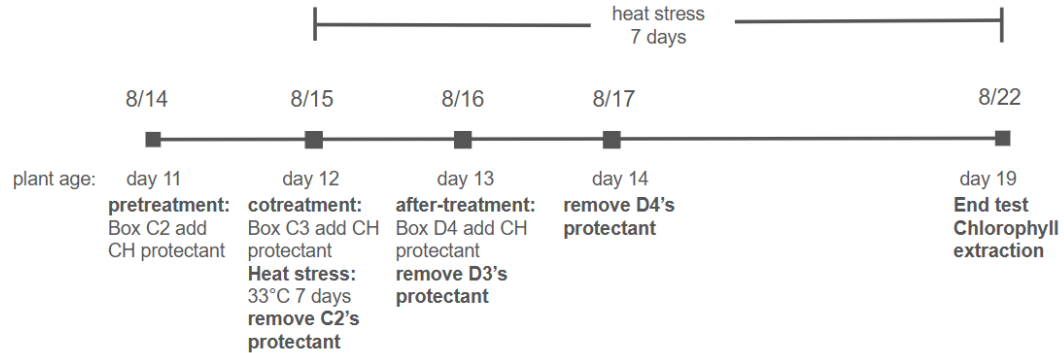


Hydroponics

- Heat stress 33°C (set C)
 - we placed the boxes in the incubator (setting: 37°C), but the thermometer measures 33°C
 - Use CH bio peptide protectant, treat for 1 day and remove after 1 day.
 - Leaves became yellow(chlorosis), some bleached

Timeline:

*aftertreatment = post-treatment



- Heat stress 33°C pictures (4th day of heat)

C1(control):



C2(pretreatment):



C3(co-treatment):



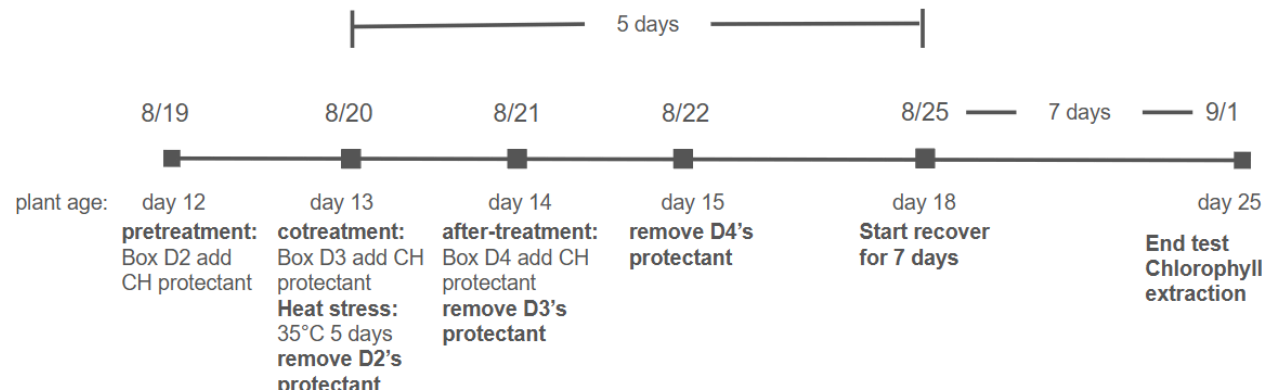
C4(post-treatment):



- Heat stress 35°C (set D)
 - added pre-treatment's protectant today
 - will add heat stress tomorrow
 - also 1 day of CH bio peptide protectant and remove after 1 day
 - threw away the smaller plants, and evenly distribute the rest of the plants into the 4 boxes (control only has 9 plants, the rest have 10)

Timeline:

*aftertreatment = post-treatment



- Set D pictures (haven't heat stress yet)
- D1(control):



D2 (pre-treatment):



D3 (co-treatment):



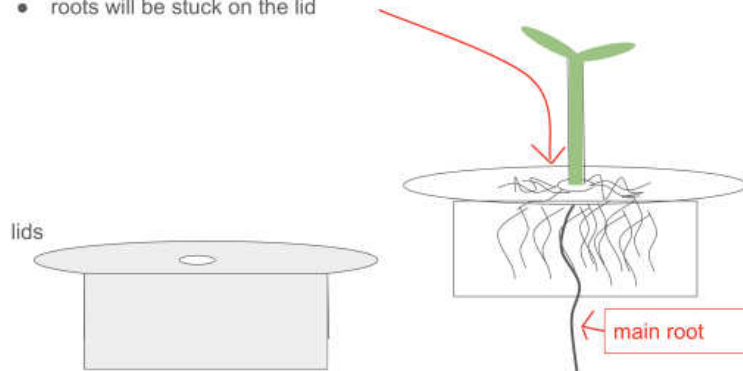
D4 (post-treatment):



Current problems:

1. hydroponics

- roots might not penetrate the agar
- roots will be stuck on the lid



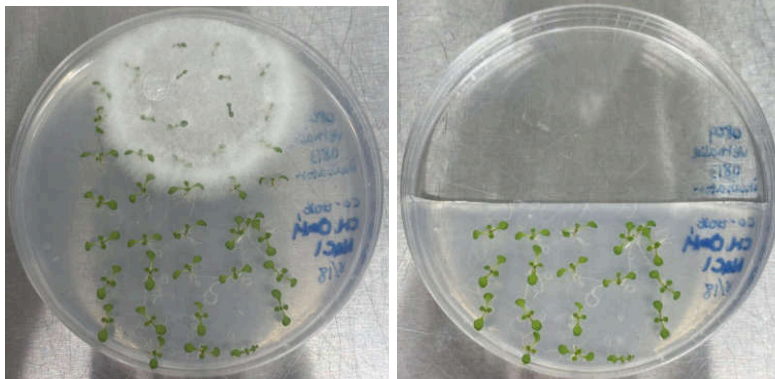
-> solution:

- lower the agar concentration (research paper shows 0.65% agar) to increase the ratio of the roots penetrating
- if the roots didn't expand-> might not grow well due to lack of nutrients or lack of exposure to oxygen

-> possible extension/validation experiments

- Transfer a few seven-day-old seedlings, originally grown in a vertical tray, and transplant them into a hydroponic setup, securing them onto a floating board
 - Goal to see whether plants whose root systems are already well-extended will exhibit better growth
 - Check if there are any suitable plants currently available that can be used for this test

2. agar(horizontal)



-> the initial plate has contamination on the top half

-> solution: cut off the top half of the agar with a sterilized knife

3. agar(vertical heat stress)



-> ants crawled into the agar and contamination of the heat stress agar

- Reason: the sucrose attracted the ants and breathable tape may not be sealed tight enough

Soil

- Tray 5,6,7(directly sowed seeds instead of transferring) seem to be growing slower than trays that transferred using 7-day-old plants
- Use plant support clips and chopsticks to support the growing stems

Tray 1:



Tray 2:



Tray 3:



Tray 4:



Tray 5:



Tray 6:



Tray 7:



2026-08-26 Progress Report + Meeting with Professor Kyle

Overall Progress:

