The Adventures Began in China: Arabidopsis, Biotechnology, and CPS.



Rylee McDermott World Food Prize Foundation 2013 Borlaug-Ruan International Internship

China Agricultural University Beijing, China

Table of Contents

Introduction		
China Agricultural University	3	
International Plant Growth Substances Association	3	
Lab Research and Analysis		
Introduction	4	
Methods and Materials	6	
The Future of this Project	10	
Independqent Research		
Introduction	10	
Results	11	
Discussion	15	
Personal Experience		
Acknowledgements		
Bibliography		
Photos	24	
	- •	

Introduction

China Agricultural University

In 1995, China Agricultural University was formed being the final product of many agricultural college merges since 1949. CAU is the oldest agricultural higher education institution in China, since one of the first key pieces of the final masterpiece was founded back in 1905 (the College of Agriculture for what is now Peking University).

Even with such a long history, CAU is still one of the top ranked key national universities in China. The motto, "Tackle Problems the People Face, Cultivate Talents the World Needs," amazingly represents their goal of becoming one of the top national and internationally renowned universities (China Agricultural University, 2011).

International Plant Growth Substances Association

While in China, I was fortunate enough to spend my second week in Shanghai at the 21^{st} Conference of the International Plant Growth Substances Association. From Just $18^{th} - 21^{st}$, I listened to some of the top researchers from around the world present their work on various aspects ranging from hormone transportation to stress responses. The Association's goal is to promote the study of plant growth substances at an international level. Meetings have been held since 1937 all over the world (IPGSA, 2013).

At the conference, it was truly overwhelming for me to be introduced to such in-depth, highquality research of the most incredibly small factors that hold an incredible working part for plant growth. In high school, I was taught of overall working systems and occasionally exposed to the minor factors at play. This was partly because of the complexity and immensity of factors, but mostly because so little as a whole is known about each and every factor that is needed for even a single pathway. Then, there I was, listening to thee researcher tell more and more about each individual factor from so many different pathways. To express this feeling as mind blowing is an understatement. The funny thing is, it became very apparent in to me how in research, one answer does truly lead to innumerable more questions. This gave me a whole new respect for all of the people working in my lab, for before this I hadn't registered how important everyone's research is at every step of every pathway of every system.

Lab Research and Analysis

Introduction

While in China, it became more evident to me than anywhere else I had been that the human population is pushing its bounds on a planet that refuses to expand and grow as we do. For years now, researchers and scientists have begun to see how limited we will be in the future as generations develop swell while resources stagnate. With this seemingly inevitable fate ahead of us, questions get thrown around. Who will survive? Who will get tossed aside? Then there is also my favorite, and probably the most important - What can we do?

It is Norman Borlaug who first helped encourage my efforts getting me where I am today, but it is also Norman Borlaug who inspired and set a precedent for thousands of researchers and scientists to come. It is said that Dr. Borlaug has saved more lives than any one man in history, which is absolutely incredible (About Dr. Norman Borlaug, 2014). If he could save so many lives by gaining acceptance of his genetically modified wheat varieties, then imagine what we can do as thousands of scientists or 7 billion people. We are the first species with the opportunity to predict and possibly change our "fate". To not take advantage of that fact and work as hard as humanly possible to change it is ludicrous.

Everyone knows that things easily said are not usually easily done, and this is no exception. To be able to feed the predicted 9 billion people of 2050 seems unimaginable knowing that so many are still starving now. The answer doesn't simply lie in the "grow more food" category. Population is a funny thing: it is one of the few things we as humans can control, yet we can't control it reasonably at all. More daunting still is the list of continued uncontrollable factors, which is headed by the weather and climate. Natural disasters, flooding, drought, and wind are all catastrophic when it comes to yield productions. How can we possibly overcome these feats? What can we control? One of the saddest things to me is how much food is produced but gets wasted. The United States wasted 31% of the total food supply in 2010 (U.S., 2014)! Other countries are just as guilty. Then, there are instances where food is spoiled during transportation, or can be adequately grown but doesn't get distributed properly for sale. How much of a difference would be made if these facts alone were alleviated?

