My Summer with Bt Cotton

And other experiences in Beijing, China

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Acknowledgments

This paper is dedicated to the memory of Dr. Norman E. Borlaug who founded the World Food Prize and the Youth Institute, which gave me this exceptional opportunity.

To Ambassador Quinn for allowing me this once in a lifetime learning experience

To Lisa Fleming who guided me through this summer and answered all of my questions

To Dr. Li to making sure I was doing okay and happy during my time there

To Wu Huiling (Lily) for giving me the opportunity to see all of Beijing and taking care of me

To Yui Yuesen (Alex) for teaching me Chinese customs and history

To Lu Yao (Tina) for helping me and guiding me through the course of my project

To Tan Guiyu (Cici) for teaching and helping me with ELISA and answering all of my questions

To Bao Ruiya (Baria) for teaching me gel electrophoresis

To Liu Zhengjie (Jack) for teaching me the process of transformations

To Li Maoying (Lee) for allowing me to use his cotton and helping me with insect bioassay

To Yang Fuqiang (Tony) for helping me grow cotton and teaching me grafting

To Mrs. Tonya Nicholson for encouraging me to follow my dreams and helping me pursue them

To my parents for supporting my decision to pursue an internship in a foreign country

To Jeffrey Schott for also supporting my decision that would take me thousands of miles away

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Background

The History of China Agricultural University

China's Agricultural University (CAU) can trace its foundation to 1905 when the College of Agriculture was founded by the Qing Dynasty. In 1949, when the People's Republic of China was formed, Beijing Agricultural University (BAU) was formed with the merging of three major agricultural colleges in Beijing. These colleges were the College of Agriculture of Peking University, the College of Agriculture of Tsinghua University, and the College of Agriculture of North China University. In 1952, the Beijing Agricultural Mechanization College (BAMC) was formed when the Ministry of Agriculture's Tractor School, BAU's Department of Agricultural Machinery, and North China Agricultural Machinery College joined together. Their name was changed in 1985 to Beijing Agricultural Engineering University (BAEU). Then, in 2005, CAU was formed when BAEU and BAU merged into one university, and it is a subsidiary to the Ministry of Agriculture. CAU has become one of the top agricultural universities and research institutes in China. It has over one hundred years of history and is considered one of the best agricultural colleges in China.

The History of Bt Cotton in China

Bt cotton was first introduced commercially in China in 1997, but it had been in existence many years beforehand. It began in 1991 when China Academy of Agricultural Science (CAAS) began a massive research project into investigating a line of transgenic cotton that would produce its own toxin. Within a

year and a half, CAAS had patented a gene from *Bacillus Thuringiensis.* By 1993, the first Bt cotton plant was germinated, and CAAS began field testing it in 1995 on experimental farms. The following year, seeds were given to farmers on a small-scale, and in 1997, four varieties were approved for commercial planting by the Chinese biosafety committee.

In 2006, Bt cotton accounted for 70% of all of the cotton grown in China. The farmers get the most benefits from it. They are spending less money on inputs and getting much larger yields because pests are not destroying their crops. Even though transgenic cotton seeds are a bit more expensive, farmers still make a profit because they do not have to use as much pesticide. They are also saving time and labor because most of the pesticides are applied by hand sprayers. Farmers now have more money to spend on food and healthcare than they did before. The farmers can also be considered healthier because they have less poisonings from the pesticides.

Bt cotton protects against pink cotton bollworm approximately 95% of the time, and against cotton bollworm 85% of the time. There is a concern, however, that resistance against the Bt toxin will form in these pests. It is not a question of "if"; it's a question of "when". Many strategies are implemented currently in the hopes of offsetting it. The first strategy is gene stacking. Scientists put two or more genes in the cotton, so that the pests have to form resistance to both instead of just one or the other. Another strategy that is implemented is planting refuge areas, which allows the pests to feed freely without needing to form a resistance for survival. So far, evidence shows resistance has not been formed.

