The Effect of Gibberellic Acid Biosynthesis on Salt Tolerance in Lima Beans

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Abstract

This paper explores the effect of Gibberellic acid biosynthesis on salt tolerance in Lima Beans. This experiment will be carried out to investigate if the biosynthesis of Gibberellic Acid will negate the negative effects of a high salinity level in the soil. Lima beans are being used because they are a common, easy to grow crop that serves as a good model organism for other types of commonly grown crops. High soil salinity levels are a major problem in certain areas of the world, namely Australia, where farmers are struggling to find ways to counteract the effects of the rising salt concentrations.

Based on previous research and known effects of Gibberellic Acid, as the Gibberellic Acid biosynthesis increases in the plants, then the salt tolerance will also increase. The results of this experiment supported the hypothesis, as actual trends in the data almost exactly matched expected trends. Plants treated with Gibberellic Acid grew faster and were overall healthier with thicker stems, broader leaves, taller growth, and a higher germination percentage. This held true in all of the solutions while the plants not treated with Gibberellic Acid in the higher salinity solutions tended to wilt and die, most not even germinating.

Introduction

There are many factors that affect the growth rate and general health of plants. These include but are not limited to Carbon dioxide concentration, sunlight, water accessibility, and soil salinity (Sela, 2008). In this experiment, all factors except for soil salinity and Gibberellic acid biosynthesis will remain constant. Soil salinity affects plant growth in a few ways. For instance, it decreases the amount of water a plant can uptake into its roots because high salt concentration results in a high osmotic potential of the soil solution, forcing the plant to use more energy to absorb water (Sela, 2008). Because of this, when there is an extreme amount of salt, plants may wilt and die, even if the environment around them is saturated. Another way salinity affects plant growth is with ion-specific poisoning. If the plant absorbs salt that contains ions of Sodium, Chloride, or excess Boron, it will result in negative symptoms. These include stunted plant growth, small leaves, marginal necrosis of the leaves, and fruit distortions (Mauseth, 1991).

There are even some indirect problems the salt will cause the plant. Since most of the essential nutrients are taken up by the roots, the imbalance of salt ions will result in a decreased uptake of other essential nutrients including nitrate, manganese, and calcium. This is because some nutrients are taken up by the roots in the water and with a decreased uptake of water there is a decreased uptake of those nutrients. In the situation where the plant is taking up water with increased salt, the high concentration of salt inside the plants roots will cause other solutes, such as the nutrients, to be unable to be taken up by the plant. This will cause the plant to have decreased growth and may cause wilting, and in extreme conditions, death (Mauseth, 1991).

An additional indirect affect increased salinity has on plants is affecting them through changed soil composition. When soil has increased salt concentration and becomes saturated, it will actually seal and will decrease the amount of water that can reach the roots of the plant. This will cause the plant to wilt and then die because lack of water results in an inability of the plant to carry out photosynthesis. In photosynthesis, water is needed in the light dependent and independent phases in the form of electrons, hydrogen ions, and oxygen. The water is split into these components through photolysis and is used to restock electrons for the electron transport chain, provide Hydrogen ions to create a concentration gradient and eventually power the enzyme Adenosine Triphosphate Synthase in the process of photophosphorylation of Adenosine Diphosphate Adenosine Triphosphate, and to reduce NADP+ and FADP+. Additionally, when dry, the soil cracks, resulting in damage to the plants roots (Sela, 2008).

The independent variable in this experiment is Gibberellic acid biosynthesis levels. Gibberellic Acid is a hormone derived from the fungus *Gibberella fujikiroi*. The fungus was originally discovered by Japanese scientists to cause a disease in rice crops that rapidly made the stems grow very long. The scientists isolated a hormone thought to cause this and referred to it as "Gibberellin A." Furthering the Japanese scientists' research, scientists Curtis and Cross isolated a related but chemically different compound known as "Gibberellin X" or Gibberellic Acid (Marth, Audia & Mitchell, 1956). Gibberellic Acid can stimulate stem elongation by stimulating cell division and elongation, stimulate bolting/flowering in response to long days, break seed dormancy in some plants which require stratification or light to induce germination, stimulate enzyme production (a-amylase) in germinating cereal grains for mobilization of seed reserves, induce maleness in dioecious flowers, cause parthenocarpic fruit development, and delay senescence in leaves and citrus fruits (Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992). It has also been shown that Gibberellic acid biosynthesis can cause a slight salt tolerance in plants by reversing all of the negative effects the high salinity levels would have on the plant (Ashraf & Iqbal, 2010).