The true madness entered the scientific world when the realization of successful transgenic crops became a reality. The amazing thing about living things is that we (almost) all have DNA, and that we all read the DNA code the same. This brings about amazing recombinant possibilities when trying work against certain environmental factors. It's easy to see that some plants grow better in certain conditions than others. This goes for drought, flooding, pest, and wind resistance. A gene that promotes better resistance can be taken from one plant and put into another, but for this to be effective, the entire plant systems have to be understood because unimaginable amounts of factors are involved in each and every step of every action a plant does. This is where the beautiful picture gets extremely foggy, as there is a scientific race to try and figure out each of the pieces as efficiently and accurately as possible. Soon every single researcher and every single project becomes just as relevant to the overall success of understanding to be grasped. I feel incredibly fortunate to be the smallest ripple in the ocean of plant pathway discovery.

One of the most prominent discussions at the IPGSA conference was that of gibberellins, defined as a group of hormones that stimulate stem elongation, germination, and flowering. A group specifically mentioned multiple times was GA (gibberellic acid). This was before I really understood what the project I was working on was about, but now I will focus on GA4, which is known to be the active GA in shoot elongation and flower initiation. This particular growth hormone is made from GGPP, a plant chemical, through a process of six enzymatic transformations. The first key enzyme in this pathway is known as CPS (*ent*-copalyl diphosphate) and is coded for by gene ga1. By understanding how CPS expression affects GA4, better growth possibilities can be achieved, which is precisely what I'm working on.

Juan Zhang, my mentor, started this project more than a year ago by isolating ga1 from cotton, maize, soybean, and tomato plants. The gene was then recombined into a P Super-1300 plasmid in a location with many enzyme loci between the Super Promoter and PAnos sites. XbaI and SpeI were the restriction enzymes used to cut in these locations. The recombined plasmid was then transformed into GV3101 agrobacteria, which was then finally incorporated into the Arabidopsis thaliana, specifically Ler strain, using agrobacterium infection.

Before testing CPS expression, three generations of the transgenic Arabidopsis need to be grown up to obtain a "pure generation". Each generation takes approximately two months to complete, so this step consumes about six months of time. This is the current level of the experiment. We are currently growing up Arabidopsis plants with 110 different strains of the CPS gene. The goal is to find 3 prime strains from each plant (a total of 12) by the third generation, A.K.A. pure generation. In the first generation (T1), the 110 different transgenic Arabidopsis plants were grown to full development with seeds and leaf samples collected. 24 tomato (SI-1 through SI-24), 18 maize (ZM-1 through ZM-18), 28 soybean (GM-1 through GM-28), and 40 cotton (GH-1 through GH-40) strains were used. During the second generation (T2), seedlings were counted in the petri dish, and only strains with a ratio of 3:1, alive: dead, or higher were selected to be transferred to soil for continuation of growth and development. For the third/pure generation (T3), only the 12 best strains of the 110 are wanted. Only the plates with 100% growth are selected for soil transfer. These are the strains that will be used for the actual CPS expressions experimentation.

Methods and Materials

Agrobacterium Infection

Agrobacterium infection is done by pipetting drops of the agrobacterium solution onto the Arabidopsis buds 20 days after soil transplant. The plants are then covered with a plastic bag for 12 to 24 hours and later tested to confirm the bacterium was taken up. The solution consists of 50 mL YEP and 100 μ L agrobacteria that has been shaken for 20 hours at 28 °C. For 50 mL YEP, combine 0.25g NaCl, 0.5g yeast, 0.5g tryptone, and 50 mL distilled water in a flask. Heat at 120 °C for 20 minutes in sterilization machine. The agrobacteria solution then needs to be tested

using a spectrophotometer to get an OD600 of 0.6 to 0.8. The spec measures the cell density in the solution, and the OD600, or concentration, needs to be between 0.6 and 0.8. Once confirmed, the solution is centrifuged for 10 minutes. The supernatant is then discarded and 20 mL of a transformation solution is added to mix with the pellet. The solution contains 2.22 g/L MS powder and 50g/L sucrose. After adding 20 mL of the solution to the pellet, 4 μ L swilet-77 is added to help the solution stick to the plant, and the solution is ready to be applied to the Arabidopsis (Clough, 1998).

Seed Sterilization

For each new generation, seeds are obtained for fully developed plants, dehydrated for 7-15 days using dehydration beads, and then sterilized. For sterilization, the seeds are separated from the beads and then further separated into 1.5 mL tubes 0.5mL full. Each tube is then filled with a 2% NaClO sterilization solution: 80 mL H₂O, 1600 μ L sodium hypochlorite, and 8 μ L Triton X-100. Shake tubes vigorously for 14 minutes. Rinse seeds by removing excess liquid with a micropipette, adding 1000 μ L H₂O, and shaking for 30 seconds. Repeat 4 to 5 times (Zhang, 2010).

