Lab 7

Plant anatomy and Physiology Lab

Effect of Salinity stress at seed germination, seedling and plant growth of glycophytes Sp.

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. Saline soils cover a substantial portion of our earth's surface, with estimates varying from 400 to 950 million ha. 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. One problem is the production of crops in arid and semi-arid regions is the accumulation of salts in the soil. Vast areas of land around the entire world have been rendered unproductive due to the accumulation of salt, either deposited from irrigation water or a natural source. 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.

Many researchers have studied seed germination and early growth under various salt environments. The research findings reveal that these are the two critical and sensitive stages in the life cycle of plants. As such, the selecting of salt-tolerant plants is necessary as different species of plants respond differently to saline environments. That is returning to diverse ionic compositions with a range of concentrations of dissolved salts in plants. The seeds of halophytes, and glycophytes, germinate best under freshwater conditions. However, the germination of halophytic seeds in saline conditions shows better results than glycophytic seeds because the former can withstand salinity. Although germination means seedling emergence, technically it is the renewal of growth activity that results in fracture of seed coat and emergence of the seedlings. Therefore the successful germination and growth depend on the amount and frequency of

precipitation, and the capability of the seed to germinate and grow when the osmotic potential is low and soil moist. 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.

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 seedling and growth of glycophyte plants (Dicot vs Monocot).
(3) To familiarize simple statistical data analysis.

Part A: Effect of salinity at seed germination of two different glycophytes plant (Wheat and Tomato).

EXPERIMENTAL WORK

The class will germinate seeds of two species of glycophytes (Dicot vs Monocots) for 3 weeks. On daily basis, the germination of seeds will be counted and recorded. The results of this experiment should be collected on an open-lab basis.

The lab instructor will split students into groups based on number of students in the session. Each group will do one kind of treatment but several replicates (at least three). PLEASE REMEMBER ANY EXPERIMENT SHOULD BE REPLICATED AND RANDOMIZED TO BE STATISTICALLY WORKABLE

Seed Sterilization:

Seeds were sterilized in sodium hypochlorite 10 % (w/v) for 10 min and rinsed several times (5-6) with sterilized deionized water according to method reported by Panuccio *et al.*[2005].

Treatments:

The experiment is one factor (Salinity) with four treatment levels: 1ppt, 2ppt, 3ppt, 4ppt NaCl and one control (distilled water).

Germination experiment:

(10 seeds) were placed on double Whatman filter paper (no.1) in sterilized petri dishes (9 cm diameter) containing with 5 ml of deionized water or each salinity solution. The petri dishes were hermetically sealed with parafilm to prevent evaporation and kept in the growth chamber at 25 ± 1°C.
The experiment was conducted in a completely randomized design with four replicates and repeated seven times. Seeds were considered germinated when radicle had extended at least 2 mm. The number of germinated seeds was recorded daily until day 14.

Calculate for each species: Total germination percentages.

Discuss the results in a formal report. Search the literature to find out two articles about the effect of salinity on glycophyts plants, this will help you present and discuss your results.

Experiment title: Germination of certain threatened glycophyte species under different salinity conditions.

Species name: …….

Day	**0**	**3**	**4**	**5**	**6**	**7**	**10**	**11**	**12**	**13**	**14**
0 **ppt** NaCl Plate 1											
Plate 2											
Plat 3											
1 ppt NaCl Plate 1											
Plate 2											
Plate 3											
2 ppt NaCl Plate 1											
Plate 2											
Plate 3											
3 ppt											
Plat 1											
Plat 2											
Plat 3											
4 ppt											
Plat 1											
Plat 2											
Plat 4											

Part B: Effect of salinity at seedling and Plant growth of glycophytes plant Sp. (Wheat and Tomato).

EXPERIMENTAL WORK

The class will be studied the effects of salinity at two different species of glycophytes (Dicot and Monocot plant) for 4-6 weeks. On daily basis, the plant growth will be evaluated and recorded. The results of this experiment should be collected on an open-lab basis.

The lab instructor will split students into groups based on number of students in the session. Each group will do one kind of treatment but several replicates (at least three). PLEASE REMEMBER ANY EXPERIMENT SHOULD BE REPLICATED AND RANDOMIZED TO BE STATISTICALLY WORKABLE

Seed Sterilization:

Seeds were sterilized in sodium hypochlorite 10 % (w/v) for 10 min and rinsed several times (5-6) with sterilized deionized water according to method reported by Panuccio *et al.*[2005].

Treatments:

The experiment is one factor (Salinity) with four treatment levels: 1ppt, 2ppt, 3ppt, 4ppt NaCl and one control (distilled water).

Plant Growth Experiment:

1- Seeds of the two different plants were sown in plastic buckets of 40 cm height and 20 cm diameter. To ensure adequate soil fertility throughout the experiment period, NPK (15:15:15) was added during irrigation.
2- The planted seeds were irrigated with the test solutions for the 5 treatments, replicated three times in a randomized split plot design with salinity as the main plots.
3- The following physiological and yield parameters were studied: plant height, number of leaves/plant, dry weight, number of pod/plant, seeds/pod, seed/plant, 100 seed weight and

yield per plant. The physiological studies included; Chlorophyll concentration, number of stomata per leaf area, leaf area and water contents.

4- Discuss the results in a formal report. Search the literature to find out two articles about the effect of salinity on plants, this will help you present and discuss your results.

Plant Parameters:

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

2- Leaves area was measured according to (Li *et al*., 2007) by using chart book (graph paper), whereas each square equal $1mm^2$

3- Measurement of Chlorophyll *a*, *b* and Carotenoid:

4- Number of stomata per leaf area.

5- Fresh /dry weight.

References:

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[3] Atkinson, N.J. and P.E. Urwin, 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of experimental botany, 63(10): 3523-3543.

[4] Babu, M.A., D. Singh, K.M. Gothandam, 2012. The effect of salinity on growth, hormones and mineral elements in leaf and fruit of tomato cultivar PKM1. J Anim Plant Sci, 22(1): 159-164.

[5] Barrs, H.D. and P.E. Weatherley, 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences, 15(3): 413-428.

[6] Basak, U.C., N. Gupta, S. Rautaray, P. Das, 2004. Effects of salinity on the growth of mangrove seedlings. Journal of Tropical Forest Science, 437-443.

[7] Bewley, J.D. and M. Black, 1994. Seeds: Physiology of Development and Germination. Plenum Press-New York, 1-33.

[8] Taneenah, Ayat, Rosimah Nulit, Umi Kalsom Yusof, and Mohammed Janaydeh. "Tolerance of Molokhia (Corchorus olitorius L.) seed with dead sea water, sea water, and NaCl: germination and anatomical approach." *Advances in Environmental Biology* 9, no. 27 (2015): 106-117.