Lab 7: Effect of Salinity stress at seed germination, and plant growth of glycophytes Sp.)

OBJECTIVES

- (1) To investigate the effect of different salinity conditions on seed germination of glycophytes plants (Dicot vs Monocot).

- (2) To investigate the effect of different salinity conditions on growth of glycophyte plants (Dicot vs Monocot).

- (3) To familiarize simple statistical data analysis.

Background

- Salinity and drought are the two major environmental and abiotic stress factors that can reduce productivity and development of plants.

- The salinity of soil and water is caused by soluble salts due to **the deterioration and dissolving of rock,** as well as concentrated as a **result of evaporation.**

- The problem in the utilization of the vast areas of saline soils and the abundant sources of saline water sources around the world is a long-standing one.

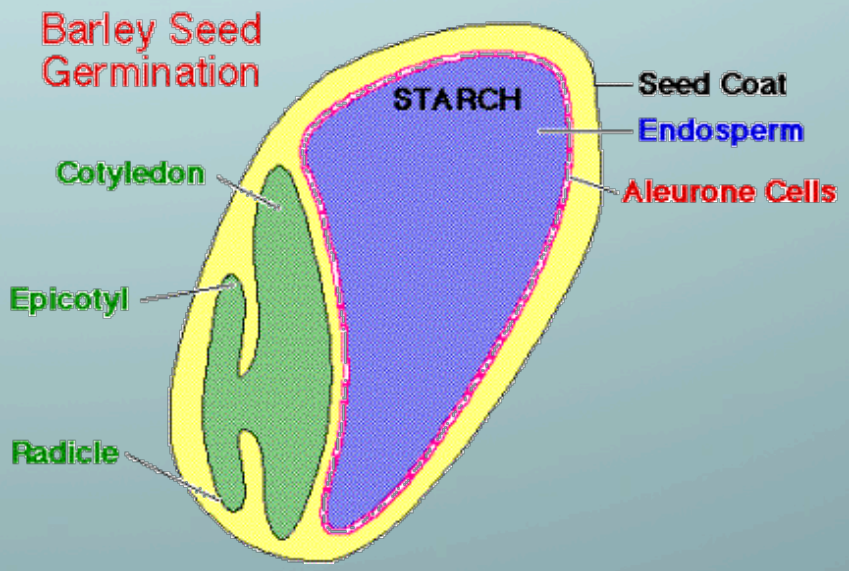
Part I: Seed Germination

Seed Germination Defined

The process by which a dormant seed begins to grow into a seedling under the right growing conditions.”

Seed Anatomy

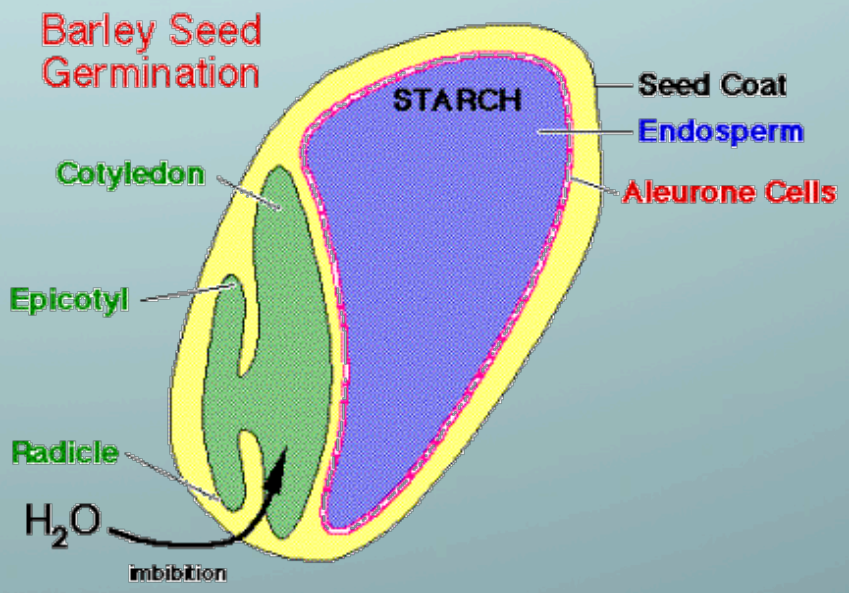
- Seed coat provides protection
- Endosperm = food (STARCH)
- Aleurone cells = store abundant protein



