The Effect of the Degeneration of the Testa by Different Periods of Cellulase Exposure on Germination Period and Growth of *Solanum lycopersicum*

Allyson Bazer, Rachel Weinstein, Aakansha Kumar, Julianna Mastropierro

Molecular and Agricultural Biotechnology

Abstract

The purpose of this experiment was to determine if exposing *Solanum lycopersicum* seeds to cellulase prior to planting them would help to decrease the germination period and increase the growth of the resulting seedlings. By using the cellulase to degrade the cellulose in the outer seed coat (testa), the radicle would have less of a barrier to protrude through. A decreased germination period and increased growth would help to improve the net profit and efficiency for tomato cultivation. Several lengths of time of the cellulase soak were tested: from zero hours to thirty hours, at five hour increments. The research question is: “What is the effect of a cellulase soak for different time intervals on the germination period and growth of *Solanum lycopersicum* seeds?” After soaking five seeds for each of the designated time intervals, the thirty five seeds were planted. Measurements of growth and germination were recorded. It was concluded that the seeds soaked for fifteen hours resulted in the greatest growth and shortest germination period. The final growth of the plants, averaged for each soak time, exhibited a bell shaped curve: the ideal soak time was fifteen hours, with the least effective soak times being zero and thirty hours, when not including the lack of growth of the seeds soaked for five hours. Using these results, it can be determined that a cellulase soak does increase growth and decrease the germination period of *Solanum lycopersicum* seeds, and can thus be utilized as an effective treatment versus standard protocol.

Introduction

Germination refers to the time when a plant embryo resumes growth and metabolic activity - the seedling sprouts from the seed. Cutting down the time it takes for plants to germinate may allow farmers and others planting the seeds to be more productive and spend less money taking care of the plants before they’ve sprouted. In mass production, this reduction of germination period may increase profits for farmers and lessen the market prices of crops that have lower germination stages.

The tomato, or *Solanum lycopersicum*, is a major crop in the United States. According to the National Agriculture Statistics Service (NASS), the tomato was the highest ranked fresh-market vegetable in the U.S. with its production in 2010 valued at $1.4 billion (Boriss & Brunke, 2011, para. 7). Tomatoes were chosen for this experiment because they are a significant food source and export for the country. The purpose of this study was essentially to discover a way to make tomato farming more profitable and efficient. Tomato seeds take approximately 5-10 days to germinate. During germination, the radicle, or root apical meristem, which will produce the roots, pushes through the seed coat first. The seed coat, or testa, is a hard, highly resistant structure that protects the plant embryo within it. Some seed coats are so hard that water and oxygen cannot reach the plant embryo until the testa is broken down. There must be a weakening of this external tissue to allow the radicle to emerge from the seed (Black, Bradford, & Vázquez-Ramos, 1999, p. 232). The weakening of this tissue involves the breaking down of the cell walls of the cells found in the restraining tissues (Black, Bradford, & Vázquez-Ramos, 1999, p. 232). The degrading of the seed coat and endosperm is naturally carried out by several enzymes produced by the plant, including cellulase (Black, Bradford, & Vázquez-Ramos, 1999, p. 235). Cellulase refers to a group of enzymes that hydrolyze cellulose (Worthington Biochemical Corporation, 2014, para. 1). The major component of plant cell walls is cellulose (Mendu, Stork, Harris, & DeBolt, 2011, p. 1638). Cellulose is found in the seed coat and endosperm of almost all seeds, including tomato seeds, which were used in this experiment (Mendu, Griffith, Persson, Stork, Downie, Voiniciuc, Haughn, & DeBolt, 2011, p. 111). The basis of this study was the idea that if the seed coat and endosperm could be dissolved or partially dissolved by cellulase before the seeds were planted, germination would occur more quickly because the radicle would not have to break through as thick of a seed coat and endosperm as it normally would. Cellulase already plays a role in the breaking down of the restrictive tissues surrounding the plant embryo. Enhancing this natural process by soaking the seed in cellulase prior to planting should shorten the germination period. The biochemical processes that occur to disintegrate the seed coat and other protective tissues would be minimized - possibly even made unnecessary - if cellulase broke down the cellulose in the testa early on, again, leading to the sprouting of a seedling much more quickly.

