Summer 2015
Borlaug-Ruan
International
Internship

Silencing Genes Associated with Salinity Stress in Cotton

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Table of Contents

Acknowledgements

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Background

My childhood was spent growing up on a farm, drinking out of a hose, and making forts out of hay bales. Agriculture seemed to be the only life I knew. In high school, I enrolled in agriculture education classes where I soon became an FFA member. I was learning to do, doing to learn, earning to live, and living to serve. Learning about myself was one of the most important lessons I learned. I found that communication was one of my strong suits. My FFA advisor encouraged me to write a research paper for the Iowa Youth Institute in the spring of 2013. My communication came into play when discussing hunger and global agriculture with other students from Iowa.

In the fall of 2013, I attended the Global Youth Institute where I met with other young adults from across the world. We shared ideas and my mind was overwhelmed with the idea of how many people were truly effected by food insecurity. It was eye-opening, almost disturbing, to think of hunger on this large of a scale. In my hometown of New Hampton, the extent of improving food security is donating to the local food pantry. I had never given thought to fighting food insecurity on a global level.

There were many eye opening moments at the Global Youth Institute as I listened to speakers from across the globe. These speakers were humans just like me who gave examples of poverty they witnessed on a daily basis. I realized how poverty was not a condition in a closed-off island on a far-off world. Poverty is a problem right now in our world. There are solutions that can be reached. All that is needed is someone to be the change.

The Borlaug-Ruan Internship was introduced to me at the Global Youth Institute. Past Borlaug-Ruan Interns lead the groups around and took time to get to know us better. They were introduced to us students as ‘the change the world needed’. The interns who talked with me seemed very knowledgeable and much more intelligent than I could ever be. They spoke of different cultures, much different than America’s, and going to a different country where English was not spoken. I was in awe of all of the work they did and how cultured they were. I thought of how wonderful an experience it would be to apply for such a prestigious opportunity. On the other side, it was hard for me to believe that these young people left their family and friends for such a long time. All of these thoughts, good and bad, were pushed aside when I realized I was too young to apply. I placed the whole thought of the internship out of my mind.

I wanted to continue spreading information about struggling people in third-world countries to others in my own community. I took advantage of my determination and communication by organizing a group of classmates to write a grant to buy water filters for Haiti.

The next year, I encouraged several underclassmen to write a research paper for the Iowa Youth Institute. By encouraging them to write the paper, I talked myself into applying for the internship. After I sent in my application, I realized how this was a once in a lifetime experience and that I wanted to help solve world hunger more than anything. I tried my hardest with the application and interview process. My hard work paid off when I found that I would be going to China to work in a science lab during the summer of 2015.

Before the spring of 2015, I had never been outside of Iowa or any bordering states. Growing up on a swine and bovine farm greatly impacted my life. Vacations never lasted too long and we never traveled too far as chores would need to be done in the evening. Going out with friends meant busting your tail to be done working by the time 7:00 came around. These minor hindrances were greatly outweighed by other values I gained. My work ethic, endurance, patience, and courage all came from how I was raised. These values have very close ties to the work the World Food Prize supports.

World Food Prize Goals

The desire to grow more crops on less land is a standard goal in the world today. I wrote a research paper in the spring of 2013 regarding water sanitation in Haiti. In my eyes, clean water meant healthier people who could grow high yielding crops with the knowledge they gained from attending school. It was easy for me to see how clean drinking water would help the world be a healthier place. At the Iowa Youth Institute, my eyes were opened to many other problems- infrastructure, woman inequality, and climate volatility- but how would fixing these factors help feed the world? The students in my group opened my eyes to see that reliable infrastructure allows goods to be delivered to markets before they spoil. Allowing women to find a job will provide families with a greater income. Adapting to the climate in the region will provide farmers to grow more crops. Food scarcity is not solely fought by growing more crops with less land, it is providing greater opportunities for the less fortunate. This may include developing a plant able to grow in a wider array of environments, offering a greater level of education, or providing government subsidies to those who encourage greater yields.

China Agricultural University

My summer internship was held at the China Agricultural University. This university can be traced back to the year 1905. At that time, three agricultural college/universities joined as one university to form Beijing Agricultural University (BAU). At the same time, three engineering colleges joined to form Beijing Agricultural Mechanization College (BAMC). In 1995, these two schools came together to form China Agricultural University. China Agricultural University (CAU) works directly with China's Ministry of Education. The president of the university is chosen by the Central Government ("Brief." Web).