A Little Background About the Author

I have lived in the small town of New Hartford, Iowa my whole life on the same street in the same house. Looking back at my life, I realize that I was destined for my internship in China in the summer of 2009.

I have always been a smart child. I could read and write before kindergarten and was always one step ahead of everyone in math. When it came to science, my favorite word was, "Why?" Whenever something captured my interest, I would do additional research to go more in depth and learn more about it. It was due to these reasons that I entered in the Talented and Gifted (TAG) program at my school in the fifth grade.

It was this program that would put me on course to where I am today. One year in elementary TAG, although I do not remember what year specifically, we had to create a poster promoting genetically modified food. This was the first time I had heard of GM foods, and I ended up linking them to just being bigger-than-normal vegetables and fruits. The best poster would be selected at the school, district, and state levels. The best one would be used as an ad slogan for the company sponsoring it. We had to make a poster that spoke for itself because we would not be there for the judging. I remember my slogan was, "Eat big food because it's really good." My poster ended up making it all the way to the state level.

A few years later, when I was a freshman in high school, I went to an honorary dinner for those students who had won at the district level for Know Your Constitution. It was a program that I had become

involved with while in high school TAG. At the dinner, two students would be randomly chosen to go to Washington D.C. as the "grand prize". During the dinner, someone came and spoke about her summer in a different country. I now realize that she was a Borlaug-Ruan intern. I cannot remember the specifics anymore, but I do remember thinking that a summer abroad would be a great opportunity. My teacher and I decided to find out more about it, but it slipped out of our minds due to the other time consuming programs with which I was involved.

During my junior year, a few friends and I implemented a recycling program in Dike. I had always been interested in conserving natural resources, so it was perfect for me. I learned much about it. When I announced that I was going to the international community problem solving contest, one of the residents at the retirement community I worked at thought I was a Borlaug Intern. I was confused because I had never heard of it, but I just let it pass. This was also the year for college visits and deciding my major. I chose Agricultural Engineering due to its natural resource conservation emphasis, but I was not sure if it was right for me. Near the end of the year, my TAG teacher got a packet in the mail about the Youth Institute. After reading through it, she realized that it was perfect for me. Everything I had experienced over the past few years fell into place, and it just made sense to do it. I signed up that day. That summer, New Hartford experienced massive flooding due to a number of water management issues. It was during this time that I decided I would pursue Agricultural Engineering.

I loved the Youth Institute. I went to every speech and seminar that was offered and had time for. It was extremely interesting. After talking to the past years' interns, I made the decision that I would apply. I never dreamed that I would actually get it. I only knew that if I did not apply, I would always ask myself, "What if?" Looking back I realized that my life had led up to this summer and the pursuit of agriculture as a career. It was destiny for me to come to China and work with GM cotton. Now I know that GM plants are more than just large food items!

My Project

Topic: The evaluation of Bt cotton in order to determine if it is still effective in China from all possible angles using transformations, grafting, enzyme linked immunosorbent assay (ELISA), polymerase chain reaction, gel electrophoresis, and insect bioassay.

My Evaluation of Bt Cotton

Materials and Methods

Transformations

Since I was only in China for eight short weeks, I could not perform all the steps of Transformations. Instead, I focused on one step, which was the embryo callus step, and simply learned about the others. The entire transformation process begins with cutting a cotton plant into 5-7mm pieces for explanting. It

has been found that using hypocotyls or cotyledons is ideal. The explants are cultured in an MSB medium, which consists of Murashige and Skoog medium plus B_5 vitamins for calli stimulation. The explants need to be subcultured into MSB medium that contains KNO_3 for calli creation. The explants should be subcultured approximately two to three times for optimum calli growth.

The viable calli are yellow, loose, finely grained particles. The black and white calli should not be used. The viable calli were carefully removed from the MSB medium and inoculated with *Agrobacterium* for twenty minutes with gentle agitation. The excess *Agrobacterium* was drained from the calli, and the excess liquid was removed by blotting them with filter paper. The calli were transferred to a co-culture medium, covered, and stored at 28C for 48 hours in the dark.