In this experiment Lima beans will be treated with Gibberellic Acid and grown from seed while watered with solutions with differing salinity levels. The growth and health of these Lima beans will be compared to the growth and health of Lima beans grown from seed and watered with the same solutions, but not treated with Gibberellic Acid. Based on the previous research, as Gibberellic Acid biosynthesis increases in plants from normal to enhanced, the salt tolerance of those plants will also increase.

This research is very applicable to modern events. Soil salinity levels are high in all coast line areas and are increasing due to winds bringing ocean salts inland and farmers are finding it much harder to grow their crops. One of the main affected areas is the West Coast of Australia. Over many years the salinity level in this area has become so high that the Australian farmers' crops are not growing and the crops that do grow are short, unhealthy plants with a small yield of usable biomass (Sarre, 2004). The farmers have been searching for ways to increase their plant production because if they don't, the Australian people will go hungry and the nation's gross income from agriculture will continue to decline. Gibberellic Acid treatment could be a viable solution to the presented dilemma. If the seeds are treated with Gibberellic Acid, they will not only germinate sooner, but a larger percentage of them will germinate. Also, the effects of the high salinity levels will be counteracted by the hormone, allowing the crops to grow as they once did when the salinity levels were normal. Further, this can be applied to other areas with conditions unsuitable for healthy crop development due to salinity levels.

Materials

The materials used in this experiment were: 5 plant trays, a plant rack with UV light, 60 wild-type Lima beans, Gibberellic Acid in solution form, Sodium chloride (NaCl), potting soil, deionized water, 30 small sized pots, weigh boats, scoopulas, a standard table balance, 2000 mL Erlenmeyer flasks, magnetic stirrers, hot plates with a magnetic stirring function, lab tape, lab markers, a camera, a ruler, and a 10% bleach solution to kill fungi.

Methods

To make 2 liters of the 0.1 M Sodium chloride solution, 11.68g of NaCl was added to a 2000 mL Erlenmeyer flask filled with 2000 mL of deionized water. A magnetic stirrer was placed into the flask and the flask was placed onto a hot plate with a magnetic stirring function. The magnetic stirrer was turned on and left on until the NaCl was completely dissolved. This solution would later be used to water one set of Lima beans as the first level of independent variable.

For the second level of independent variable, the Lima beans watered with a 0.5 M Sodium chloride solution, a 0.5 M NaCl solution needed to be made. To do this, 58.44g of NaCl was added to 2000 mL of deionized water in a 2000 mL Erlenmeyer flask. A magnetic stirrer was added and the flask was placed on a hot plate with a magnetic stirring function, which was turned on until all of the NaCl was dissolved.

To plant the Lima beans, 30 small planting pots were filled three quarters of the way with potting soil. Two Lima beans were added to each of the pots. The pots were put in groups of 5 replicates and labeled 1 through 5 with lab tape. 10 pots were placed in 3 large plant trays each and separated into two groups. One group of 5 was labeled Gibberellic Acid and the other was labeled Normal. One planting tray was labeled Deionized Water, which would serve as the control, with both Gibberellic Acid treated plants and non-Gibberellic Acid plants in normal conditions. The next planting tray would be labeled 0.1 M NaCl and would hold the Gibberellic Acid and non-Gibberellic Acid treated plants watered with the 0.1 NaCl solution. The final planting tray would be labeled 0.5 M NaCl and would hold the Gibberellic Acid and non-Gibberellic Acid treated plants watered with the 0.5 M NaCl solution.

To plant the Lima beans, a depression was made in the soil about 2 inched deep and 2 Lima beans were dropped in. The hole was then filled with extra potting soil. The pot was placed back into the plant tray with the others. For the Gibberellic Acid treated plants, 50 mL of 50g/L Gibberellic Acid was poured evenly over each of the pots in the Gibberellic Acid groups. The plant trays were then filled one quarter of the way with their corresponding solutions and placed under a plant light.

Every other day the plants were taken out from underneath the plant light and each was measured. The height of each plant was recorded to be analyzed later. This, along with the overall health of the plant, was considered the dependent variable. The overall health of the plant was assessed based on height, stem thickness, broadness of leaves, color, and lateral growth. Pictures of each level of dependent variable were taken along with qualitative observations of the previously mentioned qualities of plant health. This continued for 3 weeks.