1. Finished collecting exp set #7 trial 3 (0mM, 75mM, 100mM NaCl vs. CH bio peptide protectant pre, co, and post- treatment)
 - a. On 8/20, cut the moldy agar plate (co-treatment, control) in half to remove the mold
 - b. Sustained for one day without mold, next day (8/23) the mold had covered the entire surface
 - i. (Originally planned to finish observing on 8/25, we are two days early)
 - c. Measured fresh weight on 8/23, chlorophyll content on 8/24
2. Have a new set of stratified seeds from 8/18, in growth chamber in 8/22
 - a. Pre-treatment 08/26 (today) using new ACCD
 - i. Removing water using a pipette to prevent it from diluting the ACCD
 - ii. Changing application method to spraying 3 sprays on the agar surface at around 15cm away from the plate
 - b. Will collect data on 9/2 or 9/3
3. Have a new set of vertical testing plates currently stratified/in the growth chamber
 - a. Planning to try a new set of vertical plates with better lighting/sealing

Standards for picture taking:

1. Black background
2. No overexposure
3. No reflections
4. The conditions of every picture needs to be the same (exposure timing, white balance, IOS all need to be consistent)
5. Clear focus
6. Don't take the pictures at a slight angle or the plant leaves will differ in size due to difference in distance

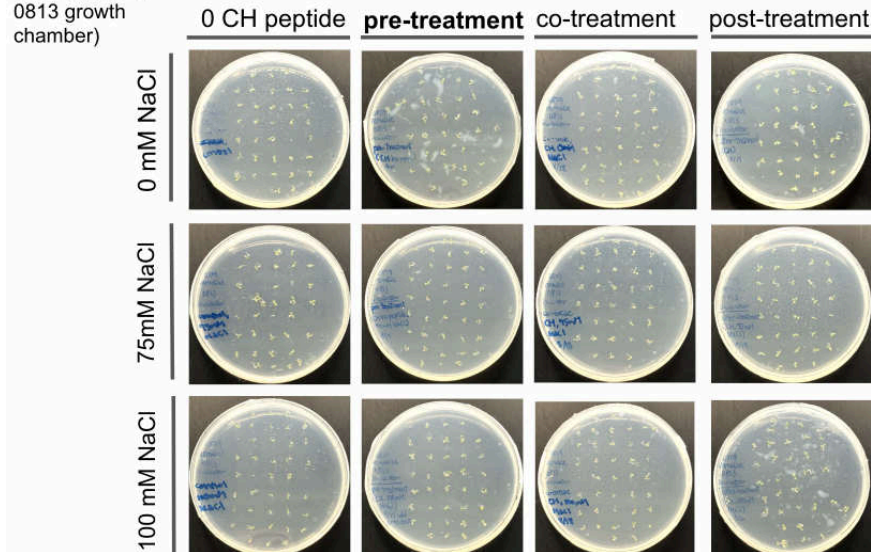
Exp set #7 trial 3

(0mM, 75mM, 100mM NaCl vs. CH bio peptide protectant pre, co, and post- treatment)

- Need to repeat this trial again for more consistent data collection and due to contamination less effectiveness shown
- Place the agar plates under a paper or black background to better show the roots and leaves phenotype and have consistent lighting for better comparison

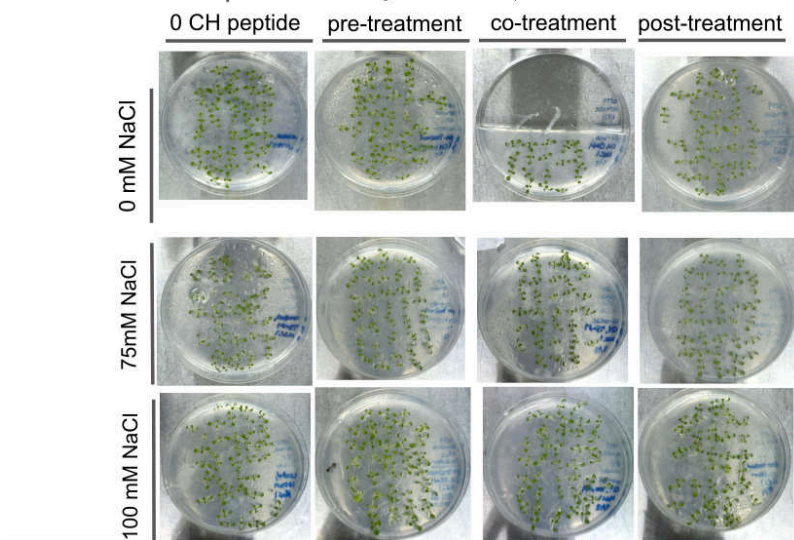
260817 circular plates (Added CH peptide to pretreatment)

(0809 stratified
0813 growth
chamber)



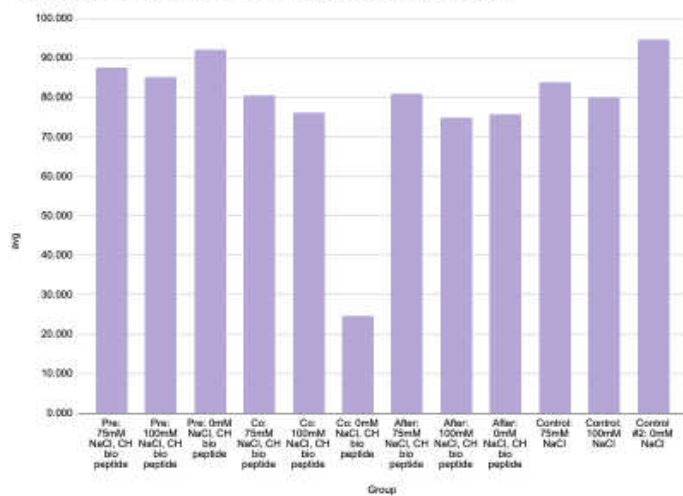
260822 circular plates

(0809 stratified
0813 growth chamber)



-> Chlorophyll data

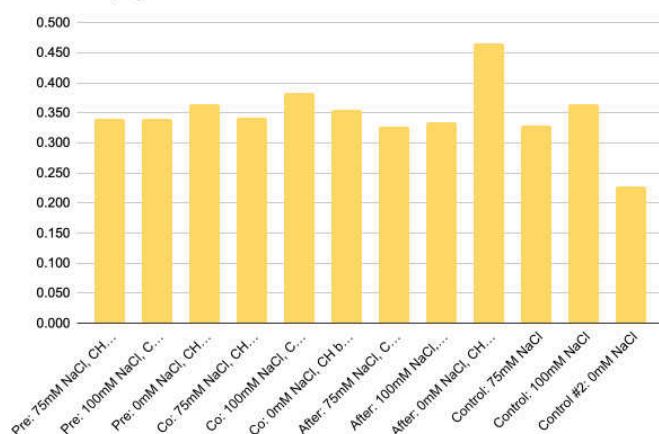
Chlorophyll content without fresh weight normalization (mg/g)



Without normalization (*aftertreatment = posttreatment):

- Chlorophyll concentration of co-treat 0 mM NaCl is especially low because of the moldy seedlings and the seedlings that we didn't include
 - For others, we took all 40 seedlings and added 10 metal beads and shake until the leaf fibres are white)
- Rescue effectiveness is shown in the co-treat, 75mM NaCl and post-treat, 75mM NaCl groups (CH peptide has better rescue effectiveness at 75mM NaCl rather than 100mM)
- Overall In this trial, CH bio peptide on pretreatment seems to have better rescue effectiveness than co-treatment
 - Opposite than the results shown from trial 1

Chlorophyll Content



With normalization (*aftertreatment = posttreatment):

- Out of the three co-treatment groups, 100mM NaCl had the highest chlorophyll content
 - When taking pictures, we noticed larger leaf area and also a brighter green