After 48 hours, the calli were washed three times in sterilized water, and sterilized filter paper was once again used to blot the calli dry after the third wash. They were placed in a culture dish with selective medium making sure that they were not over crowed or too far apart for optimum calli preservation. The dish was covered and incubated in the dark for two weeks at 28 C. The calli were subcultured two more times every two weeks removing the dead, black calli each time.

After six weeks of subculturing, the viable calli can be removed from the selection medium and placed in growth medium. Each callus is considered an individual occurrence and is cultured separately. Once the plants have a practical root system, they can be transferred to soil. A bag should be placed over the plant, so it can recover. After two to three days, the plant can slowly be exposed to the light. If the regenerated plant does not produce a root system, grafting can be used. The same method of light deprivation must also be used to allow the plant time to recover.

Grafting

Grafting can be used to salvage regenerated cotton plants that would otherwise die due to a lack of a viable root system. Native cotton seeds were surface sterilized with 9% aqua hydrogen dioxide for 30 minutes, washed three times in distilled water, and planted in a sandy culture at 25C. When the plant is between 10-15 days old, it can be used for grafting. The native plant is cut just above the cotyledons. The top half is disposed of and the cotyledons, stem, and roots are retained. A vertical cut is made in the top of the stem to a depth of 2 cm. The bottom of the regenerated plant is cut, so it forms a “V”. The regenerated top is then inserted into the native bottom. Grafting tape is used to secure them together. A plastic bag with holes, protecting the need for fresh air exchange, needs to be placed over the grafted plant and placed in low light. After about a week, the bag can be removed gradually to expose it to more direct light.

DNA Analysis

I first extracted the DNA from the cotton plants by using the standard procedure as follows. I ground the samples in a liquid nitrogen cooled mortar and pestle, which was then transferred to a test tube. 600 micro liters of CTAB buffer were added, and the samples were put into a 65C water bath for 20 minutes with occasional vigorous shaking. The samples were centrifuged for 10 minutes at 12000 rpm. The aqueous phase was transferred to a new test tube, and 600 micro liters of Chloroform and Isopropranol

(24:1) were added and mixed with gentle inversion. The samples were put into the centrifuge for 10 minutes at 12000 rpm. The supernatant was again transferred to a new tube, and the same amount of Chloroform and Isopropranol were added, mixed, and centrifuged. After the supernatant was again transferred to a new tube, 500 micro liters of cold Isopropranol were added and mixed with gentle inversion. It was centrifuged for 5 minutes at 12000 rpm to resolve the phases. This time, the supernatant was discarded, and the precipitate was retained. The inside of the test tube was dried with a paper towel without touching the precipitate. 1,000 micro liters of 75% ethanol were added, and the samples were centrifuged for 5 minutes at 12000 rpm. The supernatant was discarded, and 1,000 micro liters of 100% ethanol were added and centrifuged for 5 minutes at 12000 rpm. The precipitate was retained, and the inside of the test tube was again dried with a paper towel. The excess ethanol was removed with a pipette, and the samples were put in a dryer for 8 minutes. Finally, 50 micro liters of distilled water were added to the test tube.

I then prepared my samples for polymerase chain reaction (PCR), which creates duplicates of the existing DNA through a series of heating and cooling while adding and separating the DNA chains. I put one micro liter of the individual DNA samples into a microtitration plate. Then, I added to each: 2 micro liters of Buffer, 2 micro liters of DNTP, .4 micro liters of Forward Primer, .4 micro liters of Reverse Primer, .3 micro liters of Taq, and 13.9 micro liters of distilled water. I made a master mix of these ingredients because the values were so small by adding 19 micro liters of the mix to each sample. Then, I covered the samples and put them in the PCR machine. I used the following cycle sequence: 94C for 5 minutes, (96C for 30 seconds, 57C for 30 seconds, 72C for 1 minute), 72C for 10 minutes, and 4C for storage. The sequence in parentheses was repeated 30 times.