The seeds in this study were soaked in a cellulase solution for different periods of time ranging from 0 to 30 hours. The variable that was tested was how the length of time the seeds were soaked in cellulase affected the germination and growth of the tomato plants. Overall, the goal was to determine whether or not soaking the seeds in cellulase would affect growth at all. The seeds were exposed to the cellulase for varying time spans in order to find the optimal duration to soak the seeds to achieve maximum growth. The hypothesis was that the tomato seeds that had been in cellulase for 15 hours would germinate the fastest and, in the end, would be the plants that experienced the most growth. Studies have been done on the absorption of cellulase by cellulose. It was discovered that the maximum absorption of the enzyme by the cellulose was between 15 and 24 hours after initial exposure (Lee, Shin, Ryu, & Mandels, 1982, p. 2150). Past this time frame, the absorption rate appears to level out and stop increasing. The 15 hour seeds were predicted to have the shortest time before germination. The seeds that were not soaked in cellulase (0 hours - the control seeds) would germinate after the typical 5-10 days for tomatoes. The seeds that were soaked for 30 hours might have been exposed to the enzyme for too long. The entire plant contains cellulose so if the seed coats were broken down too much, the embryo itself would begin to deteriorate, which could lead to these particular seeds not germinating at all. Fifteen hours was the middle of the two extremes in this situation so it was expected to produce the best results. This hypothesis was also created based on the fact that the previous study demonstrated that the optimal cellulase adsorption by cellulose would be between 15 and 24 hours.

Materials

The materials that were used in this experiment were 4 g of cellulase, distilled water, 35 tomato seeds, microtubes, conical tubes, potting soil, pots, plant tray, metric ruler, lab tape, lab marker, and a notebook to record data.

Procedure

A cellulase solution containing 4 g of cellulase and 200 mL of distilled water, for a concentration of 200 g/L, was prepared in six conical tubes. Seven microtubes were labeled with 0, 5, 10, 15, 20, 25, and 30 hours. One mL of cellulase solution was added to each of the seven microtubes. Five seeds were placed in each microtube, because there were 5 replicates for each time trial. All seeds were removed from the microtubes once their soaking time was up. The seeds were then immediately dried and placed in the microtube once the solution was rinsed out. All seeds were then planted at the same time. One seed was planted in each pot. The seeds were planted approximately ¼ inch below the surface if the soil, which is the ideal depth to plant seeds when growing tomato plants. The plants were kept in a plant bed and were given free access to 1L of water in the plant tray. They were given more water in the plant tray as they ran out - usually every two or three days. All seeds were grown until germination. Observations were recorded every other day, when possible. The heights of the seedlings were also recorded.

The independent variable was the length of time each seed was soaked in the cellulase (0 hours, 5 hours, 10 hours, 15 hours, 20 hours, 25 hours, 30 hours). The dependent variable was the germination period of each plant. The control was the seeds that were not soaked in cellulase (0 hours). Some constants included: the concentration of the cellulase solution, the type of seed planted, the technique used to plant the seed, the depth the seed was planted, soil type, and the water and light availability. There were five replicates for each time trial. The length of the seedling was recorded in millimeters with a metric ruler. Statistical analysis included mean, median, standard deviation, graphs and t tests.

Results

Over the course of the twenty day experiment, the heights of each plant were recorded on seven different days. Using a metric ruler, careful measurements were taken from the base of the stem to the apical bud. The measurements were recorded in Tables 1.1 to 1.7. Table 2.1 averages the growth of the five plants in each treatment group, and the standard deviations are shown in Table 2.2. The means are displayed graphically in Figure 3.2. All of the standard deviations, other than the seeds soaked for five hours, which did not germinate, and the seeds soaked for thirty hours, of which only one germinated, were very similar. There is a range of only 1.3. Looking at the means, the seeds soaked in cellulase for fifteen hours had the highest recorded growth by the end of experimentation. These seeds, on average, grew to 61.3 millimeters. Only noting the final measurements, the plant growth appears to exhibit a bell shaped curve, as shown in Figure 3.1. Disregarding the seeds soaked for five hours, the seeds soaked for zero hours and thirty hours exhibited the least amount of growth. Growth gradually increases as the soak times approach fifteen hours, and then peak at fifteen hours and gradually decreases for the seeds soaked longer than fifteen hours.