2. This time the control groups did not grow as well, had a lower chlorophyll concentration compared to the ones with NaCl
3. Overall, post-treatment had the least amount of rescue effectiveness and co-treatment had the highest average chlorophyll content

Common problems:

1. Chlorophyll content of experiment set 7 trial 3 still doesn't exactly align with the visual observation of the recorded pics of agar plate pictures
 - a. Possible reasons
 - i. Lost of samples during the process of grinding the leaves
 - ii. Volatilization(evaporation) of acetone
2. Control group receives no volume of liquid while 1mL of protectant is added to other testing groups-> inconsistency in moisture level

Solutions:

1. Take pictures of each centrifuge tubes after finish extracting chlorophyll and compare with the ELISA reader results to ensure the results match (such as the color and intensity of green)
 - a. Add a lid/plastic wrap onto the rack with samples to avoid sample solution from evaporating
2. When applying protectants, need to ensure the control group also receive equal amount of liquid as it also affects growth conditions
 - a. E.g. adding 1mL of protectant to treatment groups and also adding 1 mL of sterile ddH₂O to the control group-> same for the spraying method(consistency)

Hydroponics

- Heat stress 33°C Results
 - Pre-treatment has highest freshweight
 - Control has highest chlorophyll content
 - Doesn't match with the results from salinity test
 - All leaves are very yellow (doesn't have a big difference)-> chlorosis

Control:



Pretreat:



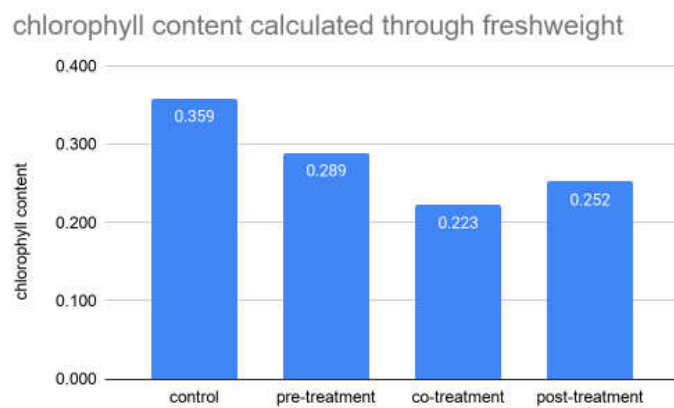
Cotreat:



Post treat:

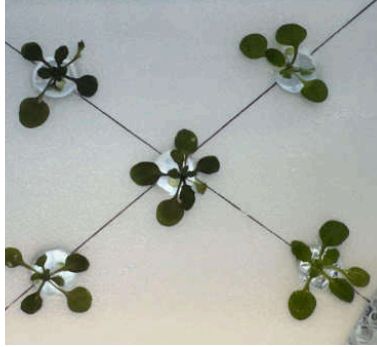


Chlorophyll graph:



- Oxygen and medium testing
 - Boxes with oxygen are growing much better
 - Plants that aren't adding oxygen have Anthocyanin accumulation
 - Leaves at the top left box became yellow and white
 - Contamination in boxes with oxygen

Box $\frac{1}{2}$ MS + O₂ Date: 8/25



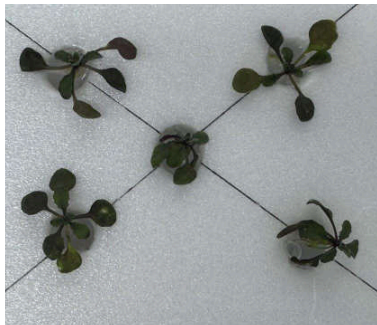
Box 0.1 MS + O₂ Date: 8/25



Box $\frac{1}{2}$ MS without O₂ Date: 8/25



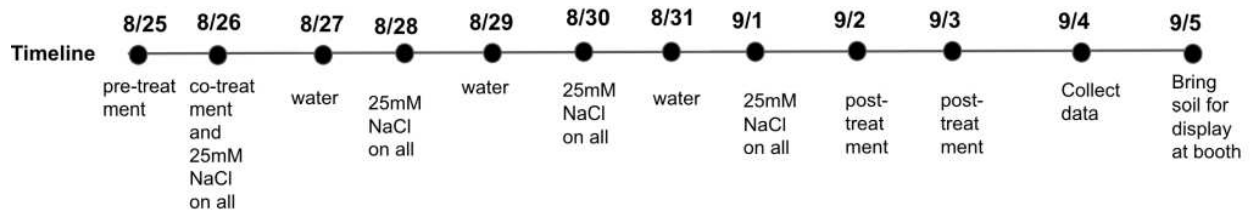
Box 0.1 MS without O₂ Date: 8/25



Soil

- **Progress**

1. (Pretest + for green forum showcase) Purpose: want to demonstrate results at 9/5 forum event so referenced a paper that uses a month old arabidopsis for experimenting
 - a. build up of salinity(25mM) per day until it reaches final concentration of 100mM (suggested by Dr. Verslue)
2. Originally two day pre-treatment (8/23, 8/24), did Western blot and found out there's no ACCD in the *B Sub*.
 - a. Changed to 8/25 start pre-treatment(originally 2 days to 1)
 - b. 9/4 data collection:
 - i. Rosetta area, morphology, survival rate, amount of seeds collected



- **Protectant (ACCD) application on soil method**

- Dilute the 3M NaCl stock to 100 mM NaCl → 1.65ml of 3M NaCl stock + 48.35ml of sterile ddH₂O = adds up to total volume of 50ml
- Pour the 50 ml of 100 mM NaCl to the surface of the soil evenly, let the NaCl soak in the soil until reaching the bottom of the tray
 - We tested how much of NaCl added is able to sink to the bottom until it leaks a little bit out today, typically around 50ml for the majority of pots but some pots soaked in faster with only around 30ml)



-> Ensuring bottom has a little leakage of NaCl solution, meaning it has soaked in the soil

2026-09-01 Progress Report + Meeting with Professor Kyle

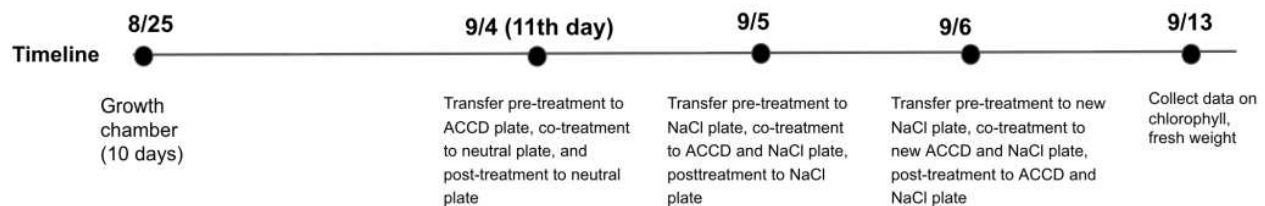
Overall Progress:

1. Problems

- a. The agar with ACCD added all are contaminated by bacteria, only control and control+salinity are not contaminated)
 - i. Reason: only removed some impurities in ACCD
 - ii. Solution: need to filter again in laminar flow hood with 0.22 μm filter

2. Planned trials:

- a. 0825 in growth chamber, 0904 transfer and 0913 collect data
 - i. Transferring instead of adding directly into the agar plate



Problems:

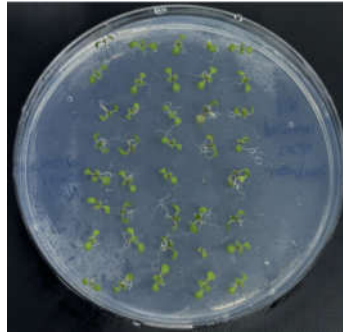
1. Water Droplets Issue

- problem: water droplets accumulate on the lid, slight movements easily cause the droplets to submerge the plants
- We tried to blow dry the agar in the bsc with the lid open, and flick the lid using finger to get rid off the droplets
 - Feedback: this will cause water loss of agar and cause shrinkage in agar size so do not leave agar exposed without a lid inside the BSC for too long(around 30 seconds to 1 minutes is fine)
- solutions:
 1. When water droplets are still small:
 - a. Can leave the lid opening facing upward to slightly dry out
 - b. However, must simultaneously cover the plants and agar with another sterile lid to prevent direct exposure
 2. Use humidity chamber
 - a. First test whether which conditions reduce the most water droplets - using 3 agar plates containing same size of plants, with different sealing methods
 - i. Parafilms with holes poked, inside humidity chamber(transparent box)
 - ii. Without parafilm, inside humidity chamber(transparent box)
 - iii. Double breathable tape wrapped around the plate, inside growth chamber
 1. goal: Determine which environment results in less transpiration to reduce water droplet condensation issues

ACCD pretest on agar results:

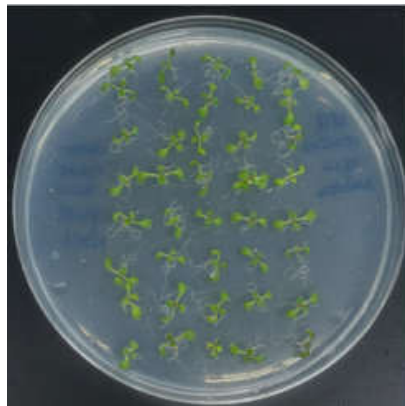
- valuable result: Post-treatment showed better growth compared to control

- Current plates are contaminated too severely, so we can only use results before the contamination and bacteria grew -> **8/30 results**
- Despite ACCD having contamination issues itself, pre-treatment and co-treatment have signs of inhibiting plant growth (plants not growing well)
- But post-treatment groups grew better than control groups despite just 2 days of treatment → suggesting that post-treatment ACCD does have a significant and observable rescuing effect
- Verified that NaCl inhibits plant growth: 0 ACCD and 100mM NaCl has anthocyanin accumulation on the center of the plants

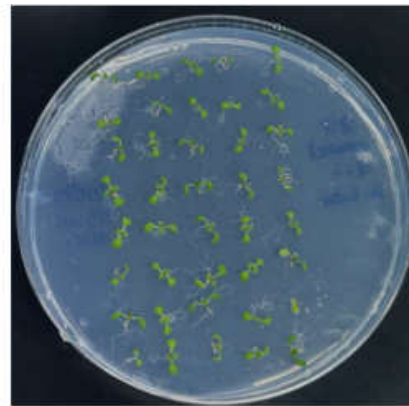


(control, 100mM)

- ACCD post-treat rescue obviously has larger leaf area and root length



(Post-treat ACCD, 75mM NaCl)



(Control, 75mM NaCl)

Current ACCD testing experimental design problems

- Transferring 10-day-old seedlings from agar is not very feasible
 - Roots may have already penetrated the agar, making transfer likely to damage roots
 - By ~7 days, roots are already tangled together
- Other method suggestions (but both involves re-planting a new set)
 1. Apply a mesh on the surface of the circular horizontal agar before planting new seeds, so when transferring, lift all of the plants together
 2. Stratified seeds and incubate plants in growth chamber through vertical planting, as the roots grow along the agar surface → transfer is easier and won't damage roots
- Solution for now: observe if 10-days-old seedlings have serious tangle issues, and if too tangled, use the same application method as previous CH bio peptide trials (drench the surface by adding 1ml of protectant/treatment)

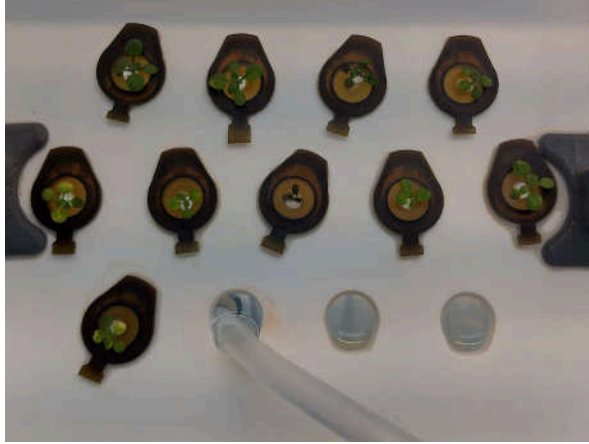
Hydroponics

- Contamination reason testing
 - We tested the contamination reason for hydroponic boxes (10 conditions in total)
 - Transparent box vs opaque box
 - The presence of floating board
 - Adding Oxygen vs without oxygen
 - Growth chamber vs BSC (to check if growth chamber is dirty)
 - Results (contamination status determined by the cloudiness/opaqueness of the medium)
 - transparent box + medium: contaminate
 - transparent box + board + medium: clear
 - transparent box + medium + O₂: contaminate
 - transparent box + board + medium + O₂: contaminate
 - box with black tape + medium: contaminate
 - box with black tape + board + medium: a little bit contaminate
 - box with black tape + medium + O₂: contaminate
 - box with black tape + board + medium + O₂: contaminate
 - medium in beaker: contaminate
 - transparent box + medium + BSC: clear
 - transparent box + board + medium + BSC: a little bit contaminated(not much)
- Set F (11-day old arabidopsis)
 - All uses 0.1x MS medium and add oxygen
 - serious contamination
 - not growing well
 - not doing any testing on these

F1:



F2:



F3:



F4:



Soil



1. Adjustments in groups

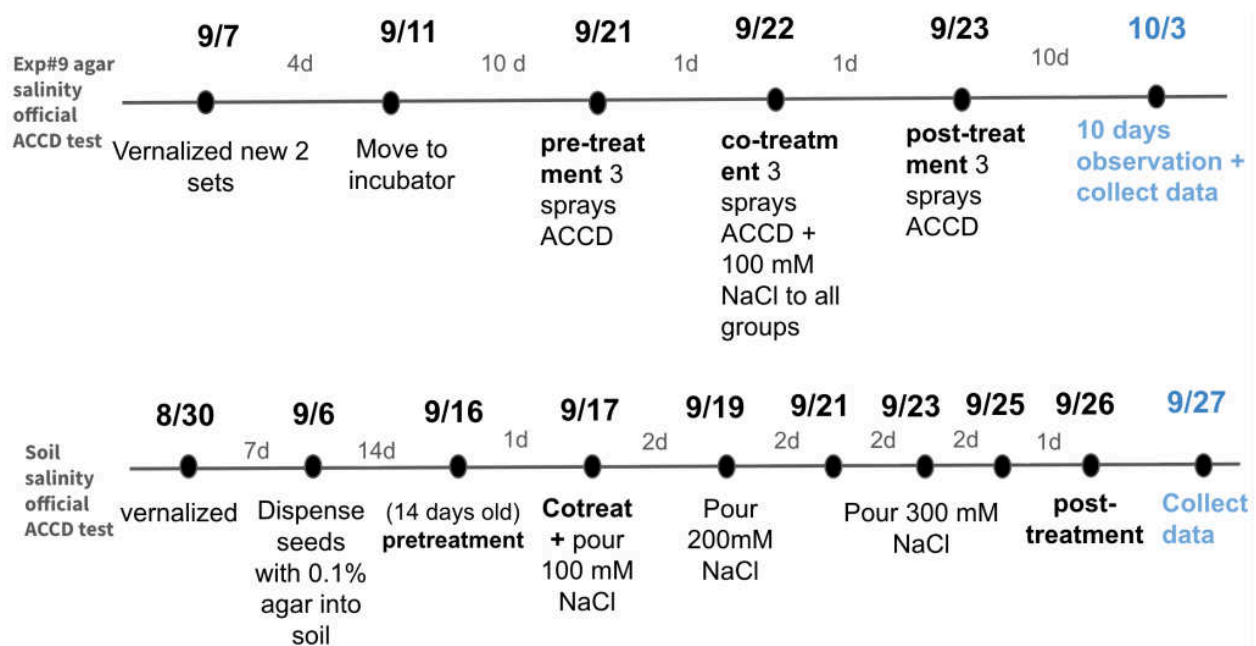
- a. original soil ACCD pretest-> not able to have significant visual differences and timeline