I made the gel for electrophoresis while the samples were in PCR. Gel electrophoresis allows scientists to see the DNA profile. I created a 1% Agarose gel. I mixed 100mL of TAE Buffer and 1g of Agarose in a beaker and put it in the microwave for about 2 minutes on medium high. I added 5 micro liters of red dye and poured it into a mold with preplaced combs. The gel set up in 20 minutes. I hooked the negative and positive electrical cables up to the electrophoresis machine and tank and filled the tank with TAE Buffer. I carefully removed the combs and the mold around the gel and placed the gel into the tank so the holes were on the negative side. I added 1 micro liter of loading buffer to the DNA samples and mixed it. Then, I loaded the samples into the gel with the DNA marker first and the controls last. I ran the electricity at 100 volts for 20 minutes. Since different parts of DNA run at different speeds, the Bt gene can be located with the Bt marker gene. I tested all 11 different lines, but I was only interested in the 5^{th}, 6^{th}, and 7^{th} samples because testing all the samples was not feasible. I chose Lu 35, DP99B, and Lu 28 respectively because DP99B was from Monsanto, Lu 35 was new, and Lu 28 had been on the Chinese market for awhile. The controls were lines 10 and 11.

Enzyme-linked immunosorbent assay (ELISA) Analysis

ELISA is a test that detects certain molecules that have antigen properties, such as proteins. In this case, *Bacillus Thuringenesis* is the protein. I tested three lines and a control. They were DP99B, Lu 35, and Lu 28. The control was Shi Yuan 321. Three samples from each line were used. The procedure I used was as follows: .5g of sample leaves were placed in a cooled mortar and pestle. 3mL of coating buffer and a

small amount of Silicon dioxide were added. The leaves were ground to a homogenous liquid and put in a test tube. 2mL of coating buffer was added to the mortar and pestle to clean it, and this was added to the test tube. The samples were incubated at 4C for 4 hours, and then put in the centrifuge at 4500 rpm at 4C for 20 minutes. The supernatant was transferred to a new test tube. A standard curve of known protein values was created by putting 1mL of coating buffer in the first test tube and .5 mL in the next seven. 1 micro liter of known protein concentration was added to the first test tube and mixed well creating a concentration of 100ng/mL. Then, .5 micro liters were taken from the first test tube, added to the second test tube, and mixed well, creating a concentration of half the first. The pattern was repeated for the rest of the test tubes except for the last since this concentration was zero.

100 micro liters of each known protein concentration were added to a microtitration plate with the duplicates next to each other vertically. Then, each sample was added to three wells next to each other horizontally. The microtitration plate was incubated at 37C in a covered metal box lined in wet newspaper for 3 hours. The plate was washed with washing buffer 3 times from low to high concentrations and dried. 6mL of dilution solution and 1.5 micro liters of antibody were mixed in a new container and 100 micro liters were added to each well. The plate was incubated again in the same conditions for 30 minutes and again washed with washing buffer 3 times and dried. 6mL of dilution solution and 3 micro liters of enzyme labeled antibody were mixed in a new container. 100 micro liters were added to each well, and the plate was incubated again the same as previously. The plate was once again washed 3 times with washing buffer and dried. Then, .02g of Phenylenediamine, 10 mL of substrate buffer, and 4 micro liters of hydrogen peroxide were mixed in a test tube. 100 micro liters were added to each well to induce a color change. After the yellow color appeared in all concentrations, 50 micro liters of H_2SO_4 were added to stop the color change. The microtitration was analyzed in a spectrometer at a wave length of 492, and the results were printed.

Inspection of Bt cotton plants

We obtained bollworm eggs from China Academy of Agricultural Sciences on two separate occasions. Both times, we hatched the eggs. Our first attempt at insect bioassay failed because we did several things wrong. We started the bioassay too early; they were only two days old. We also failed to feed them first, so they were already starving to death when we began the experiment. The last mistake that we found was we did not add water to the dish, so the leaves dried out leaving the larvae without water. The second time we hatched the eggs, and they survived for 6 days. I believe they died this time because we did not feed them until the fifth day, and we fed them an artificial medium obtained from CAAS.