Another measurement that was recorded was germination period. In order to determine how the different soaking times affected germination period, it was decided that ten millimeters would represent the point at which germination occurred. Table 1.8 depicts the raw data for each plant's germination. The medians of the germination periods for each soak time were recorded in Table 2.3 and are shown graphically in Figure 3.3. Median was chosen rather than mean because, since many of the seeds never germinated, this would heavily skew the mean towards zero, giving an inaccurate representation of the data. The seeds soaked for ten, fifteen, twenty, twenty five, and thirty hours all germinated at either Day 6 or Day 8. The control germinated at Day 13.

Several t-tests were conducted in order to determine if the different cellulase soaking times resulted in seeds whose growth was statistically different from that of the control. These t-tests are shown in Table 2.4. The t-tests were one-tailed, as growth in the positive direction was the only anticipated direction of growth, and type 2, as the data is unpaired. All of the t-test values that show a statistical difference are highlighted. The seeds soaked for five, fifteen, and twenty hours show a statistical difference in growth when compared to the seeds soaked for zero hours.

Additional t-tests, also one-tailed and type 2, were conducted between the different time intervals, as seen in Table 2.5. Every time trial had a significant difference with the 5 hour trial, because none of the 5 hour plants grew, which is most likely due to error. The majority of the time trials, excluding the 10 and 20 hour seeds, had a significant difference from the 15 hour trial as well, because it was by far the most effective treatment. Because many time trials had a significant difference when compared to the 15 hour time trial, the effectiveness of the 15 hour treatment is supported and the bell curve is confirmed.

Table 1.1: Day 6* Growth of Tomato Seedlings (in mm)

Trial Number	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
1	0 mm	0 mm	0 mm	11 mm	7 mm	0 mm	13 mm
2	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
3	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
4	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
5	0 mm	0 mm	0 mm	10 mm	6 mm	0 mm	0 mm

*Measurements of growth began to be taken once the first seedling was visible above the soil, which occurred six days after planting. The number of days refers to how much time passed after planting when these measurements were taken.

Table 1.2: Day 8 Growth of Tomato Seedlings (in mm)

Trial Number	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
1	0 mm	0 mm	0 mm	40 mm	41 mm	0 mm	19 mm
2	0 mm	0 mm	0 mm	15 mm	0 mm	0 mm	0 mm
3	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
4	0 mm	0 mm	0 mm	0 mm	0 mm	20 mm	0 mm
5	0 mm	0 mm	17 mm	35 mm	30 mm	17 mm	0 mm

Table 1.3: Day 11 Growth of Tomato Seedlings (in mm)

Trial Number	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
1	10 mm	0 mm	0 mm	47 mm	43 mm	20 mm	33 mm
2	0 mm	0 mm	0 mm	48 mm	0 mm	0 mm	0 mm
3	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
4	0 mm	0 mm	0 mm	0 mm	0 mm	29 mm	0 mm
5	0 mm	0 mm	39 mm	46 mm	41 mm	38 mm	0 mm

Table 1.4: Day 13 Growth of Tomato Seedlings (in mm)

Trial Number	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
1	35 mm	0 mm	0 mm	55 mm	45 mm	25 mm	34 mm
2	0 mm	0 mm	0 mm	50 mm	0 mm	0 mm	0 mm
3	4 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
4	0 mm	0 mm	0 mm	0 mm	0 mm	35 mm	0 mm
5	0 mm	0 mm	42 mm	47 mm	47 mm	40 mm	0 mm

Table 1.5: Day 15 Growth of Tomato Seedlings (in mm)

Trial Number	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
1	49 mm	0 mm	0 mm	56 mm	46 mm	26 mm	34 mm
2	0 mm	0 mm	0 mm	50 mm	0 mm	0 mm	0 mm
3	14 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
4	0 mm	0 mm	0 mm	0 mm	0 mm	39 mm	0 mm
5	0 mm	0 mm	44 mm	50 mm	50 mm	44 mm	0 mm

Table 1.6: Day 18 Growth of Tomato Seedlings (in mm)

Trial Number	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
1	55 mm	0 mm	0 mm	60 mm	46 mm	45 mm	35 mm
2	0 mm	0 mm	0 mm	52 mm	0 mm	0 mm	0 mm
3	34 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
4	0 mm	0 mm	0 mm	0 mm	0 mm	40 mm	0 mm
5	0 mm	0 mm	46 mm	66 mm	52 mm	41 mm	0 mm