2026-09-08 Progress Report + Meeting with Professor Kyle

Overall Progress:

- Finished exp set#7 trial 4 (0mM, 75mM, 100mM NaCl vs. CH bio peptide protectant pre, co, and post- treatment) + exp set#8 ACCD pretest (0mM, 75mM, 100mM NaCl vs. ACCD pre, co, and post- treatment)
- Majority of 0821 and 0823 (exp#9 official ACCD trials) stratified sets in the growth chamber has contaminated
 - (reason: high humidity stored inside the plates)
 - adjustments
 - Use the remaining 4 plates (2 of 0821, 2 of 0823) to repeat post-treatment vs. control experiments
 - restarting exp#9, stratified x2 new sets today (7 of 0.8% and 0.6% each) → 2 repeats
 - will make sure to dry plate lids inside laminar flow hood during incubating
 - Stratified new sets every week → multiple trials

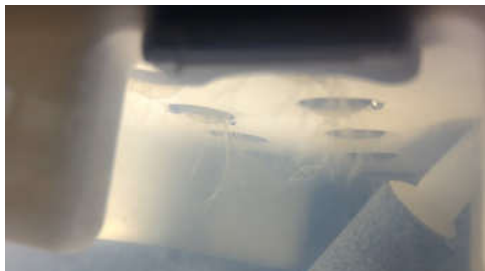
Timelines:



Hydroponics:

- Set F (0.6% agar) (19-day old)
 - Almost all of them have accumulated anthocyanins (the leaves are very dark)
 - The contamination is extremely severe
 - Only three plants look to grow somewhat better
 - The ones that have grown larger have also developed roots

F1:



roots

F2:



roots

F3:



roots

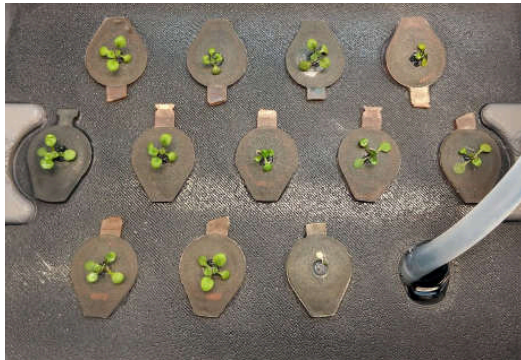
F4:



roots

- Set G (0.6% agar) (11-day old)
 - No aeration initially; aeration was introduced only when plants reached 7 days old
 - At 7 days old, they were transferred to an opaque container
 - The contamination issue in the medium has improved somewhat
 - The G3 plants are growing particularly poorly
 - The plants are slightly smaller than those from the previous batch

G1:



G2:



G3:



G4:



2026-09-15 Progress Report + Meeting with Professor Kyle

Overall Progress:

- Ongoing trials
 - group 1(0911 in growth chamber): test control experiment(if the buffer has effect on plants alone) → the plants are still too small
 - 2x agar (buffer/ACCD)
 - group 2(0911 in growth chamber) CH peptide experiment → canceled
 - 7x 0.6 agar(retry experiment set 7 trial 5-> data not align with images)
- Planned trials
 - group 3(0917 in growth chamber)-> remain 4 days in growth chamber
 - 12x for ACCD testing (trial 1 same conditions as pretest)
 - group 4(vertical plates(0917 in growth chamber->7 days old)
 - n=2
 - yes heat stress: control, pre,co,post-treat
 - no heat stress: control, pre,co,post-treat
- Discussion(possible data collection aspects)
 - circular plates 40 plants
 - x10 for freshweight/chlorophyll
 - x10 for MDA
 - x10 for total protein
 - x10 for relative expression level

Problems and Solutions:

1. 2x 0.8% and 6x 0.6% agar planned to do experimental set 7 trial 5 and testing whether buffer has effect on plant growth-> shown to be contaminated and the plants are growing very inconsistently
 - a. Solution: keep the two that are not contaminated and let them grow in growth chamber for a few more days since they are too small for testing
 - i. test for control group (x2)
 - ii. stratify new plates as backup for future trials if anything similar happens
 - iii. abandon experiment set 7 trial 5 since CH peptide is baseline tuning (should target on ACCD testing now)

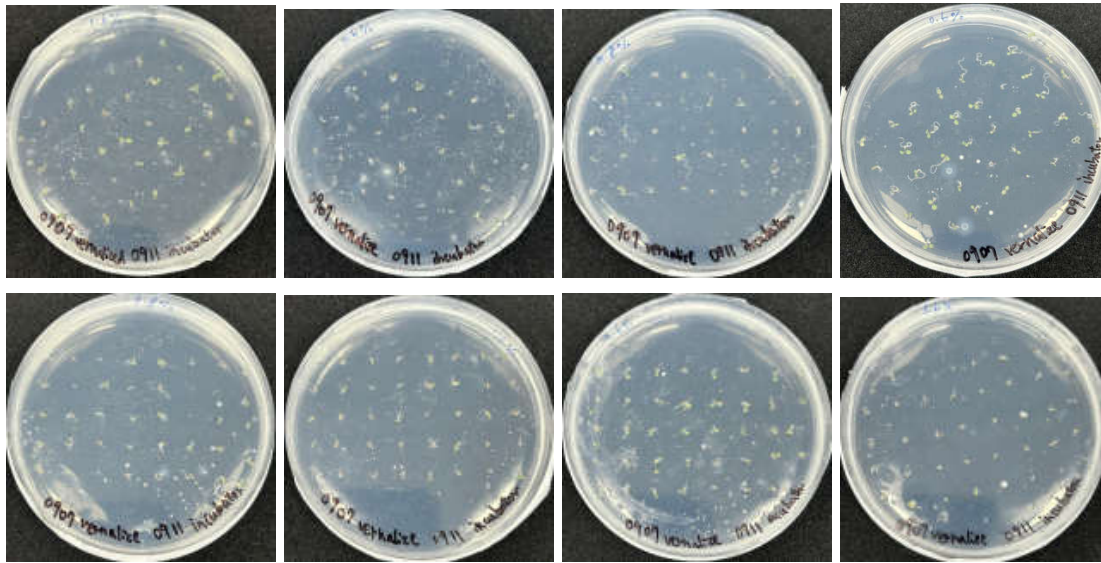
Contaminated plates:

- Possible reasons:
 - Looks like the cover(cap) of the agar plate or the outer layer may already have bacteria
 - There are signs of water spreading out-> it's because of the water vapor and droplets in the plates that brought the bacteria onto the plants