The CAU campus has an area of 388 acres. There are 14 different colleges with 60 departments. There are currently 70,000 students enrolled in classes. Of these students, 50,000 of them are part-time students. There is an east campus, west campus, ("Facts." Web) and Jianshe campus ("About." Web). The College of Life Science Building was completed by 2007 on the west campus ("Facts." Web). Most students on the west campus are graduate students while the east campus is home to many undergraduate students. College departments include life sciences, engineering, economics and management, and humanities and development ("About." Web)

Research

Abstract

Virus-induced gene silencing is a common method to knock-down a gene in plants for a short period of time to advance research on that particular gene. At China Agricultural University, I had the opportunity to perform a research project that analyzes salt stress in cotton. My laboratory partner, Zhou Lin, helped me throughout the extent of this project.

To measure cotton's resistance to saline conditions, we grew cotton plants that had specific genes silenced. Sixty cotton plants were grown under my supervision and inserted with bacteria. These plants were allowed to grow for approximately one week before salt was added to their nutrition solution. Phenotypes were recorded during the following four weeks. When we were done observing the plants, we photographed them.

By sequencing the bacteria, we found that the four plants that showed phenotypical differences were not solely influenced by the saline. None of the genes I silenced in my data set seemed to be influenced by saline water conditions. The results were then mapped into the data base.

By completing this experiment, we have furthered the understanding of salinity stress on cotton. Optimally, the cotton with silenced genes would have shown either resistance or sensitivity to the saline solution. Being this method is relatively new, many trials will need to be completed to find the genes we are looking for. This experiment has the capability to give us a greater understanding about growing cotton in salty soils.

Introduction

Cotton is grown to produce a wide array of products for people around the world. Not only is cotton grown for clothing, but also diapers, high quality paper, nets and ropes, tents, and upholstery. Cotton plays a key role in many everyday activities. Still, the area of cultivated land is diminishing. With cotton consumption rising, it is important for this crop to be diverse in its growing regions. Desirable land is hard to acquire. Land for planting often has saline and alkali characteristics, which cotton struggles to grow in. I was assigned to research the salinity resistance aspect of cotton to better understand how to regulate the physiological mechanism.

Virus-Induced Gene Silencing (VIGS) is becoming a common way to silence specific genes in plants. Drought and salinity stress have been further investigated with VIGS technology. Using viruses to silence genes can determine an individual gene's functions through observations and sequencing. The new cells with vectors of viruses are transferred into *Agrobacterium tumefaciens* to stimulate dsRNA growth and kick-start the forming of siRNAs for silencing of the expression of the targeted gene (Ahmed. 44 Print). This method of post transcriptional gene silencing uses a virus vector that is inserted into a cotton bacteria cell. The bacteria is inserted into the plant after transcription using liquid YEP and antibiotics (Pandey. 4 Print). The virus is accepted by the host plant generating siRNAs. The siRNAs then silence the viral gene expression and the virus is no longer in existence. (Ahmed. 44 Print.)

Over the course of one week, the genes are more completely silenced. This is evident in the CLA 1 infected plant. Recording the physical description of each plants growth showed which genes are detrimental and which are provocative towards cotton's growth. The genes were sent

to a company to be sequenced. By mapping our data results in to the base, our experiment can be referenced in future studies.

Methods and Materials

My internship at CAU took place for 60 days. With this amount of time, some of the steps in my experiment were not done by myself. I was unable to help soak the cotton seeds in 100% H2SO4 for several seconds (until the pericarp is black) to enable the pericarp to be penetrated during germination.

At this time, I was able to take over my experiment until some of the final steps. My mentor, Zhou Lin, was doing the same experiment in the same room, but with different genes being silenced. She helped me understand many of the processes by having me help with her experiment and later allowing me to work independently. The procedure that I am describing below includes sufficient supplies and materials for both of our experiments as we often prepared for them together.

From the community cotton seed bag, we selected enough seeds to fill a flask 400 milliliters full. After being soaked in ion-free water from 24 hours, the seeds were further classified. We classified them by their physical characteristics as ‘able to germinate’ (seeds beginning to sprout) and ‘unable to germinate’ (seeds with an impermeable pericarp or were mushy and moldy).

The seeds classified as ‘able to germinate’ were planted in a tub with 3 centimeters of sand. The sand was previously rinsed two or three times to remove any particles from previous experiments. We wet the sand until it stayed in a clump in your hand, but easily fell apart when dropped back into the bucket. The seeds were planted in the sand by pressing the seed into the sand, sprout down, until the top of the seed was flush with the top of the sand. Seed spacing was approximately one centimeter away from another seeds in rows and columns.