Table 1.7: Day 20 Growth of Tomato Seedlings (in mm)

Trial Number	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
1	55 mm	0 mm	0 mm	60 mm	47 mm	49 mm	36 mm
2	0 mm	0 mm	0 mm	53 mm	0 mm	0 mm	0 mm
3	43 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
4	0 mm	0 mm	0 mm	0 mm	0 mm	42 mm	0 mm
5	0 mm	0 mm	56 mm	71 mm	58 mm	43 mm	0 mm

Table 1.8: Time Tomato Seeds Took To Germinate (based on a minimum height of 10 mm)

	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
1	Day 11	n/a*	n/a	Day 6	Day 8	Day 13	Day 6
2	n/a	n/a	n/a	Day 8	n/a	n/a	n/a
3	Day 15	n/a	n/a	n/a	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	n/a	Day 8	n/a
5	n/a	n/a	Day 8	Day 6	Day 8	Day 8	n/a

*This was not applicable to these plants because they did not germinate.

Table 2.1: Average Growth Over Time for Cellulase Soaked Seeds

Day	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
6	0 mm	0 mm	0 mm	10.5 mm	6.5 mm	0 mm	13 mm
8	0 mm	0 mm	17 mm	30 mm	35.5 mm	18.5 mm	19 mm
11	10 mm	0 mm	39 mm	47 mm	42 mm	28.5 mm	33 mm
13	19.5 mm	0 mm	42 mm	50.7 mm	46 mm	26 mm	34 mm
15	31.5 mm	0 mm	44 mm	52 mm	48 mm	31 mm	34 mm
18	44.5 mm	0 mm	46 mm	59.3 mm	49 mm	43 mm	35 mm
20	49 mm	0 mm	56 mm	61.3 mm	52.5 mm	44.7 mm	36 mm

Table 2.2: Standard Deviation of Growth over Time for Cellulase Soaked Tomato Seeds

Day	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
6	0	0	0	.70711	.70711	0	n/a
8	0	0	n/a*	13.22876	7.77817	2.12132	n/a
11	n/a	0	n/a	1	1.41421	6.36396	n/a
13	21.92031	0	n/a	4.04145	1.41421	8.48528	n/a

15	24.74874	0	n/a	3.4641	2.82843	7.07107	n/a
18	14.84924	0	n/a	7.02377	4.24264	2.82843	n/a
20	8.48528	0	n/a	9.07377	7.77817	8.5049	n/a

*This is not applicable to these plants because they did not grow.

Table 2.3: Median Time Cellulase-Soaked Seeds Took to Germinate (based on a minimum height of 10cm)

	0 hours	5 hours	10 hours	15 hours	20 hours	25 hours	30 hours
Day of Germination	Day 13	n/a	Day 8	Day 6	Day 8	Day 8	Day 6

Table 2.4: T-tests Comparing Variable Soak Times to the Control

5 hours to control (0 hours)	0.006743551
10 hours to control (0 hours)	0.125051026
15 hours to control (0 hours)	0.025012964
20 hours to control (0 hours)	0.044797707
25 hours to control (0 hours)	0.294105564

30 hours to control (0 hours)	0.20767452

Table 2.5: T-tests Comparing Tomato Seedlings Soaked for Different Periods of Time in Cellulase

Time 1 (to the right)	**5 hrs**	**10 hrs**	**15 hrs**	**20 hrs**	**25 hrs**	**30 hrs**
Time 2 (below)	----	----	----	----	----	----
5 hrs	----	0.000233991	1.49957×10^{-5}	1.07014×10^{-5}	0.000231066	1.15233×10^{-6}
10 hrs	0.000233991	----	0.180078637	0.300412329	0.219089491	0.247303731
15 hrs	1.49957×10^{-5}	0.180078637	----	0.315384529	0.04064613	0.035093467
20 hrs	1.07014×10^{-5}	0.300412329	0.315384529	----	0.07762019	0.071581884
25 hrs	0.000231066	0.219089491	0.04064613	0.07762019	----	0.399051108
30 hrs	1.15233×10^{-6}	0.247303731	0.035093467	0.071581884	0.399051108	----