The results were analyzed by averaging the replicates and graphing the growth of each level of independent variable on 3 line graphs, each showing the growth of the Gibberellic Acid treated plants versus the non-Gibberellic Acid treated plants. Two more line graphs were made, one showing the average growth of all of the Gibberellic Acid treated plants and the other showing the average growth of all of the non-Gibberellic Acid treated plants. Then, a chart was made with the percentage germination of all of the levels of independent variable. Finally, an ANOVA test was run between all of the levels of variables.

Results

Figure 1 represents the average growth of the Lima beans over 15 days. Measurements were taken 5 times throughout the 15 days, including the first and last day. The uncertainties are due to the ruler measuring to the millimeter. The plants were measured from the base to the apical meristem. If the plant was bent over, it was stretched out and then measured. Qualitative observations were taken to account for the bending over or wilting. An ANOVA test was carried out for the data in Figure E to determine if there was a statistical difference between the Normal and Gibberellic Acid treated plants' final heights when watered with the same solution, and then between the same type of plant (Gibberellic or Normal) watered with the different solutions. The p-value for the difference between the solutions was 0.0018, making the difference between the final heights of the plants watered with the different solutions statistically different. The p-value for the difference between the Normal and Gibberellic Acid treated plants was 0.0259, indicating that the final heights of the Normal and Gibberellic Acid treated plants within the same solution were statistically different.

Figure 1: Average Plant Height Over 15 Days – Plants Measured 5 Times (cm)

Solution	Day	Normal	Gibberellic Acid Treated
Deionized Water	1	1.6	7.9
	2	2.7	13.5
	3	4.3	20.8
	4	4.5	25.5
	5	4.5	38.0
0.1 M NaCl	1	3.5	3.8
	2	4.8	9.9
	3	6.6	14.0
	4	7.1	16.6
	5	7.7	21.4
0.5 M NaCl	1	2.4	5.0
	2	3.3	9.9
	3	4.5	14.0
	4	4.4	15.0
	5	4.2	15.5

*The uncertainties of all of the data points are ±0.05cm.

In this experiment, the Gibberellic Acid treated plants grew both taller and healthier in all conditions. As can be seen in Figures 2-4, the Gibberellic Acid plants not only grew taller over time, but also grew taller at a much faster rate than the Normal plants. Most of the plants treated with Gibberellic Acid were not finished growing, as can be seen by the upwards curve in the Gibberellic Acid data in Figures 2 and 3, while all of the Normal plants were either finished growing, as can be seen by a plateauing line in the Normal data of Figures 2 and 3, or beginning to die, as can be seen by a downward trend in the Normal data of Figure 4.