When all seeds were planted, the sand and seeds were sprayed with a water mist that covered all seeds at least once. This was to aid the pericarp in opening and falling off without damaging the cotyledons. The surface of the sand was covered with a tightly woven fabric. Another layer of sand was added on top of the fabric one centimeter thick. This is to give the cotton seeds some resistance to ensure that their roots grow deep and strong. The top rim of the tub was sprayed with water and cling wrap was placed over the top of the tub. By spraying water on the rim, no moisture was able to escape the tub because the top is sealed.

Place black bags on the top of the tubs to prevent light from entering and interfering with the roots’ growth. The black also attracts heat from the lighting in the room, which further aids in seed germination.

Three days later, we pulled the pericarps off. The seedlings were sprayed with water so the pericarps would come off easier. If the pericarp was not easily removed, the seedling was neglected. Neglected plants were not pulled out at this time to prevent an interference with the healthy seedlings.

The next day we transplanted the seedlings to Styrofoam lids with 12 holes placed on tubs. Before starting this step, we ensured that a worker was called to fill the water barrels with sufficient clean water. Make the nutrition solution with this clean water. For every 60 liters of water, we added 120 milliliters of concentrated micronutrient solution mix and 60 milliliters of each macronutrient. (See Appendix A for proportions)

The seedlings in the sand tubs were sorted through. First the seeds with pericarps still on them were pulled from the sand and thrown away. Then the seedlings whose leaves were obviously affected by H_2SO_4 (scorched/crinkled cotyledons) were uprooted. The rest were very gently pulled out from the sand while water was poured on the sand to loosen its grip on the cotton roots. The cotton seedlings that appeared healthy were sorted into groups based on the root length and leaf size. Groups of 12 similar sized cotton plants were wrapped in sponges at the stem directly below the cotyledons and placed in the Styrofoam lids with 12 holes in them. The Styrofoam lids were set on a tub filled with five liters nutrient solution. Zhou Lin was experimenting with 18 tubs while I observed five tubs. We kept one tub of miscellaneous sized seedlings to replace any seedlings that did not continue to grow vigorously.

The tubs were spaced out to allow room for growth. To aerate the roots, one .75 centimeters hose was inserted into each five liter tub. On the opposite end of the hose there was a needle that was inserted into the main air supply hose. We ensured that each of these hoses were functioning properly by checking each tub to see if bubbles appeared in the nutrition solution.

In the laboratory, a batch of YEP was mixed by adding five grams of sodium chloride, ten grams of yeast extract, and ten grams of tryptone to one liter of water. This did not have to be precise; a variance of .03 grams and five milliliters was allowed. We autoclaved the YEP for two hours. For the experiment I was doing, I needed to add 500 microliters Kanamycin and 250 microliters Gentamicin to one liter of YEP. The antibiotic was used to try to keep the plant healthy and growing vivaciously.

In each of 14 micro centrifuge tubes, we added one milliliter of the YEP with gentamicin and kanamycin. We then added 20 microliters of liquid bacteria from a micro centrifuge tube into a new tube with the corresponding number written on it. There were 14 different strands of genes that we are silencing: 1039, 1021, 1040, 1030, 1014, 705, 784, 1001, 761, 766, 771, 749, 813, and 720. These 14 tubes were placed in the shaker for 24 hours at 28℃ and 200 RPM.

After the YEP was thoroughly mixed with the bacteria, we started making the culture medium: 588 milliliters YEP, 12 milliliters MES ($C_6H_{13}O_4N_5 \cdot H_2O$), 120 microliters As (acetosyringone, $C_{10}H_{12}O_4$). In each ten milliliters tube, add five milliliters of culture medium and 200 microliters YEP with Gentamicin and kanamycin and bacteria. Wrap the tubes with a stretchable wax Parafilm sealer and shake for 48 hours at 28℃.

In Erlenmeyer flasks, mix one milliliter of RNA 1 with 100 milliliters of culture medium. In another other flask, mix one milliliter GFP (green fluorescent protein) with 50 milliliters of YEP culture. Set these in the same shaker as the tubes of culture media mixed with bacteria.

Make re-suspension solution by combining 271 milliliters water, 2.8 milliliters magnesium chloride, 5.6 milliliters MES, and 560 microliters As. The MES is to regulate the pH level of the liquid while the As helps the bacteria release the virus and infect the plant cell.