It was too late after the second failure to start over again, so I went to the experimental farm to inspect the Bt cotton lines for bollworms to see if any resistance to the Bt gene was developing among the pests instead. I systematically checked every plant in two student's plots for bollworms, larvae, or eggs.

Results

Transformations

When I began the embryogenic calli step, there were ten flasks of calli. The embryogenic calli had to be separated from the other non-viable calli. After we separated them, we only had two culture dishes of embryogenic calli. There were obviously more embryogenic calli in the flasks, but it would be too time consuming to try to separate them out since they were mixed in with the non-viable calli. The calli had to be subcultured every two weeks and checked frequently for overgrowth of the *Agrobacterium.* Each time the selections had to be subcultured, there was just as much, if not more, waste of potentially viable embryogenic calli. However, it would once again be too time consuming to separate every piece from the dead, unusable calli. (Appendix A)

Cotton Grafting

The cotton plants I grafted were a success. However, before I could get pictures, the plants had to be thrown out because they were infested with pests. The graduate student who was sharing his greenhouse with me had to protect his own (much more important) plants from being infested. Grafting is both simple and generally successful. It is easy for scientists to save their successfully transformed plants from an inevitable death caused by a poor root system. (Appendix B)

DNA Analysis

DNA analysis showed that Shi Yuan 321 and Lu 35 did not contain Bt, but DP99B and Lu 28 did. It is to be expected that Shi Yuan 321 would not contain any Bt because it was the control line. DP99B was purchased from the American seed company Monsato. Lu 35 and Lu 28 were purchased from China Academy of Agricultural Sciences (CAAS) in the Shandong province. Lu 35 is a fairly new line, which may explain why the seeds did not contain any Bt. (Appendix C)

Enzyme-linked immunosorbent assay (ELISA) Analysis

The results of ELISA showed that DP99B had the highest concentration of the Bt protein, then Lu 28, Shi Yuan 321, and Lu 35. The results were 9.790, 9.737, 9.595, and 9.533 respectively. Shi Yuan should have had the lowest value because it was the control line and should not have contained any Bt. However, with the results of the DNA, these ELISA results did not make sense. The control line and Lu 35 do not contain Bt, yet ELISA said they did. After discussing it with a graduate student, we came to the conclusion that Lu 35 and Shi Yuan 321 contained a protein with a structure similar to Bt. This decision was reached because the levels in Shi Yuan and Lu 35 were too high for it to just be caused by some of the Bt gene flowing from the samples that contained it to those samples that did not. A similar protein would allow the antigens to bind to them as if they were Bt but are, in fact, not Bt. This occurrence is called cross action and occurs quite often in the laboratory setting. This is why DNA analysis and ELISA both need to be done. DNA analysis detects the gene, and ELISA detects the amount.

The place of seed procurement seems to play an important role in Bt expression in cotton. The overall feeling is that Monsanto is the most reliable source of Bt cotton seeds. The Chinese seed companies are trusted less because their standards are not quite as high. This is supported by the fact that DP99B, obtained from Monsanto, has the highest Bt protein expression. Lu 28 is next, and Lu 35 does not contain Bt at all. (Appendix D)

Inspection of Bt cotton Plants

When I first looked at the cotton plots, I felt my chances of finding bollworms were quite high. Many of the plants were obviously damaged and had large holes in their leaves. In some plants, only the veins remained on a couple of leaves. However, after two hours of searching, I still had not found a single bollworm, larvae, or egg. I even called over the students who were with me to help me look, but they could not find any either. Then, one of the students informed me that the plants had been treated with pesticides the day before because they were infested with pests.