Figure 3.1

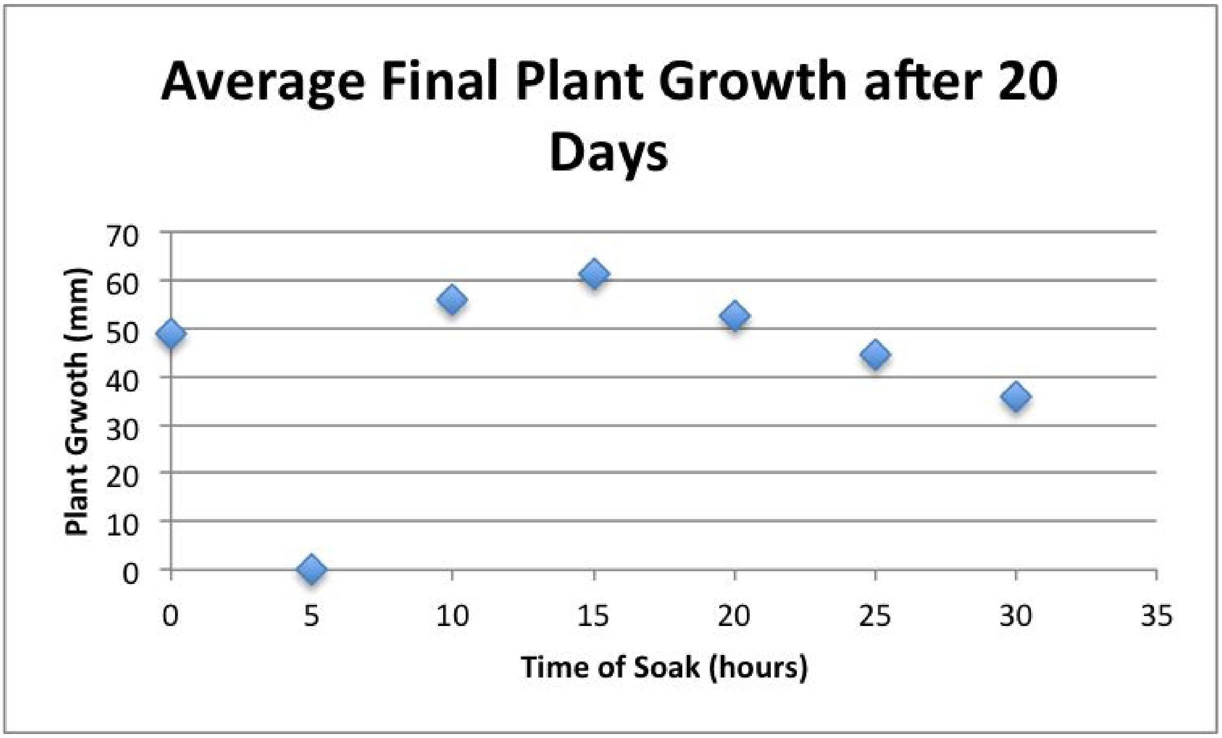


Figure 3.2