- Cotyledon → leaves
- Epicotyl → shoot
- Radicle → root

Process: Seed Germination

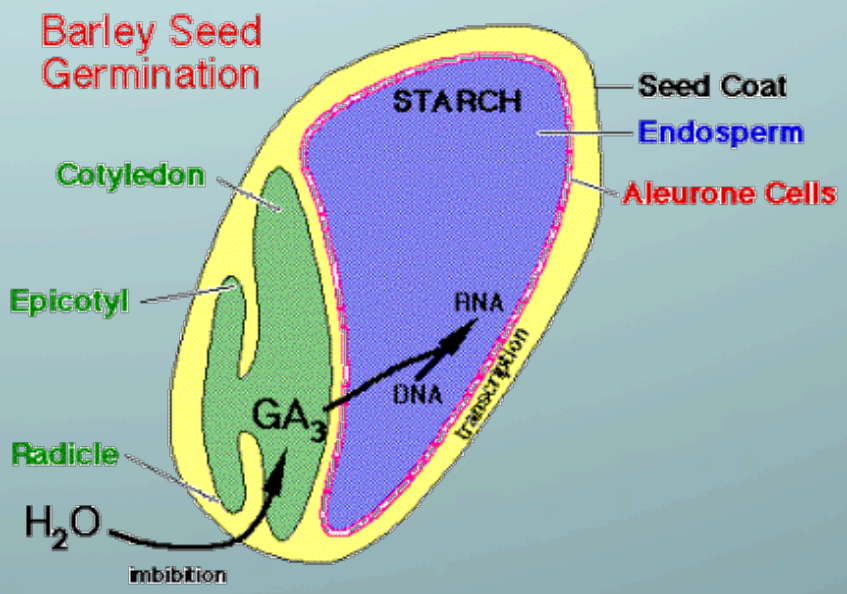
1. Imbibition
   - water uptake, softens inner tissues
   - causes swelling and seed coat rupture
   - more water uptake



