The Effects of Salinity on Mung Bean (*Vigna radiata*) Seed Germination

Authors: Emily Fong, Jimmy Huang, William Lee, Claire Weng

Abstract

Vigna radiata, also known as “the green gram” or “mung bean,” is a plant species in the legume family. Mung beans typically germinate within 2-5 days, but factors such as temperature, salinity level, pH of water, and more affect germination and growth rate (Overhiser 2019). Knowing this, we aimed to determine how the salinity concentration present in the growth medium would affect the germination rate of mung beans. In this experiment, members performed the same experiment with the same measurements. Each group member exposed 25 mung bean seeds to varying salinity treatment groups: 0, 40, 80 and 120 mM/ L. The amount of seeds germinated were recorded each day. Our study found a general decreasing germination rate with increasing salinity concentrations, which can be associated with ethylene production and osmotic stress. Average germination rate after two days was determined to be 73% in the 0 mM/L, 67% in the 40 mM/L, 53% in the 80 mM/L, and 43% in the 120 mM/L; all values are significantly different from one another receptor for 0 and 40 mM/ L groups. This affirms our initial hypothesis that an increase in salinity would cause a decrease in germination. Although only mung beans were tested, results can be applied to many other dried legumes and seeds, allowing us to test more advanced hypotheses in the future. For future studies, this could be replicated with other types of beans or include greater sample sizes.

Introduction

Crop plants are exposed to environmental stresses which can limit their productivity and growth. This is important because in 2011, 82% of the global energy supply consisted of plant-based foods with demand only continuing to rise (Alexander 89). Germination and early seedling stages are crucial in the plant life cycle, so it is important to identify an ideal growth medium to ensure crops are efficiently yielded to meet this increasing global demand. When environmental conditions cannot support healthy growth, seeds enter a dormant stage to avoid premature germination and will only continue to germinate if conditions become ideal again (Wolny 2). Further in the life cycle, the most typical effect of salt stress on plants is stunted growth as salinity reduces cell division and the synthesis of RNA, DNA, and proteins (Sheoran 171). Salinity is one of the most impactful environmental factors, and approximately 20% of all cultivated lands around the world contain high enough salt concentrations to cause salt stress to crop plants (Kaymakanova 326). By determining the factors that contribute to an ideal growth medium, the dormancy stage can be shortened or avoided altogether to render faster

germination, and plants will be able to reach their maximum growth potential to yield larger volumes of harvestable crop.

The objective of our research was to study the effects of salinity on mung bean (vigna radiata) germination by observing the result of increasing salt concentrations on the germination of mung bean seeds. As supported by Sheoran's study, we hypothesized that increasing the salt concentration of each growth medium would slow or even completely halt the germination stage in a respectively increasing degree (172). Our experiment was conducted using common mung bean seeds and four different salt concentration treatment groups. The experiment lasted a total of four days, and the findings from this study can be used to identify an ideal growth medium for mung beans.

Methods

In total, we conducted four identical experiments; each team member was responsible for their own experiment. Experiment set-up, data collection, and materials were synchronized between all four team members to minimize the effects of extraneous variables. As outlined in figure 1, we first distributed 100 green mung bean (*Vigna radiata*) seeds to each team member from the same commercial bag. Each group member then split the 100 seeds into 4 groups of 25 and wrapped each treatment group (25 seeds) with a single paper towel before placing it into a clear ziplock bag. The four treatment groups were subjected to four different water salinity concentrations: 0 mM/L, 40 mM/L, 80 mM/L, 120 mM/L. These concentrations were made using measured amounts of tap water and Windsor table salt, and are based upon similar previous experiments to allow for easier comparisons between results (Kaymakanova et al.). Once the four water salinity solutions were made, each member transferred two spoonfuls of each solution into their respective ziplock bags. Ziplock bags were closed leaving a two-centimetre remaining gap to allow for some aeration, and paper towels were replaced every two days and

resupplied with a fresh supply of the salt solutions. We then placed the four ziplock bags beside each other and counted the number of seed germinated every day at the same time.