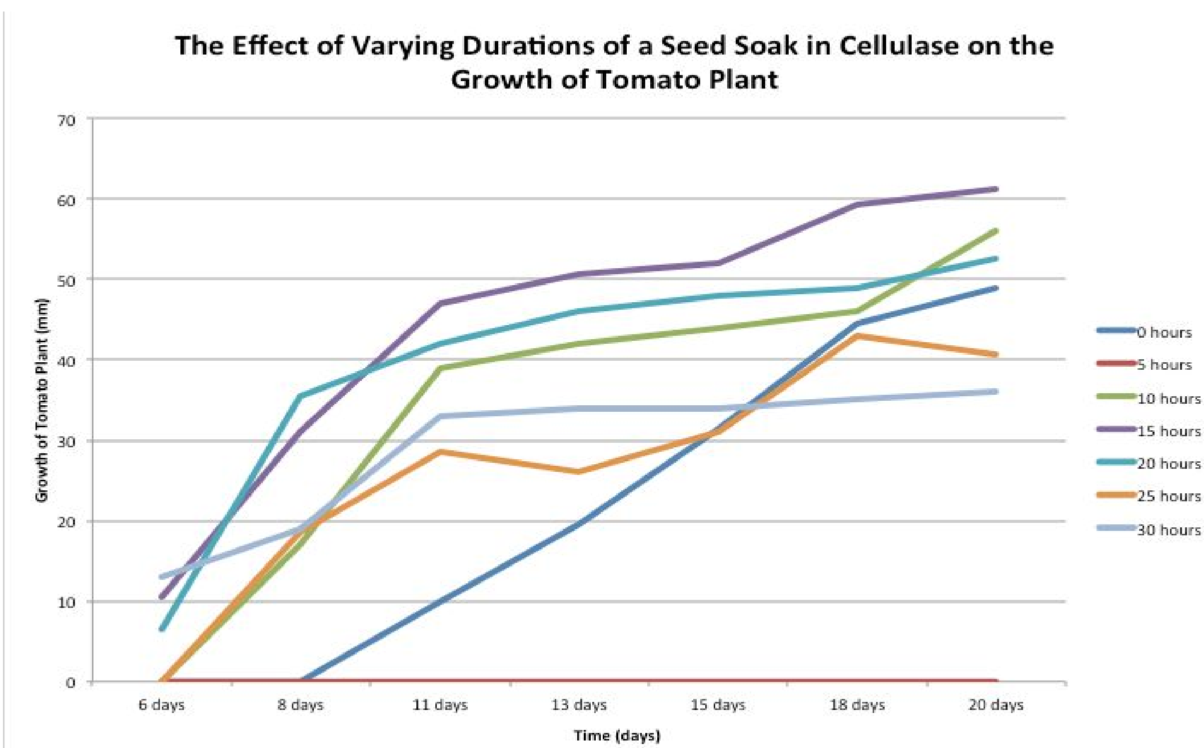
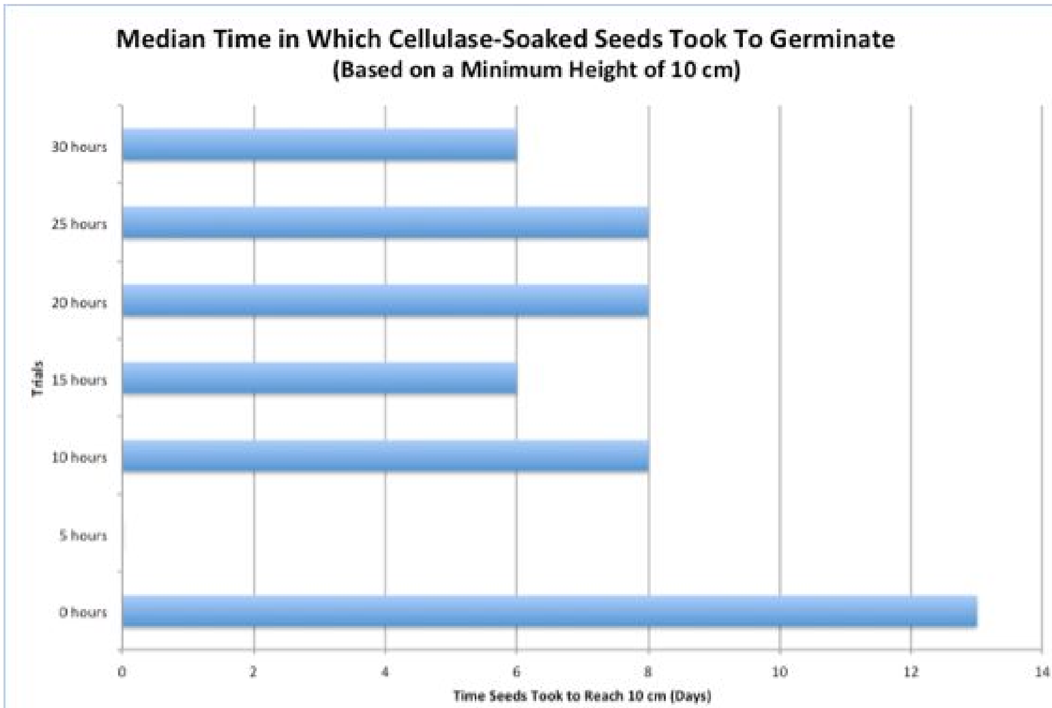