Figure 2: Average Growth of Lima Beans Watered with Deionized Water Over 15 Days

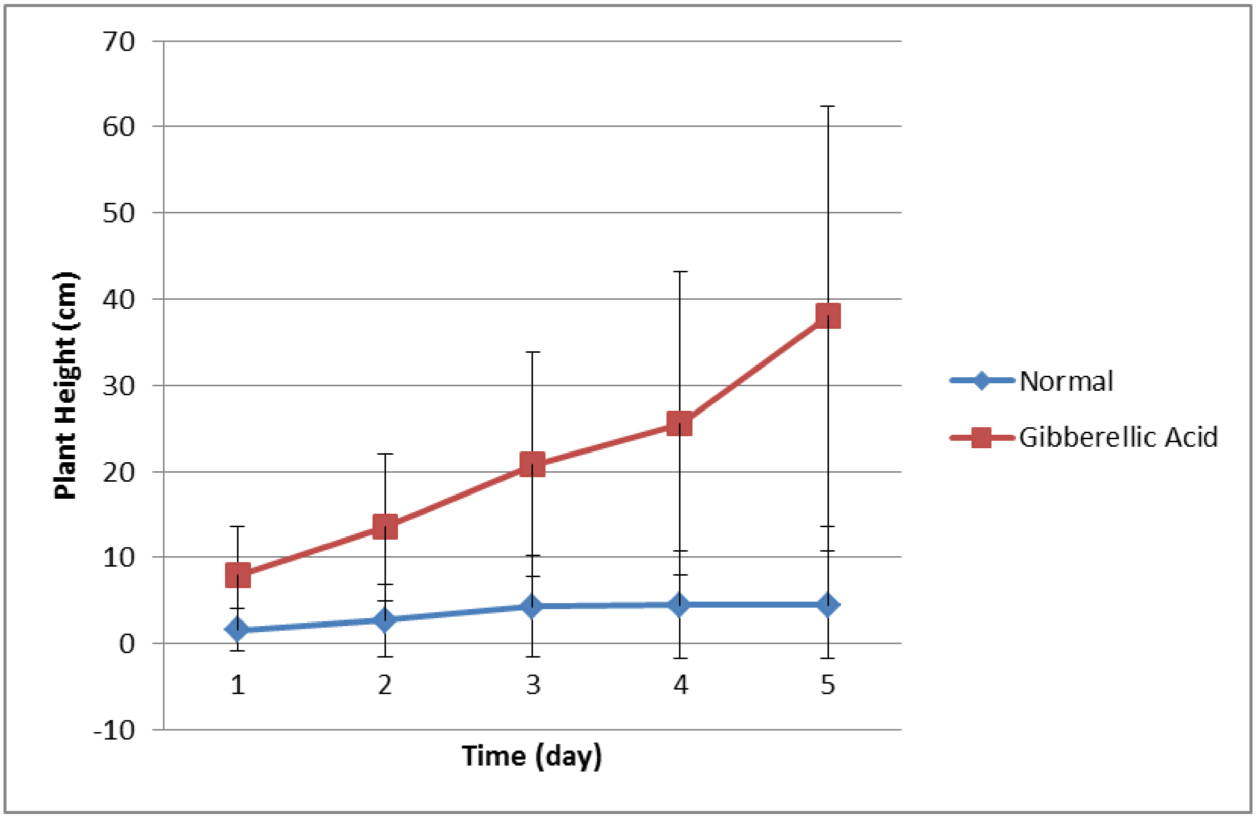


Figure 3: Average Growth of Lima Beans Watered with a 0.1 M NaCl Solution Over 15 Days