Process: Seed Germination

1. Imbibition
   - water uptake, softens inner tissues
   - causes swelling and seed coat rupture
   - more water uptake

2. Gibberelic Acid
   - Plant hormone (similar to steroids)
   - Dissolved & distributed by water

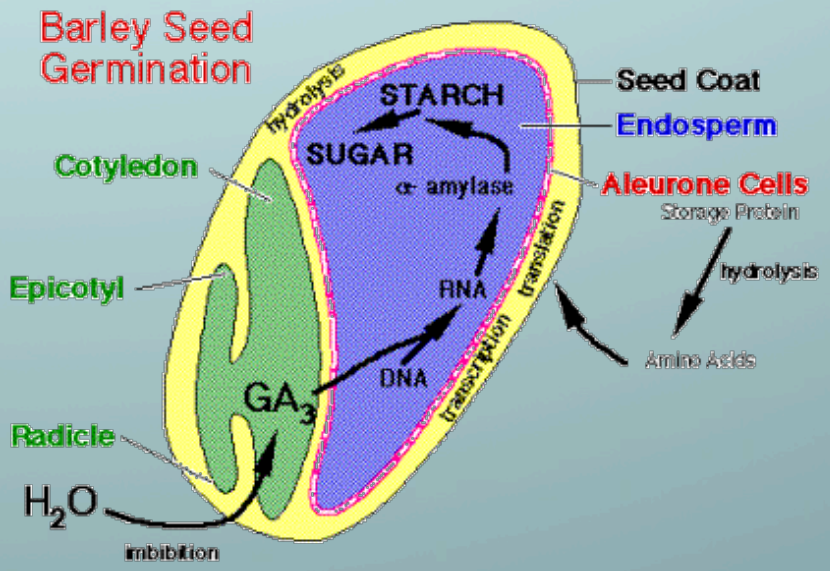


Process: Seed Germination

2. Gibberelic Acid
   - Arrives at aleurone cells
   - Activates certain genes

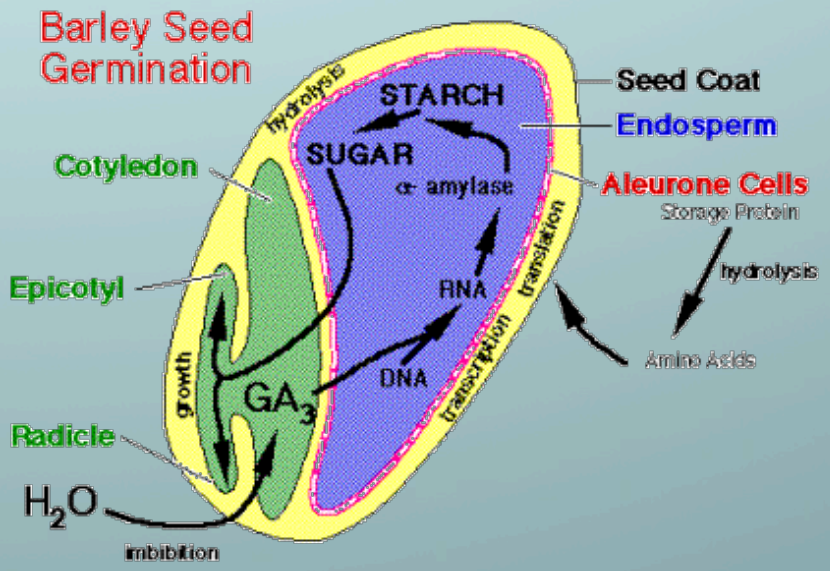
3. Transcription→ Transportation→ Translation → amylase

4. Amylase accelerates hydrolysis of starch



Process: Seed Germination

5. Hydrated starch moves to the cotyledon and radicle to initiate growth



Process: Seed Germination

Factors Affecting Germination

- Temperature:
- Water
- Oxygen
- Light/darkness
  - Forest seeds will not open until hole in canopy

Lab work

The requirement for seed germination and plant growth as follows

- 1- Distilled water
- 2- 0.05 % NaCl
- 3- 1 % NaCl
- 4- 2 % NaCl
- 5- 3% NaCl
- 6- Petri Plates
- 7- Filter paper
- 8- Forceps
- 9- Tomato and Wheat seeds
- 10- Seeds that can resist salt partly
- 11- 1% hypochlorite
- 12- Tissue paper
- 13- Markers
- 14- Parafilm
- 15- Growth chamber
- 16- Total of Plates (30 plates for each Lab)
- 17- Pots
- 18- soil