Figure 3.3



Discussion

This experiment was successful in allowing for the determination of the effect of a cellulase soak and the length of the soak on *Solanum lycopersicum* seeds. The hypothesis was supported by the results. It was predicted that tomato seeds soaked in cellulase for fifteen hours would germinate the fastest, and, as a result, would experience the most growth. The seeds soaked for fifteen hours germinated within six days (Figure 3.3), the shortest germination period of all the trials (except thirty hours, which also germinated in six days). These seeds also grew to an average height of 61.3 millimeters, as seen in Figure 2.1. These results support the original hypothesis. A t-test also shows a significant difference between the growth of the control group (zero hours) and the fifteen hour group (Table 2.4). Based on these results, it was established that the optimal time period in which *Solanum lycopersicum* should be soaked in cellulase was 15-20 hours, as that range allowed for rapid germination and increased growth. After 20 hours, the germination period and average growth decreased, with 30 hours having the least amount of growth, other than the seeds that were soaked for five hours, which did not grow at all. This may indicate that after a certain amount of time, the embryo inside the seed became damaged by the cellulase. For example, the 30 hour trial did not develop any leaves, even as it grew. This indicates an inherent problem with the embryo, which may be attributed to excessive cellulase exposure.

The results of this study are consistent with previous findings. Cellulase is known to reach its peak absorption by cellulose between fifteen and twenty-four hours (Lee, Shin, Dewey, Ryu, & Mandels, 1982, p. 2150). The results showed that cellulase was most effective between fifteen and twenty hours, as this was the only period during which the seeds soaked in cellulase had growth that was statistically different from the control. Although the seeds soaked for five hours showed statistical difference, this was most likely due to error, as discussed in detail in further paragraphs. The idea of peak absorption and a gradual decrease in efficiency as the time increases or decreases is represented by a normal model, or a bell-shaped curve, as seen in Figure 3.1.

There were several sources of error in conducting the experiment. Initially, the seeds were soaked in cellulase with the intention that the seed coat would disintegrate or become weaker. It was later realized that since the embryo also contains a high amount of cellulose, the cellulase could potentially kill the embryo as well. This is what may have caused the decreased growth in the 30 hour trials. Additionally, this may have caused a high number of the seeds to not germinate at all. There was also an issue with the process of soaking itself. Since each group member took individual conical tubes home to soak overnight, there was little consistency with how the seeds were soaked. Some seeds may have had better coverage by standing the conical tube upright or on its side. However, since each group member may have oriented their tubes differently, some seeds may have had a weaker seed coat or a damaged embryo. Perhaps seeds used in the “5 hour” trials were damaged in transportation to and from school, as each group member used different methods of soaking and transporting their seeds. Finally, there was also an issue with how much light and water each plant was receiving. Due to the issue of limited accessibility on the weekends, one liter of distilled water was poured into the plant tray as free access for the plant to soak up. It was assumed that once the soil was saturated, it would stop soaking up water. However, it was later observed that some plants had damper soil, while others were dry. This shows that some soil was oversaturated, while other soil didn’t have enough water for the plant. This may be due to the fact that the middle of the tray was slightly lower than the edges of the tray, causing water to pool in the middle. The plant tray also needed to be placed in such a way that the middle portion of the tray (the “10 hours” - “20 hours” trials) was getting the most light due to a space issue. Meanwhile, the trials on the ends of the tray (“0 hours”, “5 hours”, “25 hours,” “30 hours”) were not receiving as much light.This may also account for why growth was so much more successful in the 10 hour-20 hour trials. All of these factors may have contributed to the lack of growth in the five hour trial.

There are many extensions that may be performed to further explore this study. Other methods of decreasing the germination period of seeds and increasing their growth could be tested. For example, instead of breaking down the seed coat, methods such as soaking seeds in water or sulfuric acid could be used to break down chemical inhibitors in the seed (Missanjo, Maya, Kapira, Banda, Kamanga-Thole, 2013, p. 2). Additionally, seeds do not germinate in the colder temperatures, as they are dormant. Using a similar idea to ours, of inducing germination, steps could be taken to simulate winter-like conditions in which the seed is dormant. This way, the seeds are only dormant for a month, and can be transferred into warm conditions to simulate changes in weather, essentially breaking the seed dormancy. This technique is called cold stratification (Chin, Blanche, Bachireddy, 1992, p. 689). Another way to induce germination is to introduce plant hormones. Gibberellic acid is a potent plant hormone that, when administered in the right dosage, can quicken seed germination, quicken root and stem growth, and encourage rapid mitosis in leaf cells (Chin, Blanche, Bachireddy, 1992, p. 689). These are all well-known ways of decreasing the germination period and it would be interesting to compare these methods to each other and to the cellulase method to see which would be the best option for faster germination. Farmers may then be able to apply these techniques to grow crops more quickly and efficiently for increased profits and production.