Take the Erlenmeyer flasks and 10 milliliter tubes out of the shaker. Pour RNA 1 into two 50 milliliter tubes and the same amount of GFP into two 50 milliliters tubes. It is important to keep the amounts equal to keep balance in the centrifuge Spin the 50 milliliters tubes and ten milliliters tubes in the centrifuge for 12 min at 4℃ and 6000 RPM

Dump out the supernatant and leave the bacteria pellet in the bottom of the tube. Add two to three milliliters resuspension solution to GFP and RNA 1 (50 milliliters tubes). Using a pipette, spray the bacteria pellets with resuspension solution until the pellet is dissolved. If using a pipette to suspend the solution is too complicated, close the tube and shake vigorously.

Add two milliliters resuspension solution to each of the gene silencing bacteria. Flush bacteria pellet using a pipette or shake by hand to dissolve. If the gene silenced bacteria was made in the proper proportion, the OD_{600} should be 1.5 in each of these bacteria.

Before balancing the OD_{600} of the GFP and RNA1, pour approximately .5 milliliters of each in a separate tube in case too much re-suspension solution is added. Add resuspension solution until the OD_{600} of the GFP and RNA1 are 1.5 to match that of the gene silencing bacteria.

Measure the optical density of all of the bacteria to ensure they have the same amount of bacteria. Rinse a quartz cup with resuspension solution, dump it out, and then fill it with just the resuspension solution to zero out the spectrophotometer. Every time I put a solution in the spectrophotometer, I put three milliliters in the quart cup to maintain accuracy.

Let the bacteria set for three hours. Add two milliliters re-suspended solution RNA1 to each numbered tube. Now there should be four milliliters in each ten milliliters tube of gene silencing bacteria. (The RNA 2 is in the bacteria already.)

Add GFP and RNA 1 solutions together in an equal ratio. RNA1 was the limiting reagent in this step.

Gather the gene silencing bacteria, GFP bacteria, 35-40 disposable syringes, 3-5 metal needles, and masks and prepare to bring them to the greenhouse.

In the greenhouse, we looked through each of the cotton plants in all 24 tubs. We took out the 12 plants that had bad leaves or looked weak. We were left with 276 plants: 216 plants were Zhou Lin's, 60 were mine. Scratch the back of each cotyledon leaf in four spots (one in each section between the veins) with a metal needle. If at any point this needle came in contact with any kind of gene silenced bacteria, the needle was discarded in fear of contamination.

Use a needleless syringe to inject the bacteria solution into cotton. Each different gene silencing bacteria gets a new syringe. When inserting the bacteria, press the syringe perpendicularly against the cotyledon and put your finger on the opposite side so the syringe applies flat pressure

and the bacteria does not leak. Slowly push the bacteria solution in to the leaf until the whole leaf is saturated internally. The leaf will show an apparent color change when injecting. Cover each of the tubs with black bags to block the light, and put them under the table so the black bag does not attract heat from the overhead lights.

One day later, remove the plastic bags from the cotton, put the tubs back on the table, and reinsert the air tubes into the Styrofoam lids.

Record the phenotypes of the plants that are still unaffected by the salt. Record the leaf size, height, color, and stem width. I have also decided to record the phenotypes of the roots. (See Appendix B for phenotype observations)

After 13 days, add one mole of sodium chloride to each tub over the course of 24 hours. Add the salt in three different batches, 0 hours, 12 hours, 24 hours: 14.61g, 20.454g, 23.376g respectively. Before adding the first batch of salt, we had to change the nutrition solution. Therefore, we added the salt directly to the large barrel of nutrition solution. This was more convenient than adding salt to each tub individually. At this point, the plants should be receiving .2 molar sodium chloride in addition to fertilizer.

The nutrition solution was changed and the first batch of salt was added at 5:00 pm. In each large water barrel, there are 60 liters of nutrition solution. Each plant's tub was five liters. Twelve tubs per water barrel multiplied by 14.61grams of sodium chloride per tub equals 175.32 grams of sodium chloride in each water barrels. There were two barrels, so we weighed out 24 servings of salt due to the fact that some water dribbles out the side of the pitcher when adding it to the tubs.

That evening in the laboratory, we measured out 23 servings of 20.454 grams of sodium chloride into plastic bags. Due to other projects in the laboratory, we also weighed the third batch of salt; 23 servings of 23.376 grams of sodium chloride into clean plastic bags. Plastic zipper bags were used to ensure the sanitization of the bag because it is submerged in the nutrition solution when rinsing them out. We were careful not to let these bags touch the counter in the laboratory for this reason.

The second batch of salt was added to the plants at 8am on July 15. Each plant's nutrition solution had 20.454 grams of sodium chloride added. Likewise, the third batch was added at five pm on July 15. We added 23.376 grams of sodium chloride into each plant's tub.