Plate Seeds

Once sterile, plate the seeds. Empty the entire contents of 2 tubes onto one MS medium plate using a micropipette. Swirl the contents around the plate and remove excess liquid, avoiding the seeds. Sterilize the plate by running the lid over an open flame, put the lid on the plate, and then run the edges of the plate over the open flame. Seal plate shut with Para film. Freeze plates for 2-3 days (Zhang, 2010).

MS Solution and Growth

The MS growth medium for the plates contains 4.43 g/L MS powder, 30 g/L sucrose, and 8g/L agar. Add the MS powder and sucrose to the water; adjust the pH to 5.8 by adding a NaOH buffer. Add agar. Sterilize in high temperature sterilization machine for 20 minutes at 115 °C. When cooled but still liquid, add 600 μ L hygromycin antibiotic. Within the carrier plasmid contains a gene for hygromycin resistance, so by testing for the gene right away using the

medium, untransformed plants can be eliminated (they won't grow). Pour 20 mL into each plate. Seeds can be added when medium solidifies.

After 2-3 days, the seed plates are taken and stored in a greenhouse with constant light at 18-20°C. The seedlings grow for 10 days before they are counted as alive or dead; this is based on the root development. The ones with the best roots are then transferred into soil pots and grow for 20 more days before leaf samples are collected. Two months after the transfer, the Arabidopsis is fully grown and seeds are collected (Zhang, 2010).

DNA Extraction

The leaf samples are collected for DNA extraction. DNA extraction is done using the Edwards Method. Two leaves from each sample are put in a 1.5 mL tube and frozen in liquid nitrogen (- 80° C) to protect the DNA. The frozen leaves are then crushed into powder, mixed with 400 µL of Edwards buffer, and then smashed and stirred more. Centrifuge samples at 12,000 rpm for 15 minutes. Remove 300-350 µL of the supernatant, put in a new tube, and discard the pellet tubes. Add 300-350 µL (amount removed previously) of isopropyl alcohol and gently invert. Refrigerate for 20 minutes. Centrifuge again for 12,000 rpm for 15 minutes. Pour out supernatant to obtain pellet. Add 200 µL of 70% alcohol, finger flick to vortex, dump out alcohol, and turn tube upside down to dry out. Add 50 µL H₂O and mix gently. Confirm an adequate amount of DNA has been isolated using a Nana Drop 2000 Spectrophotometer. Place a 1.5 µL drop on the sensor. A computer reading of 25 ng/µL is okay, but a reading of 50 ng/µL or greater is best for PCR (Edwards, 1991).

Polymerase Chain Reaction

Polymerase Chain Reaction, or PCR, is a method used to make multiple copies of a wanted DNA sequence. In our case, we want millions of copies of the isolated CPS gene to run a gel. For a 20 microliter system for 2 tubes, 8 μ L H₂O, 0.4 μ L F primer, 0.4 μ L R primer, 10 μ L 2x Taq PCR StarMix, and 1 μ L DNA are mixed together. The F (forward) primer attaches to the 5' end, while the R (revert) primer attaches to the 3' end of the DNA fragment. The 2x Taq PCR StarMix contains an Mg²⁺ buffer, nucleotides, dye, and Taq DNA polymerase. PCR is done by a machine

in 3 repeated steps. Step one, called denaturing, is when the DNA strand is "unzipped" down the middle, which is really just heated to the point the hydrogen bond between the complementary nucleotides is broken. This breaks the double helix into 2 separate single strands. Step one is heated to 94°C for 30 seconds. Step 2, known as annealing, has the temperature reduced to 58°C for 30 seconds. In this step, the primers attach to their complementary sequences at the opposite ends of the strand. The primers can only attach to one spot because of the bases they contain, so specific primers are chosen to flank the desired gene and only get copies of that fragment. For instance, if I stood a ladder up and cut the rungs all the way down the middle, I would call the top left single side of the ladder 5', and that's where the F primer would attach, or complete the top few rungs. The bottom right single side would be called 3', and the R primer would attach to the bottom few rungs to complete those. Step 3, called the extension step, heats up to 72°C for 30 seconds. In this step, the polymerase binds to the primers and begins adding in complementary nucleotides down the lines of the single DNA strands. In other words, it fixes the ladder by making 2 new ones from the old 1 by filling in the rungs with new pieces. Now there are 2 ladders, or DNA strands where there was once one. These three steps complete one cycle, but this is completed for 35 cycles to get millions of copies of the CPS gene. These are needed for a gel electrophoresis confirmation test (Edwards, 1991).