These solutions may have a great impact on the agricultural industry. The US agriculture industry produces over 30% of the world's rice, wheat, and corn, and makes about $200 billion annually (United States Environmental Protection Agency, 2013, para. 3). As the population increases, food demand will increase dramatically as well. Farmers will soon need to find ways to be able to plant crops and cause them to germinate more quickly, even in the off season, in order to keep up with this demand. Decreasing germination time, which can be done through various methods, may allow for more of the agricultural supply to be produced to meet this great demand for food in the US and the world. If more research is poured into efficient ways to break dormancy and increase germination rates, the agriculture industry will be able to produce more crops year round, driving down costs for the consumer and increasing profits for the farmer.

Cellulase has the potential to be very beneficial to the agricultural industry. This particular experiment could be used as a technique to increase the rate of germination. Seeds may be soaked in cellulase for approximately 15 hours, as this was found to be the most effective soaking time for decreasing the time it took the plants to germinate in this study. This may cut down costs for farmers that have to plant the seeds and care for them before they have even sprouted. It could also increase profits because the farmers will be able to harvest slightly earlier, allowing them to plant more crops once these cellulase-treated plants are harvested.

Literature Cited

Black, M., Bradford, K. J., Vázquez-Ramos, J. (1999). *Seed biology: Advances and applications, proceedings of the sixth international workshop on seeds*. Mérida, México: Cabi Publishing.

Boriss, H., & Brunke, H. (January 2011). Fresh tomatoes profile. *Agricultural Marketing Resource Center*. Retrieved from http://www.agmrc.org/commodities__products/vegetables/fresh-tomatoes-profile/

Chin, K., Blanche, C.A.; & Bachireddy, V.R. (1992). Gibberellic acid and cold stratification treatments affect kiwi seed germination and root elongation. *Hortscience, 27,* 689.

Lee, S. B., Shin, H. S., Ryu, D.D.Y., & Mandels, M. (1982). Adsorption of cellulase on cellulose: Effect of physicochemical properties of cellulose on absorption rate of hydrolysis. *Biotechnology Bioengineering*, 24, pp. 2137-2153. doi: 10.1002

Mendu, V., Griffith, J. S., Persson, S., Stork, J., Downie, A. B., Voiniciuc, C., Haughn, G. W., & DeBolt, S. (September 2011). Subfunctionalization of cellulose synthases in seed coat epidermal cells mediates secondary radial wall synthesis and mucilage attachment. *Plant Physiology*, 1(157), p. 111. doi: 10.11

Mendu, V., Stork, J., Harris, D., & DeBolt, S. (November 2011). Cellulose synthesis in two secondary cell wall processes in a single cell type. *Plant Signal Behavior*, 6(11), p. 1638. doi: 10.416104

Missanjo, E., Maya, C., Kapira, D., Banda, H., & Kamanga-Thole, G. (July 2013). Effect of seed size and pretreatment methods on germination of *Albizia lebbeck. ISRN Botany*, (2013). doi:10.1155/2013/969026

United States Environmental Protection Agency. (September 2013). *Agriculture and food supply*. Retrieved from http://www.epa.gov/climatechange/impacts-adaptation/agriculture.html

Worthington Biochemical Corporation. (2014). *Cellulase*. Retrieved from http://www.worthington-biochem.com/cel/default.html

Appendix

Figure 4.1: Observations and Pictures Days 3, 6, and 20

Day	Trial	Observations
3	10h, 5 30h, 2 30h, 4	· Growth observed, but later noted that it was not a tomato seed plant · Possible implications on how the seeds in those compartments grow 
Day 6	15h, 5 15h, 1 15h, 2 20h, 1 20h, 5 25h, 4 30h, 1	· First growth observed · Soil dried out over the weekend

Day 20	15h, 5	· Leaf appears to be bent, possibly towards the light (phototropism?)