The nutrition solution must be changed once a week. Before changing the nutrition solution, make sure there are sufficient nutrients to make the solution. To make nutrition concentrates, refer to Appendix A for recipes. All recipes follow the same procedure. At the same time, record all phenotypes of plants including the leaf size, height, color, stem width, and root (see Appendix B).

On July 22th and 29th, I changed the nutrition solution. I first washed all of the equipment that I would be using. In each 60 liter barrel, I added 120 milliliters of micro nutrients and 60 milliliters of each bottle of macronutrients (see Appendix A). I then added 701.28 grams of

sodium chloride to each barrel (58.44 grams of sodium chloride per five liter tub x 12 tubs per barrel).

On August 4, I recorded the phenotypes for the last time. I looked over the observations that I had made and decided if the salt had played an important role in any of the plants' growth. Based on these observations, I choose three genes to be sequenced.

Using a plasmid extraction kit, began the steps to sequence genes 749, 784, and 720.

Label enough tubes for each of the bacteria that you are going to extract plasmid from. In each 15 milliliter tube that already has five milliliters of YEP and Kanamycin, add 100 microliters of bacteria. Shake for 16 hours at 180 RPM at 28 ℃.

Label absorption columns for each bacteria. Each absorption column needs a liquid waste container under. Wash the column with 500 microliters of BL and centrifuge for one minute at 12000 RPM. In micro centrifuge tubes, add the bacteria and YEP and Kanamycin at centrifuge for one minute. Dump the supernatant and repeat until all of the bacteria is collected at the bottom of the micro centrifuge tube.

Add 250 microliters of P1 to each micro centrifuge tube with bacteria pellet in the bottom. Using the Vortex Genie, dissolve the bacteria disks. Add 250 microliters of P2 to each tube and gently rock back and forth six-eight times. Add 350 microliters of P3 into each tube and rock gently 6-8 times until dissolved. Centrifuge for ten minutes at 12,000 RPM.

Pour the supernatant into the absorption column with care not to let any of the white flocks into the column. Centrifuge for 30-60 seconds. Dump the liquid waste. Add 500 microliters of PD to each column. Centrifuge for 30-60 seconds. Dump the liquid waste. Add 600 microliters of PW. Centrifuge for 30-60 seconds. Dump the liquid waste. Add 600 more microliters to each column. Centrifuge for 30-60 seconds. Dump the liquid waste. Centrifuge for an additional two minutes to ensure all of the PW is out. Throw away the current liquid waste container and replace with a micro centrifuge tube. Place the tubes in a fume hood for ten minutes to dry out the columns.

Add 50 microliters of water into each column directly on the plasmid filter. Do not allow for the water to touch the sides of the column. Let the tubes set for two minutes before centrifuging for one minute. Save the micro centrifuge tube of water for later use. Pipette the water at the bottom of the tube back into the column without letting the water touch the sides of the column as before. Centrifuge again for one minute. Pipette the water and bacteria from the bottom of the micro centrifuge tube into a new tube.

Prepare the NanoDrop 2000 spectrophotometer using the same water as the previous step. Gene 720 measured 30.5ng/microliters, 749 measured 32.8, and 784 measured 34.9. Pipette five microliters of the solution into a new tube. Keep these on ice. The rest of the solution is kept at -20 ℃.

Add 15 microliters of E Coli. competent cell to each tube of 5microliters of solution. Wait 20 minutes. Heat shock the tubes for two minutes at 37℃. Return back to ice. In a laminar flow fume hood, add 600 microliters of LB with no antibiotics. Shake for 30 minutes at 120 RPM. Centrifuge for one minute at 5000 RPM. Dump out the supernatant. Using the little supernatant that is left in the tube, suspend the bacteria with a pipette and place it on a petri dish of LB and Kanamycin. Use a bacteria free wand to spread the liquid until it is dry. Keep in 37℃.

When the spores have grown to be the size of a pipette tip, remove with pipette tip and eject entire tip into tube. Add one milliliters of YEP+Kan liquid. Send to company to be sequenced. When results are returned, run through several websites to find the function of the gene that was silenced.

Results

Of the plants that showed phenotypical differences, none of the genes played a role in salt stress tolerance. Gene 720 had actin-like characteristics, 749 was used as a cellulose synthase, and 784 had uncharacterized domain.

Conclusion

Although my experiment did not yield any bewildering, revealing results, I have successfully added my knowledge to gene silencing research. My labmates explained to me how different phenotypes seldom occur. Setbacks may be caused by the lack of knowledge to silence plant genes while the seed is germinating and the stability of the silencing (Senthil-Kumar. 44 Print.)

The results that I was given back state that the genes I researched had no significant impact on salt stress tolerance.