-> solution for now: Don't stick the bamboo sticks into the sterilized water(may not be sterilized enough) and change to slightly touching the agar for moisture before sowing seeds onto the agar

- Two plates that are not contaminated are especially small
 - Wait for a few more days until the plants grow larger and decide on whether to use buffer

vs. ACCD testing



Suggestions for planned trial:

- **Agar**
 - Vertical planting(heat stress): be mindful of the transfer process and prevent damaging the plant roots, plants need to align closely to the agar surface
- **Soil**
 - 2 pots from 0906 can be used to do NaCl stress evaluation
 - Concerned that the 100, 200, 300 mM NaCl accumulation will cause serious salinity stress(concentration too high)

-> plant for now: use these two pots to test 100, 200, 300 mM NaCl salinity wash application method to see if the salinity stress will be too damaging for the plants

Soil

- Ongoing trials(in growth chamber)
 - abandon tray 6 and 7(didn't grow well)
 - 2 month old tray 5 for harvesting seeds
 - harvested some already from tray 1 and 2
 - 2 pots from 0906-> test when 10 days old
 - need to plan
 - 7 pots from 0912
 - salinity testing(review paper protocol again)
- Planned trials
 - 8 pots for heat stress
 - n=2

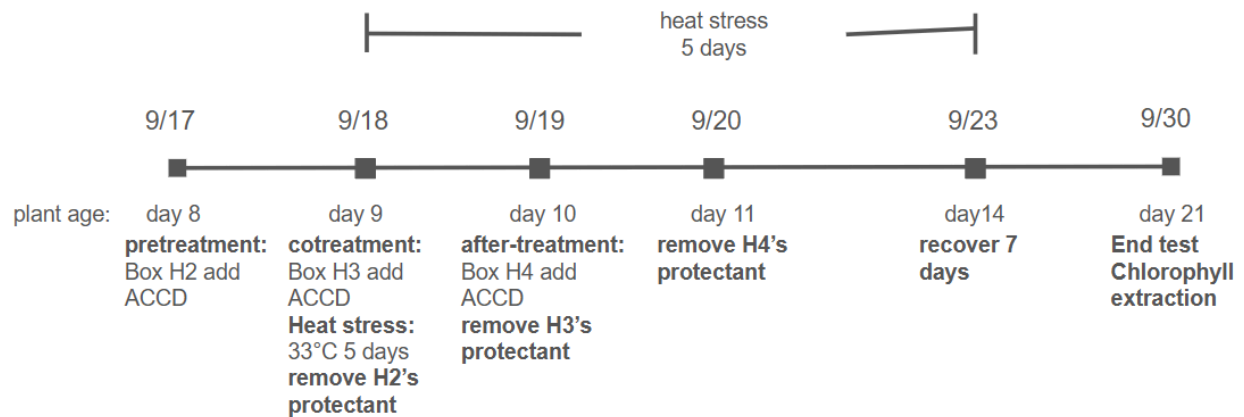
Hydroponics

- Slightly changed planting methods
 - to reduce risk of contamination in hydroponic box
 - grow plants on rack until 7 days old

- then transfer hydroponic box
- we can directly do experiments after transferring arabidopsis
- we used black tape to wrap the box for light blocking
 - significantly less contamination in the box even when pumping oxygen
- 33°C heat stress
 - immediately recover when the plant is not growing well
 - add heat stress when 9 days old (9/15: 7 days old)
 - treat with ACCD, but treatment time is still tentative

Timeline

*after-treatment = post-treatment

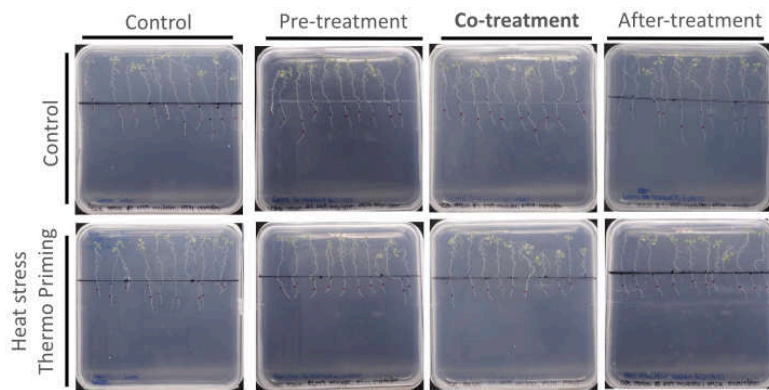


2026-09-22 Progress Report + Meeting with Professor Kyle

Overall Progress:

- (0917 in growth chamber) exp#9 ACCD salinity testing
 - 1002 collect data
- (0917 in growth chamber) ACCD heat stress testing
 - Incubator thermo-priming → 0930 post-treatment
 - 1003 collect data
- (0923 in growth chamber) 2 plates Buffer vs. ACCD redo (add salinity stress, 100mM NaCl)
 - 1002 collect data
- (0923 in growth chamber) Exp#10 LEA salinity testing
 - 0930 pre-treatment
 - 1009 collect data

→ we'll be able to collect the data by the next 10days(after 9/22)

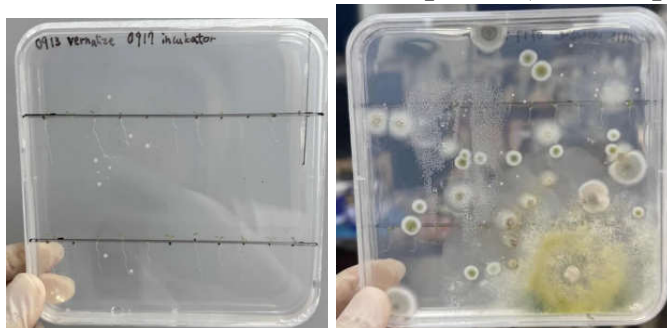


- **ongoing vertical planting heat stress ACCD testing**

heat stress condition:

- 5 days in 37°C incubator → thermo priming
- 5 days recovery in 22°C growth chamber
- adding post-treatment 1 day after taking out from the 37°C incubator

9/21 observed contaminated plates (vertical plates for heat stress)



- all the plates were contaminated-> both fungi and bacteria
 - Some of the plates only contained small bacteria colonies while the most serious is the right plate
- > Solution: we transferred the plants that had no contact with the molds to new plates (enough samples)

for a set) and conducted the ACCD heat stress testing on 9/24 as scheduled

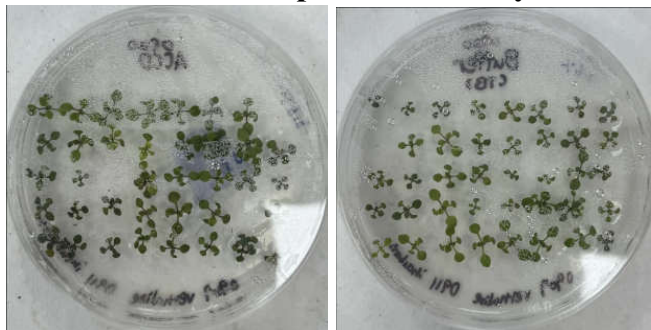
Contaminated plates (exp#9 ACCD salinity circular plates)

- Contaminated mark and trial looks like the water transported the bacteria to the plants and around the agar
- some plates have small bacterial colonies