Gel Electrophoresis

The purpose of the DNA extraction and PCR is to get adequate samples to test using a gel electrophoresis. A gel electrophoresis machine uses electric current to separate DNA fragments based on their length; the smaller fragments will be pulled furthest through the gel. This system works because the DNA has a negative charge from the phosphates in the outer structure, so when placed in an electric field, the DNA is repelled from the negative end and attracted to the positive end. The gel itself is made from agarose, which creates a porous field and can be controlled depending on the size of fragments being worked with. This becomes useful when knowing the amount of base pairs (bp), or the length of the DNA fragments. By running the samples along a ladder, or a set of DNA fragments with known lengths, the number of bp of the samples can be confirmed. Fragment lengths can be predicted because the DNA strands are cut with the same restriction enzymes, and the restriction enzymes can only cut at a designated spot every time – leaving consistent fragment lengths every time. Each of the four plants have

different length ga1 genes: cotton – 2466 bp, tomato – 2403 bp, maize – 2568 bp, and soy bean – 2448 bp.

For a 1% 30 mL gel, 0.3 g agarose is added to 30 ml TAE and microwaved until clear, swirling occasionally. Once clear and cooled add 1 μ L GelRed dye, pour into plate, add comb to create wells, and allow solidifying. Once solidified, remove comb and inject samples in to wells. Add 10 μ L of each sample but only 3-5 μ L of the ladder. The ladder used here is DL 2000 plus. Run the gel at 81 watts for 20-30 minutes. When completed, compare sample lengths with those of known ladder. If the samples have the correct amount of base pairs, then the plant from which the sample was taken can be kept and regenerated because it contains the desired gene. However, if the sample shows the gene is not there, the plant will be thrown out (Yang, 2005).

The Future of this Project

In approximately four months, pure generations of transgenic Arabidopsis will be completed. Juan Zhang will then choose 2-3 of each of the four plant varieties, reducing the different strains from 24 to 8-12. Comparisons will be made between these varieties and the control Arabidopsis plants, specifically comparing height and root length. CPS expression and amounts will be analyzed using a western blot test. By understanding CPS expression and amounts crossed with physical traits such as the root length and height, a better relationship can be drawn within the role of CPS, GA4, and plant growth. After analysis is done comparing the strengths of the CPS strains, the best ones will be tested in other crops. The overall goal is to find the CPS gene that best promotes plant growth to ideally more fit crops.

Independent Research

Introduction

On my fourth week, I was given the option to visit CAU'S WuQiao Experimental Station, since I hadn't been out of the city area, I decided this was a fantastic opportunity for me to not only see the other side of China, but to also learn about the farming community. Coming from a small farm in Iowa, I was excited to see how different farming life really was here with my own eyes. Before I left, a professor told me I would be soon seeing the "true China". I wasn't sure what to

expect from that, but I was happy to be going.

During my first couple days, I decided to really learn what these farmer's opinions were about the work they were doing and to understand the difference, I would have to ask. Once given the approval to survey 10 local farmers, I got to work thinking of what I really wanted to know and what would truly help me see the difference between here and home. Combined with a great talk when I arrived back to Beijing, I arrived at a better conclusion of what farming was really like here than I ever could have hoped for. The results astonished me.