China Water

Introduction

While living in China, I adapted to only drinking bottled water and never out of the sink. Lab work required rinsing materials with ion-free water after being washed. When changing the nutrition solution for the cotton plants, workers had to be notified to supply us with clean water. All of this work was because tap water was polluted. I decided to investigate Beijing water to further understand the precautions that we were taking.

Experiment

After finding three petri dishes that I was able to use, I washed them with tap water and soap. I dried the inside and outside of the dishes immediately using tissues. After ensuring no water had evaporated and left water marks, I labeled them as “tap water,” “ion free water,” and “drinking water.” I filled three beakers with each respective water and added 25 milliliters to each petri dish.

I set them on a counter where it would be undisturbed to prevent further contamination from polluted air. After the water had completely evaporated, I photographed the pictures to the best of my ability. The dish of tap water had a thin film of white crystals covering the surface. The ion free and drinking water disks both produced relatively clean dishes free of residue. (Photo page 15)

To find the hidden identity of the elements that composed this white film, my lab partner proposed using hydrochloric acid to see if a reaction occurred. On a small portion of the dish we added 150 microliters of .14M hydrochloric acid. There were no exuberant reactions, but it seemed as if there was a minuscule reaction happening. After adding 150 microliters of 1M hydrochloric acid, the acid made the crystals start to bubble and dissolve.

This reaction can be written as:
$CaCO_{3(s)} + Mg^{2+}_{(s)} + 2HCl_{(l)} = CaCl_{2(l)} + MgCl_{2(l)} + CO_{2(g)} + H_2O_{(l)}$

This reaction shows how the evaporated water leaves behind a film which is partly made of calcium and magnesium deposits. By adding the hydrochloric acid, the film dissolved into calcium chloride and magnesium chloride that was suspended in water. This reaction caused the liquid to bubble and release carbon dioxide.

Conclusion

Based on how the white film began to bubble when I added the hydrochloric acid, I induced that there is an excessive amount of calcium and magnesium in the water. There may be other elements as well, but calcium and magnesium left the most significant deposits ("Magnesium" Web).

Personal Reflection

Walking away from the baggage claim at the Beijing airport was a moment I will never forget. What if no one is here to pick me up? Will someone know how speak English if I need help? Is there another airport in Beijing? What if they went to the wrong airport? All of these questions were answered within several minutes. I had nothing to worry about. Someone was there waiting to pick me up, and they spoke just enough English that I could communicate with them. From that moment on, everything I did required trusting someone else. There were many times I did not know what I was eating, but I was willing to try something new. I went sightseeing on the weekends and immersed myself in Chinese culture, but I was never told what exactly we were going to see or where we were going, in the morning.

Not knowing what the day was going to bring was an adventure. I knew that I would be back in the apartment to sleep that night. I could not help but imagine a day like this from a rural farmer's standpoint. This sense of not knowing what the future will bring is not adventure, it is anxiety. However, my sixty day trip of not knowing what the day would bring will never be comparable to the anxiety of a small Chinese farmer trying to raise enough money to provide for a family.

By researching cotton growth in a saline environment, my goal was to allow cotton growth in a wider variety of land types. Although, my 60 days of research will not cure the world, but I was able to experience what it is like to make small improvements to research.

Before I went on this internship, I had only taken basic science classes in high school and had not taken a math course in six months. I felt very worried that my knowledge would be

insufficient to work at a graduate student science laboratory. The China Agricultural University students made sure I was not left behind. The knowledge that I already had needed to be translated by using the internet. When I did not know a term or method, I was able to look it up on the internet. So it did not matter if I knew the terms in the first place or needed to look them up to understand. The students at CAU took me under their wing and were very willing to answer all of my questions. By the end of my experiment, I knew many more science related terms.

I was only able to conduct research on one complete set of cotton plants, but I helped with portions of other projects. By seeing the same project repeated, I was given the sense that what I researched was just an ice crystal on the tip of an iceberg. I never realized how patient one must be with science. My project only proved that I have to repeat the experiment at least once more to make any findings. This internship has showed me the highs and lows of working in a lab. All in all, I am very happy to have this experience because it has solidified my choice to pursue a career in the science field in the future.

By going to a foreign country, I also experienced an almost antithetic culture. From the first day I arrived, the aberrancies overwhelmed me. Of course there was a language barrier, but that was anticipated. I began recording the differences: squatting toilets, no cheese or milk, beautiful umbrellas, no drinks with meals, and high heels. An open mind was necessary if I wanted to have any fun while I was there.