-> quickly stratify new sets of agar for replacement

Buffer vs. ACCD experiment: 5 days after adding TB and ACCD



(left) ACCD (right) Buffer

- didn't add NaCl to these 2 groups-> effects are not stress induced, meaning the results couldn't validate ACCD "rescue" effectiveness
 - Thus, we're using the two extra plates from the (0923 incubator) group and **redo this experiment again with addition of 100mM stress**, serving as co-treatment (will do experiment on 9/29→ collect data by 10/3)
- ACCD group has shown partially better growth comparing to Buffer group, but not obvious → we interpreted as ACCD effect is only induced when subjected to stress, so the effectiveness won't be obvious under no stress conditions

Soil:

- (0906 in growth chamber) 2 pots papers comparison
 - **0929 collect data**
 - pretest the 2 soil salinity protocols from 2 papers and evaluate the plants' response under stress first to see if the salt stress level would be too severe or has minimal damage to induce protectant effects

- Prof Cheng's paper/wash method: apply stress level by directly adding 100, 200, 300mM NaCl (follow dry and wet cycle) from the top of the soil surface until it washes out
- Accumulation method: accumulate 100,100,100mM NaCl (follow dry and wet cycle) by adding the liquid into the bottom container and let the soil absorb
 - We found more obvious salt stress growth inhibition shown from the plants in the soil pot using Prof. Cheng's paper protocol, whereas the accumulation method didn't cause much stress damage → thus we'll be using prof.cheng's paper for future soil salinity testing to set the baseline stress level
- (0912 in growth chamber) ACCD salinity
 - 0928 pre-treatment ACCD
 - **1010 collect data**
- (0922 in growth chamber) ACCD heat stress

- **2 pots paper comparison result**

0906 growth chamber

Tray 1 - D

Tray 1 - E



Tray 1- D Accumulation method (left)

- No obvious salt stress damage or chlorosis/bleaching
- more larger leaves and expanded rosetta area/growth

If add protectant treatment, may not have obvious rescue effectiveness vs. stressed conditions

Tray 1- E Prof. Cheng's paper protocol

- Can see bleaching and chlorosis of leaves
- smaller leaf surface area and rosette growth
- obvious stunted growth caused by salinity stress

Volume ~30ml

Tray 1- D

9/20 add 100mM NaCl

9/22 add 100mM NaCl

9/26 add 100mM NaCl

(all added to the bottom)

Tray 1- E

9/20 add 100mM NaCl

9/22 add 200mM NaCl

9/26 add 300mM NaCl

(applied directly to soil surface and let it wash off to the bottom)

Hydroponics:

Current progress:

- CH peptide and ACCD heat stress testing under 33°C
 - 9/23 put in 33°C incubator and co-treatment

Planned trials:

- 100mM salinity test with ACCD or LEA (pre-treat: 9/23)
- LEA heat stress testing

Heat stress with ACCD

- plants that sprayed ACCD and TB turned yellow

Heat stress with CH peptide

- all turned yellow
- except for pre-treat, the rest should be the same
- but plants in co-treat box grew the best

Control:



pre-treat:



co-treat:



Post-treat:



2026-10-06 Progress Report + Meeting with Professor Kyle

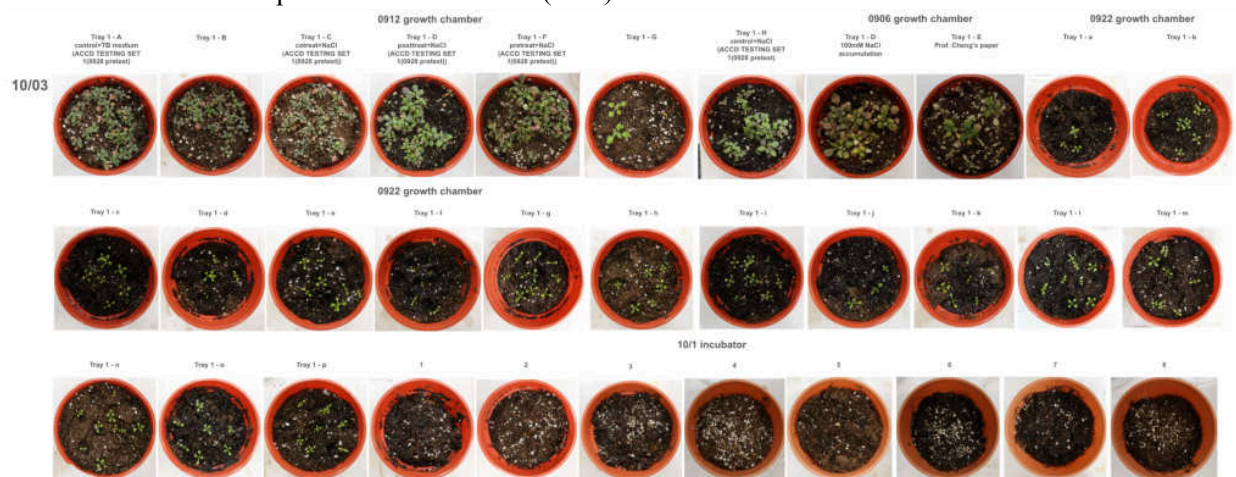
Overall Progress:

Current groups inside the growth chamber

1. (0923 in growth chamber) exp#10 Bopep4 Salinity Testing
 - a. 1004 added post-treatment
2. (0930 in growth chamber) exp#11 LEA Salinity Stress Testing
 - a. 1007 sprayed pre-treatment
3. (0930 in growth chamber) LEA heat stress testing
 - a. 1007 transferred 7 days old seedlings + pre-treatment

Soil:

- Collected two pots paper comparison data (fresh weight, chlorophyll)
- Will be collecting soil ACCD salinity data soon (10/8)
 - Added post-treatment ACCD (10/6)



Two paper comparison final result



(left) Prof Cheng wash method (right) Accumulation method

2026-10-13 Progress Report + Meeting with Professor Kyle

Overall Progress:

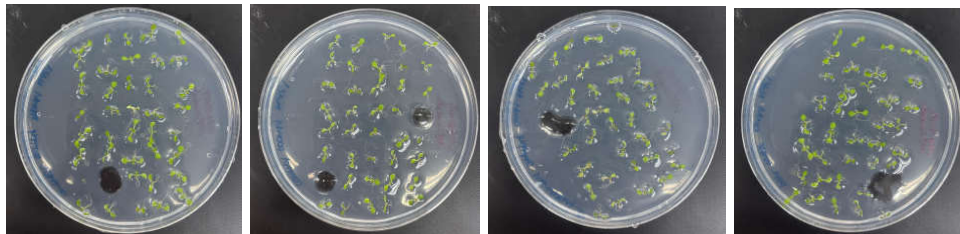
Current groups inside the growth chamber

1. Heat stress trial 2: 1014 take out of 37°C incubator —> will do 3 day observation and data on 1018
2. Bopep4 trial 1 drench: redo pre-treatment groups(initially sprayed instead of drenched)
3. ACCD salinity stress