Species

1- Wheat

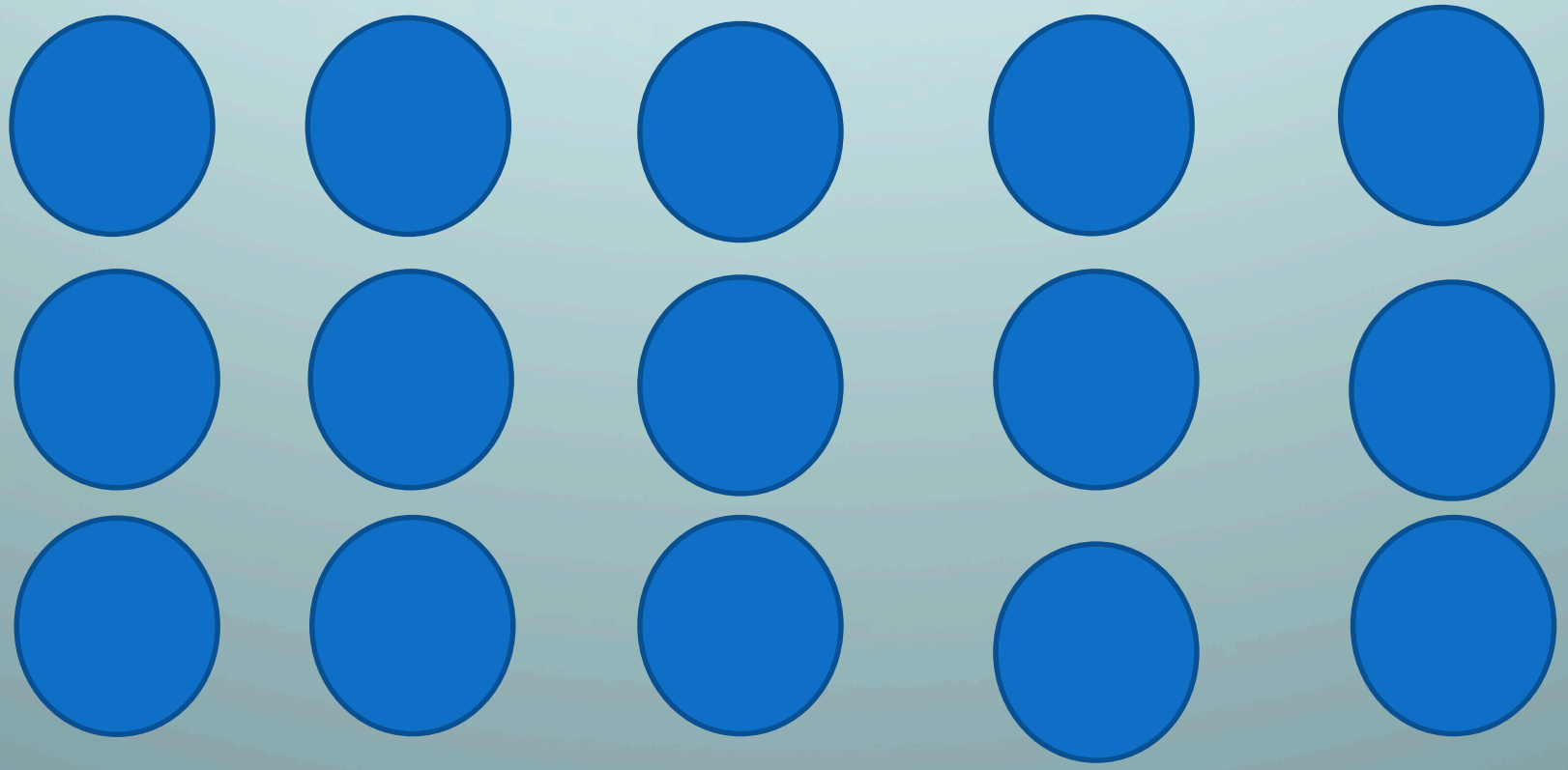
2- Tomato

Seed sterilization

Add 1% Hypochlorite

After 10 mints Wash by DW

Wheat/Tomato



- **At the End of this experiment the student will be measured the Germination Percentage.**

- Germination percentage = $\frac{\text{Number of germinated seed}}{\text{Total number of seed snow}} \times 100$

Part II: Plant growth

- One problem **is the production of crops in arid and semi-arid regions** is the accumulation of salts in the soil.

- In response to the problem of soil salinity, the crop plants show a reduction in seed germination percentage, plant growth and development stages of the plants. This response in the reduction has been observed in many species that are exposed to many differing levels of salinity and types of salt.

- The first indicator of plant response to salinity is reduction of leaf expansion. So, the anatomy and morphology of the leaves are typically affected by saline culture. Generally, salinity increased leaf succulence, delayed growth, lower quantities of specific enzymes, total protein and nucleic acids, while the specific activities and concentrations remain constant in accordance with slower growth rate.

Lab work

Methods

Irrigation 2 times/week
500 mL DW + One time
Saline water 100ml

The Students will divided into 2 groups

One seed in each pot

DW | 0.05% NaCl | 1% NaCl | 2% NaCl | 3% NaCl

Group 1

Measurements

1- Leaves area was measured by using chart book

2- Measurement of Chlorophyll a, b and Carotenoid

3- Fresh weight/ Dry weight

4- Number of stomata per leaf area.