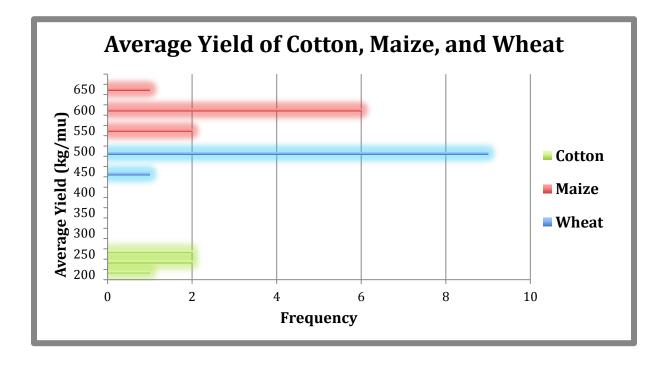
<u>W</u>	VuQiao Farmer Population Snapshot	
Age	<40	0/10
	40-49	4/10
	50-59	5/10
	>60	1/10
Gender	Male	2/10
	Female	8/10
# In Household	2	2/10
	3	2/10
	4	6/10
Education Level	Elementary	5/10
	Middle School	5/10
	High School or Higher	0/10
Other Income	Part Time in City	27.8%
(Including Spouses)	Part Time in Country	55.6%

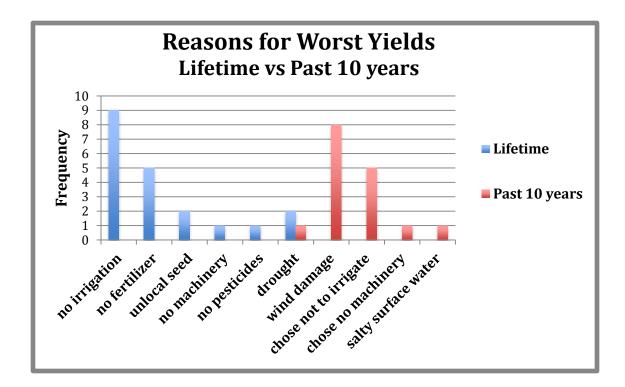
Results

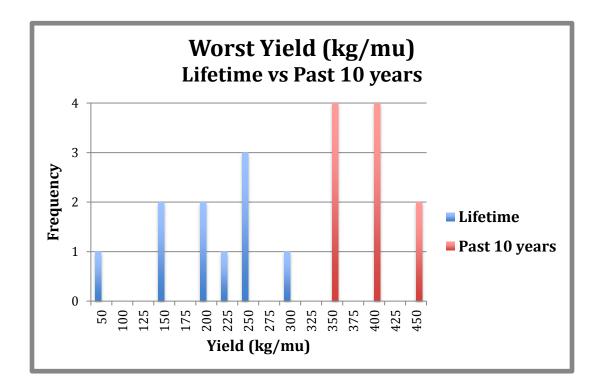
Full Time in City	16.7%
Full Time in Country	0%

Social Outlooks of Farming				
Why become a farmer?	Family farmed	10/10		
	Other	0/10		
Do you enjoy farming?	Yes	0/10		
	No	10/10		
Do yield outcomes affect	Yes	0/10		
living conditions?	No	10/10		
Do yield outcomes add	Yes	0/10		
stress you your life?	No	10/10		
How is the government	Encourages With Money	10/10		
involved in your farming?	Other	0/10		
Do you wish you had more	Yes	10/10		
help from the government?	No	0/10		
Do you feel crop research	Yes	10/10		
will affect you personally?	No	0/10		

Land and Crop Overview				
Land ownership	Own	7/10		
	Rent	0/10		
	Both	3/10		
Land Planted	<u>(mu)</u>	(Acres)		
	8	1.32		
	6.5	1.07		
	4	0.66		
	5.5	0.91		
	8	1.32		
	5	0.82		
	5	0.82		
	9	1.48		
	5.5	0.91		
	7	1.15		
Crops Planted	Wheat	10/10		
	Maize	10/10		
	Cotton	3/10		
Biggest Consistent	Wind	10/10		
Yield Decreaser	Pest	1/10		
	Rain	1/10		







Discussion

After first finishing my results, I was puzzled and unable to see the big picture of what was really happening here. I had never experienced the first-hand look at a developing country, and frankly, the Chinese farmers' methods didn't really make sense to me. It wasn't until after I returned to Beijing and was able to discuss with a professor that I understood the true situation with current Chinese agriculture.

While my sample is very small, in the snapshot it can be seen that the majority of farmers are in their 40s and 50s. In fact, this is the typical age nationally for farmers. Farmers have very little land because they lack access to modern methods that are known to boost yield. Without machinery, they do the work by hand, making it very understandable that they don't enjoy the work they have to do. While many could afford to use modern yield boosting methods such as fertilizers, tilling, pesticides, and irrigation, they choose not to because of the extra intense work

required. The extra yield means nothing to them when they have such little land and turn to other part-time or full-time jobs for primary income.