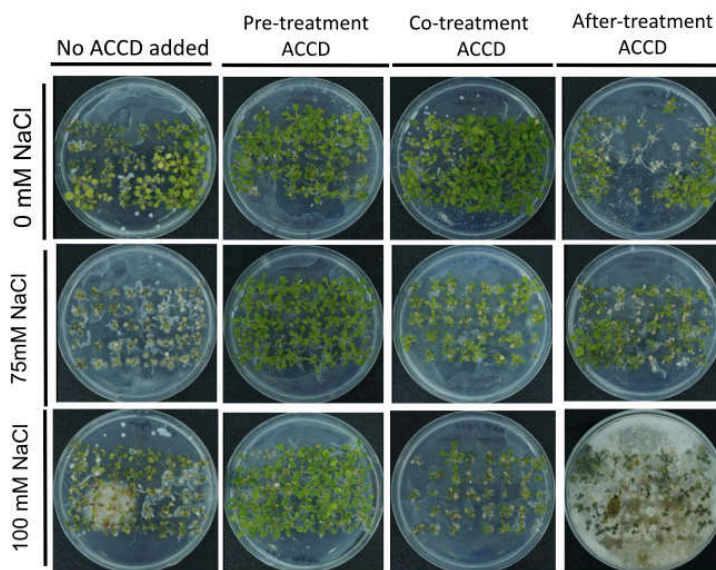
(most of our agar plates had mold on them, so we saved the ones without for ACCD salinity)

4. Bopep4 trial 2 drench: 1014 pretreatment

(we used pipette tips to remove the mold)



- Collected ACCD salinity and heat stress data (fresh weight, chlorophyll content, relative gene expression)
 - To be collected: relative gene expression(for salinity)

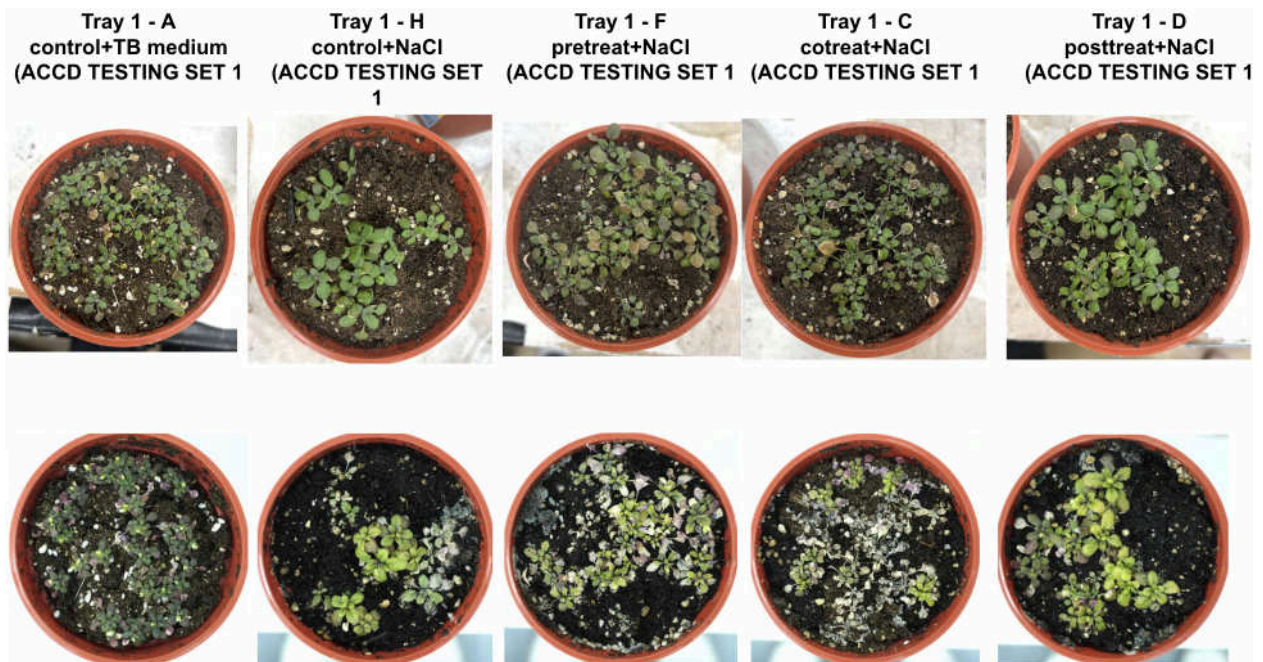


Soil:



- After heat stress, the leaves seemed to grow upwards
 - Normal symptoms of plants being stressed by heat

- **Soil ACCD salinity**

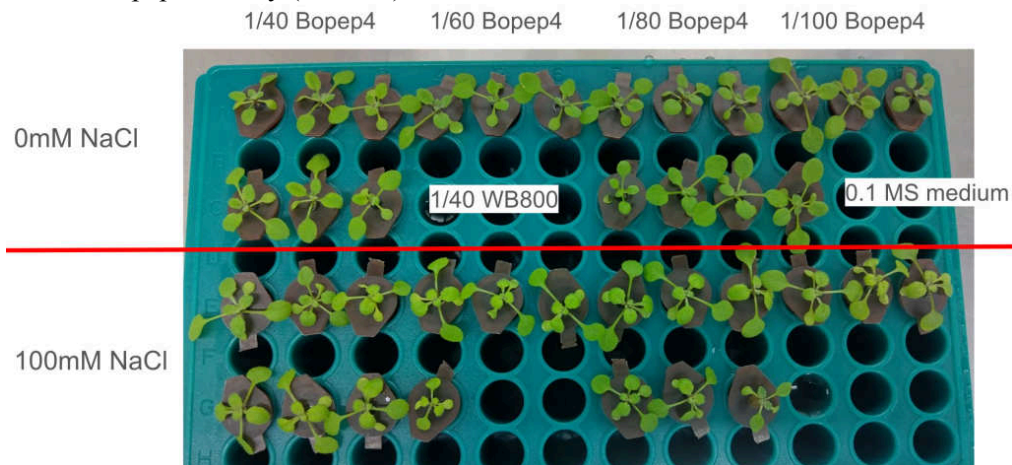


Hydroponics:

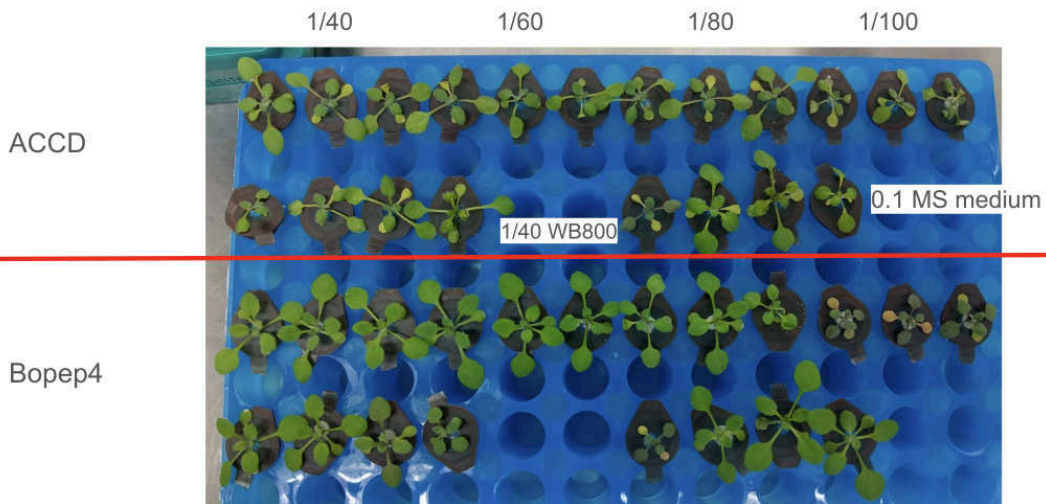
- ACCD salinity results



- Bopep4 salinity (100mM)



- ACCD and Bopep4 no heat



- ACCD and Bopep4 **with** heat