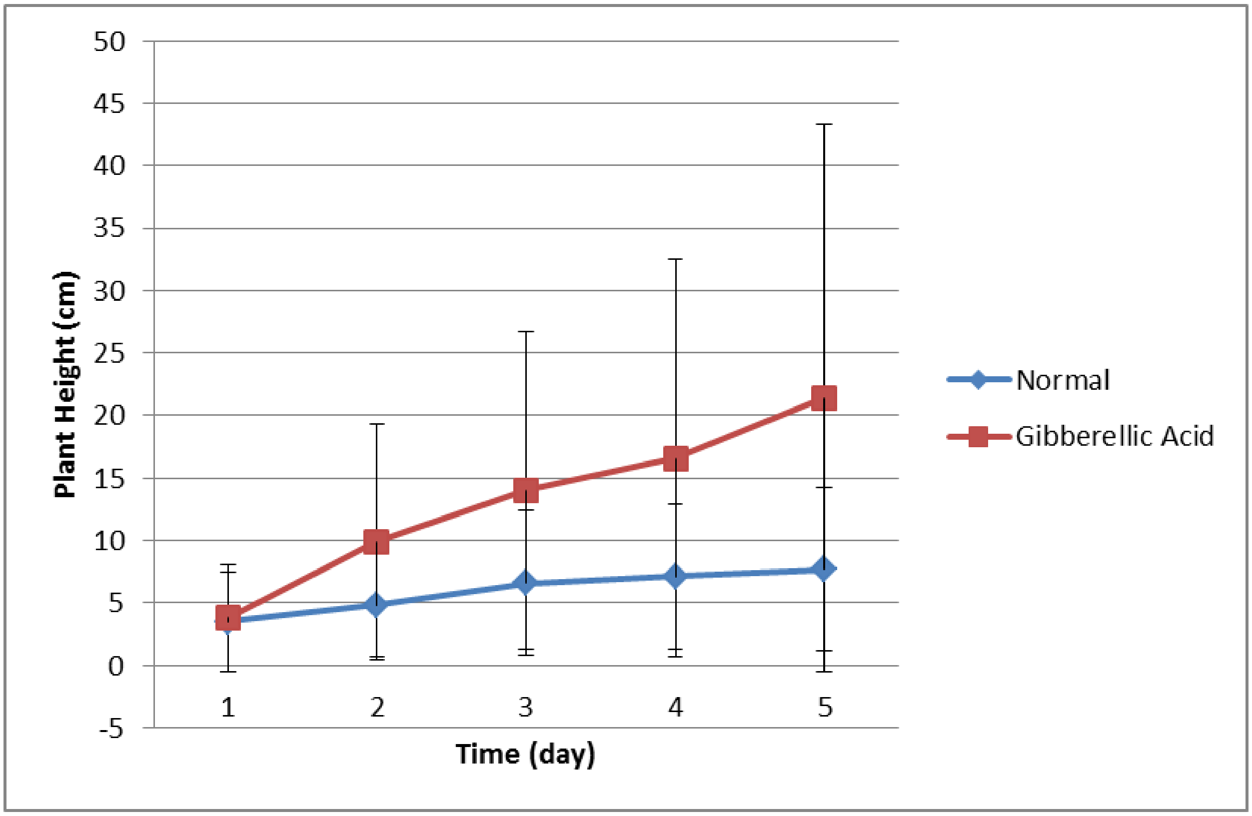


Figure 4: Average Growth of Lima Beans Watered with a 0.5 M NaCl Solution Over 15 Days

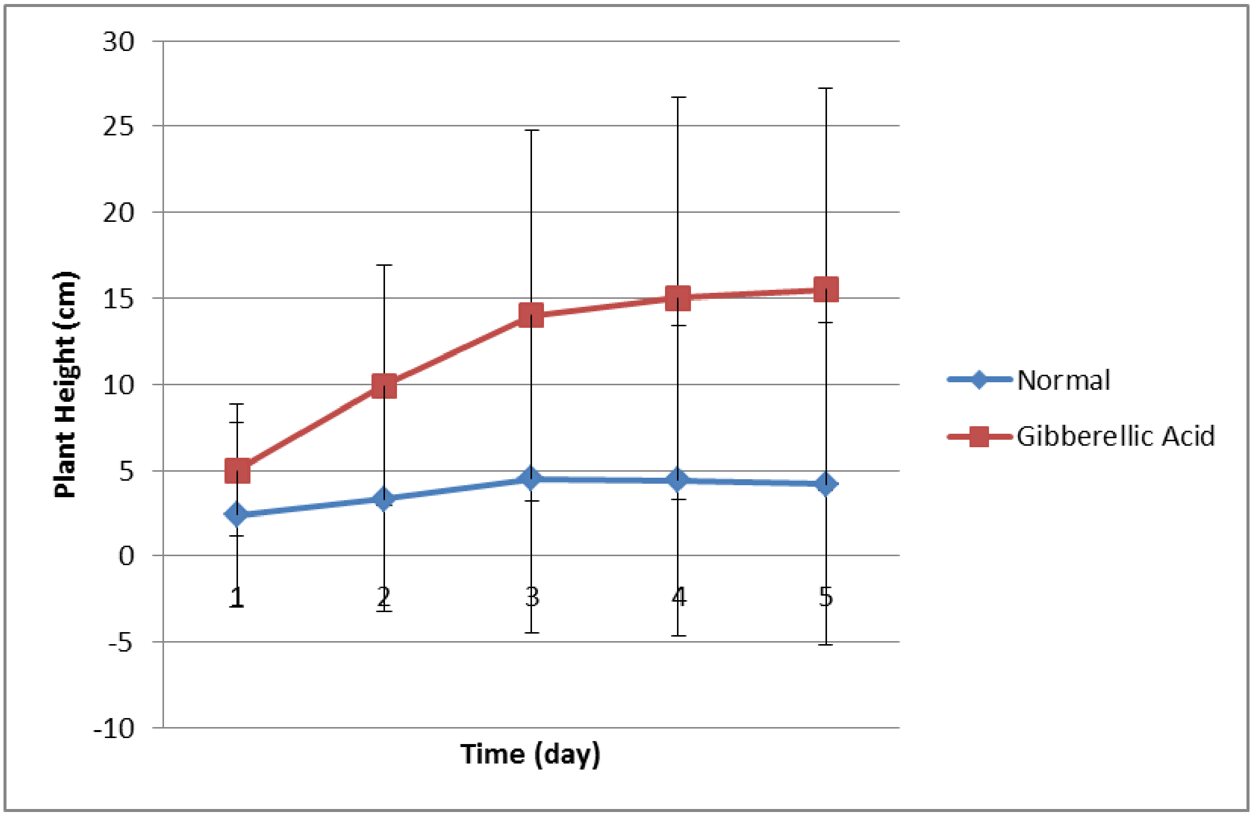


Figure 5: Overall Percent Germination After 15 Days

Solution	Normal	Gibberellic Acid Treated
Deionized Water	40%	80%
0.1 M NaCl	80%	60%
0.5 M NaCl	40%	80%
Overall	53%	73%