The other student's plot was waiting to be sprayed for the same problems, so I began looking there. Although I still could not find any of the targeted pests, I did find a wide array of other types of insects including ants, which seemed to be attacking the leaves. The Bt protein only targets certain pests not all of them, so even though the plants were damaged, it was not necessarily bollworms that were doing it. (Appendix E)

Discussion and Impact

I have found that Bt cotton is still effective in China. There have been no reports of widespread resistance among target pests, but resistance is to be expected in the future. It is not a matter of if, but of when, so scientists need to continue to make advances in order to continue to effectively stave off these potentially disastrous pests. Improvements still need to be made in the transformation process. It is very time consuming and has a very low success rate. It needs to be improved in order to continue to be a realistic way of creating Bt plants. Grafting is a very simple, but effective way to salvage the transformed plants that failed to grow a viable root system.

I found that both ELISA and DNA analyses are needed in order to evaluate Bt in cotton plants. Neither alone can be as reliable as the two put together. DNA analysis can be used to detect the Bt gene. It cannot, however, be used to determine the amount of Bt protein being expressed by the gene. It is possible that the plant can express very low, ineffective levels of Bt. ELISA can be used to determine the amount of protein that is expressed. However, as I found, it is possible to have cross action from another similar protein giving a false positive. An insect bioassay is an effective way to determine if the amount of protein expressed by the gene is protecting against pests. I did not have a chance to do this experiment, though, because the bollworm larvae died before we could start the bioassay on two separate occasions.

Even though Bt cotton plants produce their own pesticides against certain pests, this does not mean that pesticides can stop being applied altogether. Other pests, such as the ants I saw, are unaffected by Bt but can still be damaging to the cotton plants. Bt cotton can reduce the number of pesticide sprays, but it cannot eliminate them. The reduced sprays will still save farmers money and, more importantly, man power and time since pesticides are mainly applied by hand in China. This in turn can lead to better health and more income. The extra income can come from either an increase in yields due to fewer losses or from having to invest in fewer inputs like pesticides. Either way, it can increase food security.

My Cultural Experiences and Reflections

My summer experiences in Beijing are something I will never forget. It amazes me that this country has been home to mankind for over 5,000 years. There is extensive cultural history from many different dynasties and time periods that is all meshed together. There has also been an obvious Western influence, but it blends together in a way unique only to China. I believe that my cultural experiences can be related to my scientific experiences. Everything I have learned and seen in Beijing can be linked together into one long chain.

Soon after my plane touched down at the Beijing International airport after the thirteen hour flight, I was faced with my first incident. Health department workers boarded our plane before anyone was allowed off. They checked everyone's temperature with a laser thermometer. The government did not want a swine flu outbreak like the United States and Mexico had. It was similar to when we had to protect our embryonic calli from other bacteria and viruses other than *Agrobacterium* by washing our hands in alcohol. The calli were very susceptible to disease. The health workers were just protecting the Chinese citizens who would not be able to protect themselves from the unseen danger. At the time, however, I simply saw the health check as stressful and a waste of time.

As Colin, Meera, and I walked though the airport and collected our bags, I began to get nervous. Colin and Meera had both found their drivers because they were holding up signs. I had almost walked to the end of the line of people and had not seen my name. That was when Professor Li came up and asked if I was Linda. I was so relieved. He had simply recognized me and did not need to use a sign. Meera and Dr. Kong joined us soon after. It turned out that Meera and I were going to stay in a hotel for a few days before we went to our universities. Two graduate students, Bing and Yuan, were going to be staying with us. As we walked out, the two girls fought over whom was going to take our bags, as Dr. Kong and Professor Li left with our larger bags for the hotel.

As we stepped outside, I immediately noticed the chaos as drivers weaved in and out of traffic. It looked like a mess to me. I just stood there waiting for an accident to happen. Cars were cutting other cars off, and horns were an unending part of the background. It was comparable some days at the lab. Everyone was always willing to help one another in any way possible. It was like when Bing and Yuan fought over who was going to take our bags. When we would do big projects, everyone had their own job (or several), but it always seemed to me like a big unorganized mess with everyone doing many things at once. It always turned out alright, just as I never saw or was involved with a traffic accident.