One of the most consistent differences I noticed was how they cook and utilize any food they can find. When serving fish, the innards were scraped out, but the rest of the fish is thrown in the dish. Agaric mushrooms are fungi grown on trees and are part of an everyday meal. Chicken combs are a delicacy. Chicken feet are made into a candy. Flowers are not only used to add color to a dish, but also flavor to soups. Salads included the standard lettuce and spinach, but also broccoli leaves. I first thought this was crazy and uncalled for. Then I began to realize how many people there are in China. Food is food. I also noticed how food was only half the price as the same meal being served in America. It made me start thinking about all of the eating habits of Americans that I had never realized before. I will definitely be a less picky eater when I return back to the states.

When I started my freshman year of high school, I never would have guessed I would spend a summer in a science lab, let alone a foreign country. This trip has taught me an infinite number of lessons that I will be able to utilize for the rest of my life. The people I have met have showed me doors I never knew were open to me. Determination paid off.

Photos

Cotton

Gene 720 silenced

Actin-like characteristics

Gene 749 silenced

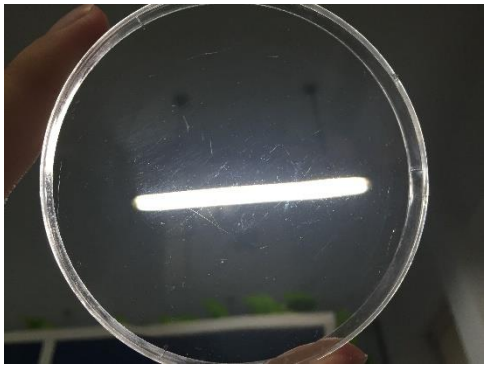
Cellulose synthase

Gene 784 silenced

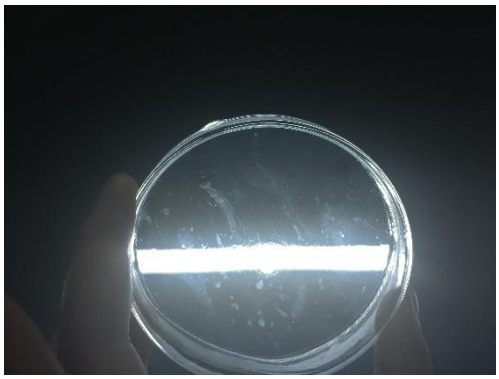
Uncharacterized domain

Water Quality

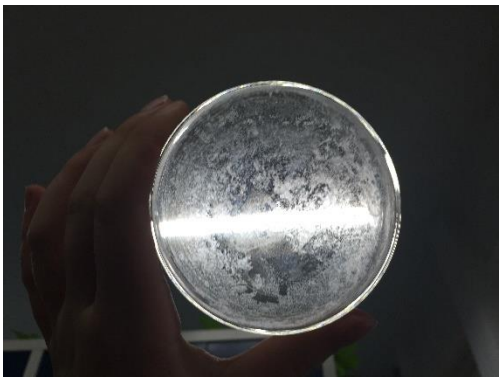
Evaporated ion-free water



Evaporated drinking water



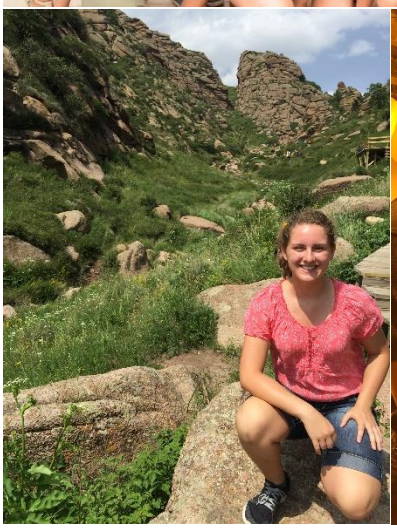
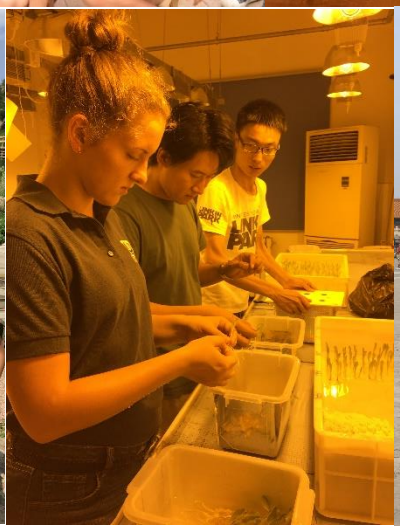
Evaporated tap water