I asked a professor, and myself, "Why do these people continue to farm if they don't like it at all, don't want to be a farmer, and don't care about yields? They obviously don't need it for the money." It turns out that the Chinese land laws make it extremely difficult for people to get rid of farmland. They can't simply sell it to whomever they want; they have to sell it to someone in their small area. Most people, however, don't want to acquire these extra pieces of land because of all the work and how small and distant each individual piece of land is. The only reason they continue to farm it as minimally as they do is because it is what they know and what they have always known to have done. It is what their family did before them. Perhaps another reason could be that some remember, or heard from their parents about, the Great Famine and can't bare the thought of the land just sitting there untouched.

Whatever the reason, Chinese agriculture is in trouble, and the government is able to see that. Younger generations are no longer wanting to take up the family farm, and kids are going to school through at least high school, as opposed to only elementary and middle school like their elders. These small pieces of land also aren't being used to their full yield potential, which is devastating when considering the number of mouths to feed alone in China. The government is trying to step in by offering incentives to families to keep farming, but mainly they're trying to encourage larger family farms that may take full advantage of modern methods, irrigation and pesticides included. As turnovers of land size and ownership take place, there is going to be a great and powerful movement into the future of Chinese agriculture. A huge revolution is in the mist, and just in time as we try to best prepare for the dramatic coming of year 2050. The talk of population rise and fear of starvation for these growing numbers will only increase as the time gets closer, but we have great investments in the education of scientists globally. It will certainly take impressive amounts of global collaboration to offset the near crisis, but it is not impossible. Developing countries everywhere are accepting biotech crops more and more every year and reaping the benefits. The work, the researchers, and the education are proving effective. The amazing part is we don't even understand the half of the true possibilities that lay ahead yet. In

my lifetime, I know I will see some incredible advancements that will change the face of agriculture forever.

Personal Experience

As a small town Iowa girl from a class of less than 50 people, being given the opportunity to travel to China the summer before my senior year was a dream come true. Even trying to imagine that it was actually happening was difficult. People would always come up to me asking, "Are you ready for China?" I always answered honestly, "No not really," because I don't know how one can "get ready" for something like that. I went in with few expectations, little preparation, but an extreme excitement to try anything. Once arriving, I confirmed my assumption that there truly is no way to prepare for an experience like that.

Getting off the plane, I was nervous about how to get out of the airport, but I was more nervous about who would be meeting me on the other side. Would they like me? Would we be able to talk easily to one another? Would these people become my friends for the summer? My fears were relieved pretty quickly when I saw these huge smiles of the two girls holding signs with my name. They greeted me happily and quickly took my bags to help lead me out. They didn't say much because all of us were nervous, yet they were just as anxious as I was to get me settled and get the summer started. We exchanged a few words in English before I was rushed into the reality of crazy Chinese traffic.

The speed, the weaving in and out, the honking – all were a huge wake up to me in a way that I could no longer ignore the actuality of where I was. While I hugged my seat belt a little tighter, I couldn't help but smile at how it all just seemed to work out with no rhyme or reason. Not once did I see a traffic accident the entire two months I was there, but believe me, I expected one every time.

I had a hard time admitting to them that I was a vegetarian when they took me out for supper. Salina (Ling Hao) and Lily (Juan Zhang) ordered us soup, fish, salad, snow peas, and another dish. At home, I loved eating "Chinese food", but that first meal I learned how much we had truly Americanized it. My lack of chopstick knowledge made eating a treat to begin with, but I certainly wasn't ready for the fish. The whole fish – it looked at me. The girls just started eating away at it while I picked at the salad for a while before finally mustering up the courage to try the fish, where I could still see the scales. While the fish was really tasty, the entire time I was in China I couldn't get past all the bones in the seafood, so I pretty much avoided it completely.

The work in my lab was a bittersweet episode. I really liked the people and loved how they all got along and were so tight-knit, but I soon realized that I didn't enjoy lab research like I thought I would. In fact, I knew I couldn't enjoy it at all for the rest of my life. It didn't take me long to realize that most of the other girls in my lab didn't really enjoy the work either. I gained a huge respect for all of the girls because of their diligent work that they not once complained about. They worked hard every day because they had to. It was really sweet to see how everyone helped each other out on their projects and referred to each other as their "lab brother" or "lab sister". Not all of the labs on campus were as special, and I felt extremely fortunate to be put in mine.