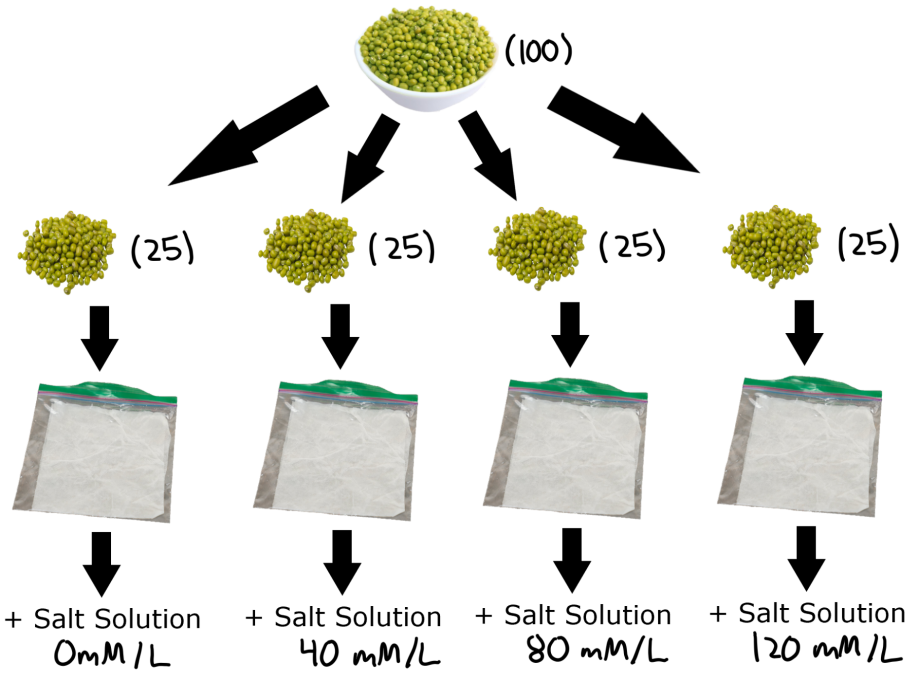

Figure 1: Flowchart of Treatment Set Up

Mung bean seed germination data and any relevant qualitative observations were collected over a four-day period, where all 25 seeds in each treatment group fully germinated by the end of the experiment. A mung bean seed germination event was defined by the cracking of the outer green shell, and the first appearance of a white root radicle. As depicted in image 1, once these two events were documented a seed was determined to have germinated. Although data was collected over a four-day period, data analysis was done only on day two germination data. Data on the second day provided us with a time frame where we had sufficient germination events (at least one seed germinated in every treatment group), but no total germination across any treatment group (less than 25 germination events). This provided the most comprehensive data to determine the rate of germination in different salinities. Once all four experiments were completed, we gathered the germination data in each treatment for day

two values, and divided that number by the total starting number of seeds to calculate an average germination event percentage: (# of germination events in specific treatment) / (25 mung bean seeds) X 100 = Germination Event (%). Percentages were calculated and averaged over the four separate experiments.

Image 1: Example of germinated seeds

We analyzed day two data using a one-way ANOVA to determine if any significance was present between the average germination event percentage of the 4 treatment groups. This test was followed up with a Tukey's HSD to determine where specifically the significant differences were. We used www.icalcu.com, a free online data analysis calculator, to complete these two tests and generate relevant results.

Results

Using compiled data for the second day, our team calculated an average germination rate for each treatment group across the four separate experiments. We calculated an average germination rate of 73 ± 5.9% in the 0 mM/L, 67 ± 3.8% in the 40 mM/L, 53 ± 3.8% in the 80

mM/L, and 43 ± 5.1% in the 120 mM/L treatment group, where error bars represent the 95% confidence interval. In figure 2, we see a general decreasing trend in germination events with increasing salinity concentrations. After conducting a one-way ANOVA test, we determined that there was a significant difference present between the different treatment groups (F-value is 33.898551, and p-value is 0.000004). Further analysis through a Tukey's HSD test revealed that results from all treatment groups are significantly different from one another, except between 0 mM/L and 40 mM/L. Average germination rates were calculated using this general formula:

$$\frac{(seeds\ germinated\ in\ Exp.1\ +\ Exp.2\ +\ Exp.3\ +\ Exp.4)\ /\ 4}{25\ seeds} X\ 100$$

.

Example Calculation for the 0 mM/L treatment group: $\frac{(17\ +\ 20\ +\ 19\ +\ 17)\ /\ 4}{25}\ X\ 100$ = 73%.