Evaporated tap water residue treated with hydrochloric acid

Experience

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Appendices

Appendix A

Nutrient Solution

	Molecular Weight (amu)	Concentration (mmol)	Per 1 Liter H_2O (g)
Micronutrients			
H_3BO_3	61.83	.02	1.2366
$ZnSO_4 \cdot 7H_2O$	287.54	.001	.28754
$CuSO_4 \cdot 5H_2O$	249.68	.0002	.049936
$MnSO_4 \cdot H_2O$	169.02	.001	.16902
$(NH_4)6Mo_7O_{24} \cdot 4H_2O$	1235.86	.000005	.006179
Macronutrients			
EDTA: FeNa	367.05	.1	36.705
$Ca(NO_3)_2 \cdot 4H_2O$	236.15	2.5	590.375
$NH_4H_2PO_4$	115.03	.5	57.515
$MgSO_4 \cdot 7H_2O$	246.47	1	246.47
KNO_3	101.01	2.5	252.525

Appendix B

Phenotype Observation

1,2,3 refer to plant #

L= leaf size, h=height, c=color, w=stem width, r=roots

1, 3,5,7,9 = degree of severity (1=minimal, 9=severe)

Gene Silenced	July 14	July 23	July 29	August 4
1021	1-3h>3	1h>3 (&45° angle) / 3L>1 / 1&3r>1	1h45° angle / 2w<1 / 1w>1	3h>1 / 1h45° / 2w<1
1040	1-3h>1	1h>5 / 2-3h>1	1h>3	1h>3 / 3h>1 / 1L>3
GFP	1h>3	1h>5	1h>3	1h>3 / 3w<1 / 2c<1 / 3L>1
CLA1	1-3c<9 (white)	1-3L wilted / 1 upright / 2 angle / 3 down	Died	Dead
749	2&3L med twist / 1L mild twist / 1-3h<5 / 1-3c<5	1-3h<7 / 1&3L<3 / 2L<5 / 2c>3 / 2r<3	1h<5 / 2-3h<7 / 2-3L<3	2-3h<9 / 2-3L<7 / 1h<5 / 1L<3
813	1-3h>3	1-3h>3 / 3L>1 / 1c<5 / 2c<1 / 3r<3	2-3L>1	3L>3
GFP (NOT #2)	2c<7 (contaminated)	1h>3 / 3r>5 / 2r<1	1h>1	1L>1
CLA 1	1-3c<9 (white) / 1-3h>1	1&3c is ½ white ½ green / 2 curled and down	"…"	"…"
705	1-2L<3 / 3L<1 / 1&3c<1 / 2c>1 / 1-3h<3 / splotchy leaves	1Ldried up / 2h<5 / 3w>1 / 2&3c<1 / 1&2r<5 / splotchy leaves	1died / 2h<5 / 2c<3 /	2h<5 / 2c<3 splotchy / 2L<5 / 3c<1 ½ splotchy
784	3h>3	3L<7 / 3c<3 / 2h>1 / 1c white edge / 3c>1 / 3r<3	3w<1 / 3h<3 / 3L<5	3 died / 1-2 necrosis on old leaves
GFP	-	-	1c<1 (necrosis) / 3c>1	1-3 necrosis on old leaves
1030	-	-	1c<3in spots on first leaf / 1c<1	1-3 necrosis on old leaves
761 (Not #1)	*1h>9 / 1L>5 / 2&3h>1 / 2&3c<1*	*(unobservable)*	*1h>5 / 3L<1*	*1h>1 / 3h<1 / 1L>1 / 3L<1*
1001	*1h>3*	*(unobservable)*	*3h<3 / 2h<1 / 3L<3 / 1c necrosis* based on 3	*2-3h<3 / 1h<1 / 2-3L<5 / 1L<1* based on 3
GFP	*(heights vary, all based on #1)*	*1&2 died / (unobservable)*		
760	*1-3h>3 / 1-3L<1 / 1-3c<1*	*(unobservable)*	*1-3h<3 / 1-3L<3 / 3c necrosis*	*1-3h<3 / 1-2L<3 / 3L<5 / 3c white on edge*
720	-	1-3h<1 / 3c<3 / 1c white edge / 1&3L<3 / 2L<1 / r<1	3hfell / 1 no leaves	1&3 died / 2L<1
1039	2&3h<3	2&3h<5 / 1h>1 / 2&3c>1	1h>1 / 2h<3 / 3h<1 / 1-3c<1	2h<3 / 3h<1 / 1h>1 / 1&3c>1 leaves curl under / 3L<1
GFP	3L>3	3h>3	3h>1	3h>1
771	1L>3		1-3L<1	3h<1