My mentor, Lily (Juan Zhang), was amazing. She was an absolute sweetheart and tried to take care of me more than I could have ever expected. I know that a lot of pressure was put on her to look out for my safety, but she also went to lengths to do a lot for me that wasn't asked of her, and that's why I can call her a true friend. She always tried to make sure I was happy and healthy, even when she wasn't herself. Lily was not my only good friend, however. I have to give credit to Dahai (my "Chinese bestie"), Liu Nian (Memory), Madeline, Miranda, Glen, and Cynthia, for helping me make all of my China memories. I couldn't have asked for better friends while I was there. The only thing I would have changed was meeting them sooner.

At first when I arrived at CAU and talked with the other China interns, I was a little jealous of their locations. I envied that Madeline had other Americans, that Glen had his own project, and that Cynthia had people who liked to take her out just for fun stuff. As I got closer to leaving, I was truly happy I wasn't placed anywhere else. I feel as if I learned so much more about Chinese culture and farming from where I was. I was able to travel to different cities for long periods of time, conduct my own social survey, gain Chinese friends who genuinely cared about me, and help my mentor work hard everyday on her project.

I will never say my lab wasn't full of spontaneity! During the end of my first week, I was told we were headed off to Shanghai in a few days for a week. Then, the Sunday of my third week I was asked to go to WuQiao the next day for a few days with someone I hadn't even met! I had no clue what to think about the whole thing, but I really wanted to see what was considered to be the "true China" outside the city, so I couldn't pass up the opportunity. Dahai, the graduate student who took me there, I knew was interesting the first little while we were traveling. He stuck out specifically because his English was fantastic, and I was so happy to be able to have a normal conversation! The girls in my lab knew some English, but we struggled to talk a lot. He, however, didn't have much trouble at all, and after our trip we became great friends.

I find it really interesting looking back because the trip was definitely filled with a roller coaster of emotions. Sometimes I was really frustrated because people would talk in Chinese for hours, and I couldn't understand a word. Other times I would be annoyed because they did things so differently. When I went to visit Madeline, though, we had a great time because we were able to share our frustrations. It's not that we were ungrateful, it was just the extreme change brings out a lot in a person – and we felt it! The part that is interesting to me is that while a lot of my feelings would be negative and prominent towards the stuff I didn't like while I was there, now that I'm home my memoires drift towards all the positives and I find myself missing it a lot. I miss my friends there more than anything.

I was incredibly fortunate that Dr. Li, my lab professor, really wanted me to get out and see the city. He highly supported my traveling, and I'm so appreciative for that support. While I didn't see him much because he was extremely busy, whenever I did he would always ask how I was and check to see if I was happy. What more could I ask for? Because of his support, I was able to visit the Great Wall, the Summer Palace, the Forbidden City, Tiananmen Square, and the Temple of Heaven in Beijing. I also was able to site see in Shanghai a bit, go hiking up a small mountain near campus, and go to an acrobatic performance in WuQiao. While there is so much more I would have liked to have seen, I know how lucky I am to have been given the experience to see so much on top of the work we did in such a short time.

I will never forget the people I met or the experiences I had. While I feel terrible when I realized I didn't like research, I now know that there is more to eliminating food scarcity than the research itself. I'm determined to find another career to contribute that also makes me happy as well. I have my experience in China to thank for so much. My life is definitely headed in a slightly more defined direction now than it was before, and sometimes, that's the hardest thing to ask for.

Acknowledgements

I would like to send my deepest gratitude to the **World Food Prize Foundation** for the incredible experiences that have driven my life for the past year, and will continue to for the rest of my years. Specifically, I would like to thank **Dr. Norman Borlaug**, **Mr. John Ruan**, **Ambassador Kenneth Quinn**, **Mrs. Lisa Fleming**, and **Ms. Catherine Swoboda** for all your hard work, amazing influences, and for giving me this chance that will do nothing less than change the rest of my life for the better. I couldn't have asked for a better opportunity, and I'm so fortunate and thankful you were able to see the potential in me. Thank you so much.

I would also like to thank **Dr. Li** for accepting me into his lab with all his amazing graduate students. I'm so thankful you wanted me to experience what Beijing had to offer. It really made my experience memorable.