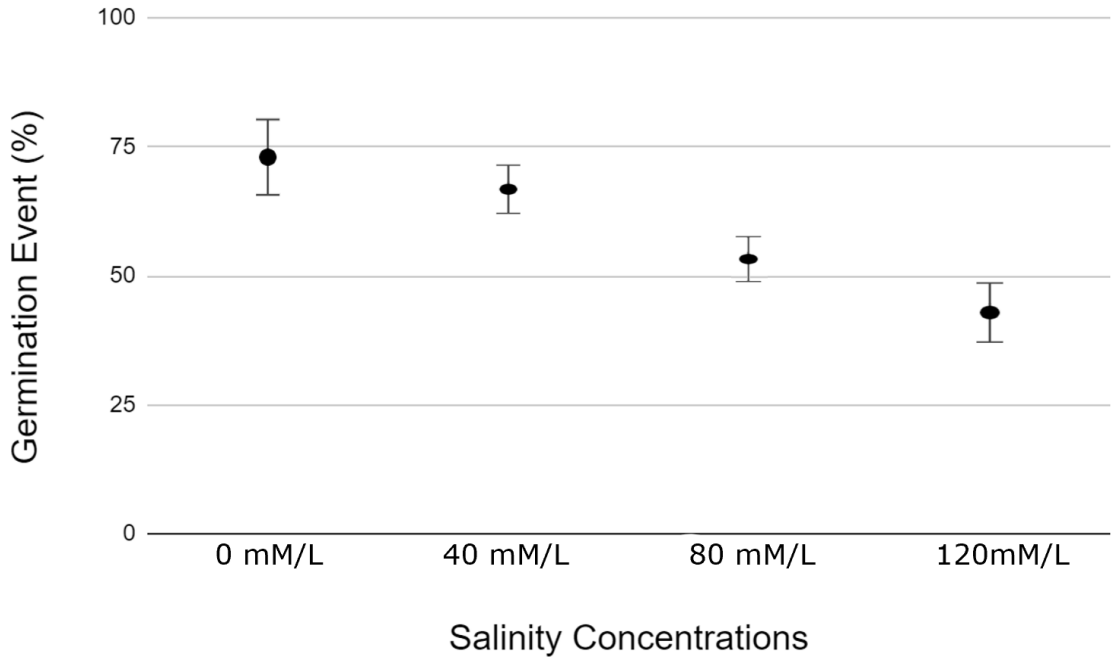

Figure 2: Average germination rate calculated in 0, 40, 80 and 120 mM/L treatment groups (n=4 for each treatment) after 2 days. Error bars represent the 95% confidence interval. One way ANOVA F-value is 33.898551, and p-value is 0.000004. All treatment groups are statistically significant from one another, except for 0 and 40 mM/ L (Tukey HSD).

Discussion

From figure 2, there is a negative correlation between germination events and salinity concentrations. The average germination rates calculated from each treatment group support our hypothesis that if we increase salinity, there will be a decrease in germination. However, an interesting note is that the 0 mM/L and 40 mM/L treatment groups were the only two treatments that were not statistically significant with each other. This raises an important question of what the lowest salinity concentration is such that there will be a statistically significant decrease in germination rate. Our paper is limited in time and resources; however, more treatments with smaller salinity increments could resolve this limitation. Currently, we conclude that any salinity concentration above 80 mM /L in the growth medium of mung beans is large enough to cause significant stress and stunt germination.

The observed decrease in germination rate of *Vigna radiata* seeds can be linked to increased ethylene production and osmotic stress (Ahmad et al). Ethylene is an important molecule centered around controlling growth by regulating the amount of CO_2 that is used for photosynthesis (Khan 72). When mung beans are put under non-optimal conditions like increased salinity, the production of superoxide radicals are biosynthetically triggered; these superoxide radicals are involved in converting 1-aminocyclopropane-1-carboxylic acid (a precursor of ethylene) through different biochemical pathways into stress ethylene (Ke & Sun, 204). Stress ethylene is formed when increased salinity concentrations in growth mediums lead to increased ethylene production inside the seed (Khan 68). Stress ethylene introduces oxidative stress, which disrupts many photosynthetic processes (Khan 68). The decreased

germination rate can also be attributed to increased osmotic stress in *Vigna radiata* seeds. Osmosis, a passive diffusion of water from low solute concentrations to high solute concentrations is an important factor in germination; sufficient water is essential for germination as it decreases the dormancy stage (Huh 136). The increased salt concentrations outside the seed cause a net decrease of water intake to the seed depleting its water stores (Huh 136). Both the stress ethylene formation and increased osmotic promote dormancy of the seed, and prevent germination.

Our experiment contains certain limitations and possible sources of errors. Firstly, with each experiment being done by different members of the group, used materials may slightly vary. These include different sized spoons, different branded ziplock bags, and varying paper towel brands. Different sized spoons will affect the amount of water placed inside each bag (which would increase or decrease osmotic stress), different ziplock bags would allow different amounts of light to penetrate through the clear layer, and different paper towels are able to uptake different amounts of water and affect evaporation. Additionally, each group member completed the experiments in different locations. Different locations vary in elevation, amount of oxygen, sunlight, and temperature which are all factors that could have affected the germination of mung beans drastically.