Figure 5 shows the final percent germination of the different levels of independent variable after 15 days of growing. Even if the plant had germinated and then died off, it was still counted in the final percent germination. Overall, the Gibberellic Acid treated seeds had a higher final percent germination than the Normal seeds.

Conclusion

The results of this experiment support the hypothesis that as the biosynthesis level of Gibberellic Acid increases in the Lima beans, the salt tolerance of the Lima beans will also increase. To judge if the Gibberellic Acid nullified the negative effects of the high salinity levels, three factors were analyzed: the height of the plants over 15 days, the overall health of the plants over 15 days, and the final percent germination of the pants.

The first way the results support the hypothesis is through the changes in plant height over the 15 days. Figure 2 shows that the Gibberellic Acid at a faster and steadier rate to the last day when watered with the Deionized water solution. Since this was the positive control group, this extreme growth was anticipated. In contrast, the Normal plants watered with the deionized water were overall much shorter and stopped growing at a certain point. This indicated that the Gibberellic Acid has a positive effect on the Lima beans growth and therefore made the rest of the data more reliable. Figures 3 and 4 show the Gibberellic Acid treated plants in both the 0.1 M and 0.5 M NaCl solutions growing at a faster and steadier rate than their counterparts; however, this growth is not as extreme as the positive control. When compared, Figure 2 and 3 show that the Deionized water plants grew at a faster rate than the 0.1 M NaCl solution plants even though the 0.1 NaCl plants do not seem to reach a maximum height either. When compared with figure 2 and 3, Figure 4 shows even the Gibberellic Acid plants reaching a max height, which indicates that there are limits to how much the Gibberellic Acid can reverse the effects of high salinity in the soil. Overall, this one method of analyzing the results supports the hypothesis, showing that the Gibberellic Acid counteracts at least one of the effects of high salinity in the soil; decreased plant height. The p-values from the ANOVA test supported this, showing that the heights were statistically different and causing the null hypothesis that there was no statistical difference in the plant heights between the different levels of independent variable, to be rejected.

The second way the results support the hypothesis if through the overall health of the plant. Overall, the Gibberellic Acid treated plants were healthier than the Normal plants. This can be seen in Figures A.1-E.3. The pictures show how over the 15 days the Gibberellic Acid treated plants were not only taller but also had much more lateral growth, had broader leaves, thicker stems, and were never withered or dying. All of the Gibberellic Acid plants were also a deep green, evidence of high photosynthetic activity and displayed phototropism. In contrast, the Normal plants, especially in the higher molarity solutions, were not as green as the other plants; yellowing in certain areas. The stems were thin and could not support the weight of the plants, causing the plants to fall over. Some of these plants were even withered and one that was being watered with the 0.5 M NaCl solution actually died.

The final way the results support the hypothesis is through the final percent germination of the Lima beans. Another effect of high soil salinity is delayed or even inhibited germination of the seeds. The Gibberellic Acid appeared to counteract this as the Gibberellic Acid plants had a higher final germination percentage than the Normal plants.

There was both systematic and random error present in this lab. The random error could have affected the overall health of the plants. This random error included changes in the room temperature or sunlight shining on the plants. The systematic error includes the way the plants were measured because not all of the plants were straightened out completely if to prevent uprooting the plants. To improve this experimental process, none of the plants should be straightened out when measuring. Also, the final plant root length should be measured to see if Gibberellic Acid has any effect on roots length and other properties.

The next step to this experiment is to research the other applications of Gibberellic Acid. For example, Gibberellic Acid's effect on the drought tolerance of Lima beans. Further, more species of plant should be tested to determine if Gibberellic Acid has the same effect on all types of plants.

Literature Cited

Ashraf, M., & Iqbal, M. (2010). *Gibberellic acid mediated induction of salt tolerance in wheat plants: Growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis*. Riyadh: King Saud University. Retrieved from http://www.sciencedirect.com/science/article/pii/S0098847210001425 on 9 April 2013.