Present your date in Graphs and discuss the result

Conclusion

1- Measurement of Chlorophyll a, b and Carotenoid:

- For the Chl extraction using acetone, the samples were ground with 2 mL of 80% acetone in combination with 0.1% CaCO3 to prevent chlorophyllase activities. After grinding, the samples were filtered, and the final volume (20 mL) was transferred to graduate tubes and characterized using a dual-beam spectrophotometer at *A*663, *A*646, *A*470, as described by Lichtenthaler (1987).

The total chlorophyll and carotenoid contents of the leaves were calculated using the method reported by:

- **Chlorophyll a** =12.21 (A663) – 2.81 (A646)
- **Chlorophyll b** = 20.13 (A646) – 5.03 (A663)
- **Total Chlorophyll** = $\frac{(\text{Chlorophyll a + Chlorophyll b}) \times 3.5}{4}$
- **Carotenoid** = $\frac{1000\,(A\,470) - 1.82\,(\text{Chl a}) - 85.02\,(\text{Chl b})}{198}$

2- Measuring Stomatal Density

- Stomata control the movement of gases in and out of a leaf, making carbon dioxide available for photosynthesis, and controlling the loss of water from the leaf through transpiration.
- Romero-Aranda et al. (2001) reported the effect of salinity on decreased of stomata density on leaves.

Measuring Stomatal Density using nail varnish Using clear nail varnish is a traditional method to measure stomatal density, since making the impression and viewing it under a microscope can be completed quickly. However, some leaves are prone to damage from the solvent in the nail varnish. The leaves absorb it, turn brown, and fail to produce any impression.

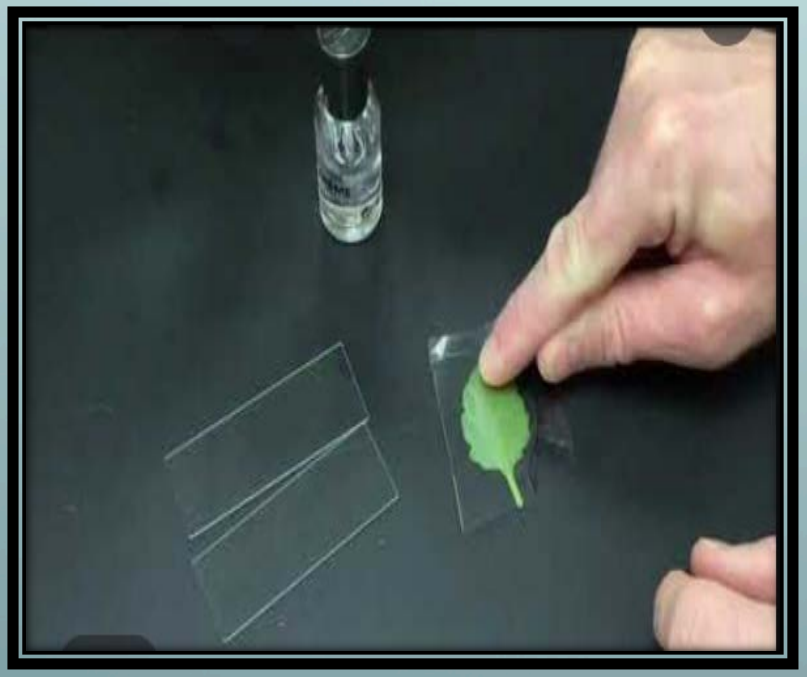
Methods

1- Prepare an epidermal impression by coating the leaf surface with nail varnish. Peel off the dried layer of nail varnish by using clear tape and stick this onto a slide.

2- Alternatively, with some plants you can peel off an epidermal strip directly, which you can mount in water on a slide and place under the microscope.

3- If you have an eyepiece graticule which you can use, you can work at a relatively low power, and you can count the number of stomata within different squares to act as replicates.

4- If you do not have an eyepiece graticule, you can work at a higher magnification and count a number of different fields - the area visible under the microscope - at any one time

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- 3-Leaves area was measured by using chart book.

- 4- Fresh weight/ Dry weight

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THANKS