Further experimentation can help reduce these sources of errors and allow for more conclusive results. Further studies should include more rigorous experimental procedures to minimize the effects of varying materials and equipment, and other extraneous variables like elevation and temperature. Further studies should also be done with a higher amount of repetitions, increased number of treatment groups with smaller incrementations, and larger sample size to provide more insight into obtained data.

Conclusion

Overall, we found that as we increase salinity from 0 to 120 mM/L, there is a decrease in germination percentage in *Vigna radiata* seeds. There was a statistically significant decrease in the germination rate from every treatment group except from 0 to 40 mM/L. Our results can be explained by ethylene production within *Vigna radiata* in high salinity, which causes oxidative stress and disrupts photosynthesis. Furthermore, the salt concentrations cause increased osmotic stress pulling water away from *Vigna radiata* seeds, and decreases water stores. Our results are limited in that we cannot determine the lowest salinity concentration which causes significant germination decrease. We hypothesized that as we increased salinity concentration, germination rate would decrease; the results of our experiment are aligned with our hypothesis and previous literature findings. Our results provide insight into optimal salinity concentrations that contribute to an effective growth medium, which can help farmers around the world maximize crop yield and produce at a level that meets increasing global demands.

Acknowledgements

We acknowledge the University of British Columbia for providing us the opportunity to enroll in an Integrative Biology Laboratory course, where this study was made possible. Further, we acknowledge that UBC's Vancouver Point Grey campus is situated on the traditional, ancestral, unceded territory of the Musqueam people, and thank them for allowing us to learn on their traditional land. Finally, we would like to thank Dr. Celeste Leander, Melanie Warren, and Tessa Blanchard for their continued support and guidance throughout the entire duration of our study; their feedback and lecture content provided us with the necessary information to plan and execute a successful experiment.

Citations

Ahmad, M. et al. “Efficacy of Rhizobium and Pseudomonas Strains to Improve Physiology, Ionic Balance and Quality of Mung Bean under Salt-Affected Conditions on Farmer's Fields.” *Plant Physiology and Biochemistry*, vol. 63, 2013, pp. 170–176.,

Alexander, Peter, et al. “Human Appropriation of Land for Food: The Role of Diet.” Global Environmental Change, vol. 41, 2016, pp. 88–98., doi:10.1016/j.gloenvcha.2016.09.005.

Huh, Yoon Sun et al. "Effects Of Altering Medium Strength And Sucrose Concentration Onin Vitrogermination And Seedling Growth Ofcypripedium Macranthossw.". *Journal Of Plant Biotechnology*, vol 43, no. 1, 2016, pp. 132-137. *The Korean Society For Plant Biotechnology*, doi:10.5010/jpb.2016.43.1.132.

Kaymakanova, M. “Effect of Salinity on Germination and Seed Physiology in Bean (Phaseolus Vulgaris L.).” Biotechnology & Biotechnological Equipment, vol. 23, no. 1, 2009, pp. 326–329., https://doi.org/10.1080/13102818.2009.10818430.

Ke, Desen, and Guchou Sun. "The Effect Of Reactive Oxygen Species On Ethylene Production Induced By Osmotic Stress In Etiolated Mungbean Seedling". *Plant Growth Regulation*, vol 44, no. 3, 2004, pp. 199-206. *Springer Science And Business Media LLC*, doi:10.1007/s10725-004-5575-7.

Khan, M. Iqbal R. et al. "Alleviation Of Salt-Induced Photosynthesis And Growth Inhibition By Salicylic Acid Involves Glycinebetaine And Ethylene In Mungbean (Vigna Radiata L.)". *Plant Physiology And Biochemistry*, vol 80, 2014, pp. 67-74. *Elsevier BV*, doi:10.1016/j.plaphy.2014.03.026.

Overhiser, A. “How to Grow Bean Sprouts.” *A Couple Cooks*, 21 Jan. 2019, www.acouplecooks.com/home-grown-bean-sprouts/.

Sheoran, I. S., & Garg, O. P.. “Effect of Salinity on the Activities of RNase, DNase and Protease during Germination and Early Seedling Growth of Mung Bean.” Physiologia Plantarum, vol. 44, no. 3, 1978, pp. 171–174., https://doi.org/10.1111/j.1399-3054.1978.tb08614.x.

Wolny, Elzbieta, et al. “Germination and the Early Stages of Seedling Development in Brachypodium Distachyon.” International Journal of Molecular Sciences, vol. 19, no. 10, 2018, p. 2916., doi:10.3390/ijms19102916.