Davies, P. J. (1995). Plant Hormones: Physiology, Biochemistry and Molecular Biology. Dordrecht: Kluwer.

Marth, P. C., Audia, W. V., & Mitchell, J. W. (1956). Effects of gibberellic acid on growth and development of plants of various genera and species. *Botanical Gazette, 118*(2), 106-111. Retrieved from http://www.jstor.org/stable/2472972

Mauseth, J. D. (1991). Botany: An Introduction to Plant Biology. Philadelphia: Saunders. pp. 348-415.

Raven, P. H., Evert, R. F., and Eichhorn, S. E. (1992). Biology of Plants. New York: Worth. pp. 545-572.

Salisbury, F. B., and Ross, C. W. (1992). Plant Physiology. Belmont, CA: Wadsworth. pp. 357-407, 531-548.

Sarre, A. (2004, January). *Monitoring the white death*. Retrieved from http://science.org.au/nova/032/032key.html

Sela, G. (2008). *Soil salinity*. Retrieved from http://www.smart-fertilizer.com/articles/soil-salinity on 9 April 2013.

Appendix

Figure A: Raw Data Table Day 1 – Starting Plant Heights (cm)

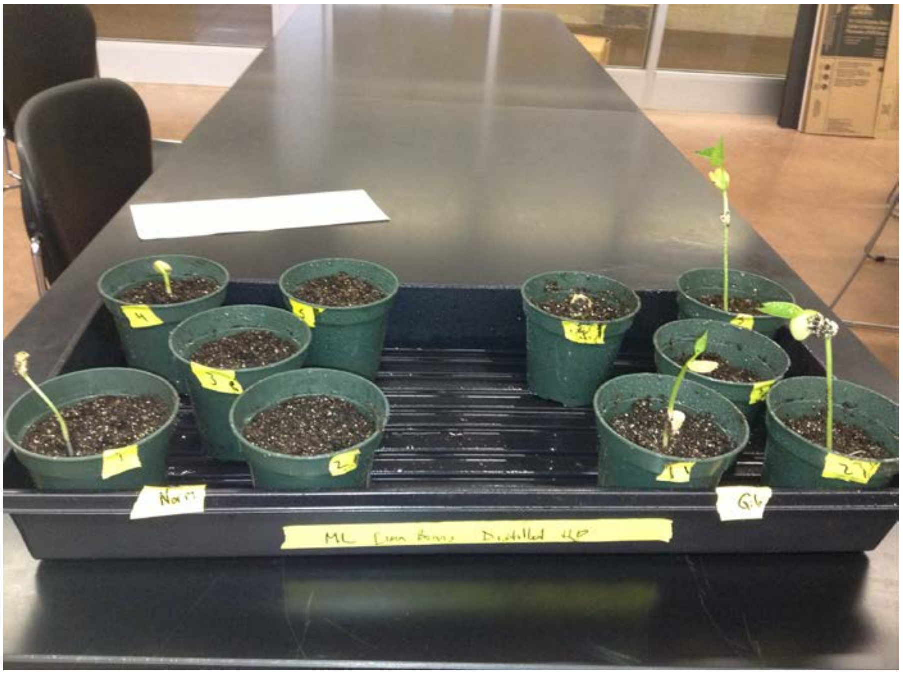
Solution	Trial	Normal	Gibberellic Acid Treated
Deionized Water	1	5.5	9.5
	2	0	10
	3	0	0
	4	2.5	5
	5	0	15
0.1 M NaCl	1	0	3.5
	2	8.0	10.5
	3	2.0	0
	4	0	0
	5	7.5	5.0
0.5 M NaCl	1	0	0
	2	0	6.0
	3	0	7.0
	4	12.0	9.5
	5	0	2.5

*The uncertainties for these data points are ±0.05cm.

Qualitative observations: The Gibberellic Acid treated seeds grew much faster and taller than the normal plants. Also, most of the Gibberellic Acid treated plants already had lateral growth. Even the tallest Normal plant did not have lateral growth yet. Further, there was a higher germination percentage of the Gibberellic Acid treated plants.