To all my friends from China:

Juan Zhang (Lily) – Thank you so much for being patient with me and teaching me your project. You greeted me every morning with a smile, and I will always hold the greatest respect for you. I'm so happy to call you a friend.

Liu Nian (Memory) – I don't know how to even express the words to properly thank you. You became an amazing friend to me with no obligations what so ever. I always looked forward to our late night walks/jogs and simply talking with you in general. Thank you.

Dahai Guan – Trouble, trouble, trouble. Thank you for showing me how easy friendship can be with anyone from anywhere once the language barrier is gone. You kept me laughing and

reminded me to enjoy life all the time. I will always admire your positive outlook, and I wish you the happiest future.

Madeline Geitz – Thank you for being the absolute best and coolest and most awesome person in the universe. (Did I get that right?) My goodness! Without you, I don't know what I would have done. We had so much fun, and with your help I really started to enjoy myself. I hope we stay amazing friends and visit each other often.

Miranda Thompson – Thank you for the most amazing adventure of my life. It will never be forgotten!

I couldn't go on without thanking the most influential teachers I've had.

Mr. Millis, thank you for being exactly who you are. Your ambition to keep learning and experiencing more to pass to your students is inspiring. I couldn't have gotten anywhere close to where I am today without your hard work and your encouragement. I will always be grateful.

Mrs. Hauser. Miss Marsha. Thank you so much for always pushing me outside my comfort zone. You have always seen the best in me and have supported me unconditionally. Thank you so much for not backing down, even when I get cranky. I know I can always count on you, and I hope more Ed-Co students realize the dedication you have to offer.

Mrs. Gast, I want to thank you for being an amazing friend and teacher. That's a hard thing to balance, but you had enough faith in me to know I wouldn't let you down with my work. Thank you for helping me with all the little things from printing to proofing to a good laugh once in awhile. Those things always matter.

Finally, to my family and friends for always supporting me on my crazy ambitions. Specifically, I would like to thank my best friend, **Brittany Young**, for always encouraging me to follow my dreams no matter how far they take me away from her. Also, to my sisters **Lindsey Swales** and **Krista Cripe** for always keeping up with what I'm doing and staying right beside me in my

adventures. I know I can come to you guys for anything, and that means the world to me. Thank you.

Works Cited

- "About Dr. Norman Borlaug." The World Food Prize. The World Food Prize Foundation, 2014. Web. http://www.worldfoodprize.org/en/dr_norman_e_borlaug/about_norman_borlaug/s.
- Bechtold, N, J Ellis, and G Pelletier. "In planta Agrobacterium mediated gene transfer by infiltration of adult Arabidopsis thaliana plants." *Proceedings of the Academy of Sciences* 316.10 (1993): 1194-99. Print.
- China Agricultural University. China Agricultural University, 2011. Web.
- Clough, S J, and A F Bent. "Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana." *Plant Journal* 16.6 (1998): 735-43. Print.
- Desfeux, C, S J Clough, and A F Bent. "Female reproductive tissues are the primary target of Agrobacterium-mediated transformation by the Arabidopsis floral-dip method." *Plant Physiology* 123.3 (2000): 895-904. Print.
- Edwards, K, C Johnstone, and C Thompson. "A simple and rapid method for the preparation of plant genomic DNA for PCR analysis." *Nucleic Acids Research* 19.6 (1991): 1349. Print.
- Hedden, Peter, and Andrew Phillips. "Gibberellin metabolism: new insights revealed by the genes." *Trends in Plant Science* 5.12 (2000): 523-30. Print.
- *IPGSA Conference 2013.* The 21st International Conference on Plant Growth Substances, 2013. Web. http://ipgsa2013.sippe.ac.cn/ep1-1.asp.
- Shuang, Yang, et al. "A rapid-simple method of trace DNA extraction from a single plant of Arabidopsis thaliana." *Europe PubMed Central* 36.1 (2005): 99-100. Print.
- "U.S. Lets 141 Trillion Calories Of Food Go To Waste Each Year." By Eliza Barclay. *food for thought.* National Public Radio. npr, 27 Feb. 2014. Radio.
- Zhang, DW, et al. "Effects of light on cyanide-resistant respiration and alternative oxidase function in Arabidopsis seedlings." *Plant, Cell & Environment* 32.12 (2010): 2121-31. Print.