Figures A.1-A.3: Day 1 Starting Growth

A.1 - Deionized Water



A.2: 0.1 M NaCl

A.3: 0.5 M NaCl

Figure B: Raw Data Table Day 2 – Plant Heights (cm)

Solution	**Trial**	**Normal**	**Gibberellic Acid Treated**
Deionized Water	1	0	16.6
	2	9.4	21.8
	3	0	0
	4	4.3	10.6
	5	0	18.4
0.1 M NaCl	1	9.5	12
	2	0	18.8
	3	3.2	0
	4	2.7	0
	5	8.8	18.5
0.5 M NaCl	1	1.6	0
	2	0	8.1
	3	0	19
	4	14.8	9.5
	5	0	12.9

* The uncertainties for these data points are ±0.05cm. There are no qualitative observations for this day.

Figure C: Raw Data Table Day 3 – Plant Heights (cm)

Solution	Trial	Normal	Gibberellic Acid Treated
Deionized Water	1	0	19.0
	2	10.0	34.2
	3	0	0
	4	11.5	22.3
	5	0	28.4
0.1 M NaCl	1	13.3	23.2
	2	0	21.0
	3	4.5	0.5
	4	3.0	0
	5	12.0	25.5
0.5 M NaCl	1	2.0	0
	2	0	7.5
	3	0	27.5
	4	20.5	14.5
	5	0	20.5

* The uncertainties for these data points are ±0.05cm.

Qualitative Observations: The Gibberellic Acid plants in all solutions have broader leaves and are generally taller than the Normal plants. The Gibberellic Acid plants also have thicker stems than the Normal. There continues to be a higher percent germination in the Gibberellic Acid plants.

Figures C.1 – C.3: Day 2 Plant Growth

Figure C.1: Deionized Water

Figure C.2: 0.1 M NaCl

Figure C.3: 0.5 M NaCl

Figure D: Raw Data Table Day 4 – Plant Heights (cm)

Solution	**Trial**	**Normal**	**Gibberellic Acid Treated**
Deionized Water	1	0	21.5
	2	10.0	47.5
	3	0	0
	4	12.5	24
	5	0	34.5
0.1 M NaCl	1	13.5	23.7
	2	0	24.0
	3	5.5	0
	4	4.0	0
	5	12.5	35.3
0.5 M NaCl	1	1.5	0
	2	0	10.0
	3	0	31.5
	4	20.5	13.7
	5	0	20.0

* The uncertainties for these data points are ±0.05cm.

Qualitative Observations: All Gibberellic Acid plants have much broader leaves and much more lateral growth than the Normal. The lateral growth on the Gibberellic Acid plants watered with the 0.5 M NaCl solution was withered and fell off when touched whereas the 0.1 M NaCl and Deionized Water Gibberellic Acid plants' lateral growth was healthy.

Figures D.1 – D.2: Day 3 Plant Growth

Figure D.1: Deionized Water

Figure D.2: 0.1 M NaCl

Figure D.3: 0.5 M NaCl

Figure E: Raw Data Table Day 5 – Plant Heights (cm)

Solution	Trial	Normal	Gibberellic Acid Treated
Deionized Water	1	0	29.0
	2	10.0	62.5
	3	0	0
	4	12.5	47.5
	5	0	51.2
0.1 M NaCl	1	14.0	25.0
	2	0	30.7
	3	5.5	0
	4	4.0	0
	5	15.0	51.2
0.5 M NaCl	1	0	0
	2	0	10.8
	3	0	31.5
	4	21.0	14.2
	5	0	21.0

* The uncertainties for these data points are ±0.05cm.

Qualitative Observations: The Gibberellic Acid plants are growing so tall that they have begun to bend over. This may be partly due to phototropism; however, the plants needed to be reinforced with sticks in order to stand up. There is some wilting and death in the 0.5 M and 0.1 M NaCl non-Gibberellic Acid treated plants.

Figures E.1 – E.3: Day 4 Plant Growth

Figure E.1: Deionized Water

Figure E.2: 0.1 M NaCl

Figure E.3: 0.5 M NaCl

Sample calculation for the average plant height per day:

- Formula: Mean
  - $(x_1 + x_2 + x_3 \ldots + x_n)/n = \text{mean}$

Sample Calculations for the Molarity of the Salt Solutions:

- Mass of NaCl = 58.44
- To make a 2 liter 0.1 M NaCl solution:
  - Molar Mass of NaCl = 58.44g/mol
  - M = moles of solute/volume
  - 0.1 = y/2
  - 0.2 = y
  - 0.2 mol NaCl x 58.44g/0.1mol = 11.69 g NaCl