As we drove through the city, I noticed that the evidence of last summer's Olympic Games was still apparent. The country was obviously very proud as it boasted "Summer 2008" and the five Olympic rings everywhere. It was written on the roads, on signs, and even still on advertisements. CAU was

proud as well. The university had built the wrestling gym on campus to host the games. All of the tables in the cafeteria advertised the games. Just as America displays its flag, Beijing was displaying the Olympics. Upon arriving at our hotel, I collapsed in bed and fell asleep.

On my first full day there, Bing took Meera and I to the Summer Palace in the afternoon. We had relaxed in our room that morning trying to adjust to the new time zone. The Summer Palace was beautiful. It was where the Emperors and Empresses of the Ming and Qing Dynasties spent their summers. The buildings were so colorful. There was a gorgeous lake bordering it. The palace also boasts the longest corridor in China. I could not think of one place in America that could match its beauty. There were statues and buildings devoted to and promoting longevity of both the individual emperors and their dynasties in general. It can be compared to transgenic cotton in that it promotes longevity in the farmers by decreasing pesticide poisonings and increasing profits which in turn promotes better health.

The next day, we went to Tiananmen Square and the Heaven Temple. Tiananmen Square was huge. In fact, it is the largest square in China. It was very busy, and there were soldiers posted at various intervals along it. It was a bit intimidating, coming from rural Iowa, because I could not help but wonder why there was so many of them. Have there been a lot of problems and crime lately? Later, I realized that there were soldiers posted almost everywhere in Beijing, including CAU. They seem to parallel our national guard and police officers. Their job is to protect the citizens and various monuments in Beijing. The fear I felt of these guards posted everywhere at first was exactly like the fear I had about failing my experiment. All of my first tries at PCR, ELISA, and insect bioassay were failures. I was very afraid of getting nothing accomplished during my eight weeks in China. It was very frustrating, but after talking with the graduate students in my lab, I realized that failure is expected in experiments. The more times you fail, the easier it becomes to succeed because you have the knowledge of what does not work. It narrows down what will work. They have all failed in their experiments, so it made me feel better. I was still learning even if I was just learning what not to do.

On the third day, Bing took us to our universities. She dropped me off first at CAU because it was the farthest away from the hotel and Peking University. A graduate student, who's English name was Alex, met me. He took my bags and checked me into my room. The building I was in was for international students; it was almost like a hotel. I was on the fourth floor and had a balcony room. I had my own bathroom with a Western toilet and shower. My room was also air conditioned and came with a TV. It was about the same size as the typical American dorm room. It had two beds, two wardrobes, and one long desk all to myself.

I went to the lab for the first time the next day, and from then on, I had a daily routine to follow. I went to the lab every day around 8:30. We would either research in the computer lab or begin an experiment. We worked until around 12:00 and went to lunch. After lunch, we would go back to our rooms for a rest. Everyone would come back to the lab around 2:00, and we would finish an experiment, start a new one, or work in the computer lab. We usually went to supper together around 6:00. Some of the graduate students would go back to the lab to work more, but I generally went back to my room when I did not have much to do. On the weekends, we did an assortment of activities. I usually went with Alex to the experimental farm to look at his cotton and some of the other crops at least one morning. If we did go

to the lab, we tended to go later in the morning and take a longer break in the afternoon. This was also when Lily and Alex would take me out to tour the city, which was usually on Sundays.

The first place Alex and Lily took me was the Forbidden City since I did not get to see it when I was at Tiananmen Square. All of the buildings were brightly painted like the Summer Palace and Heaven Temple. There were workers there renovating the paint and fixtures. The detail in the painting and decorations is exquisite, especially since it is present on such an expansive site. It is hard to imagine the sheer amount of manpower, money, and talent that it took to create such a place, especially since it was built before modern technological advances were made.

The following weekend, we got up early and went to the Great Wall at Badaling. The climb was very difficult because it is built into the mountains making the wall very steep. It was just as difficult going downhill as it was up because I had to control my speed, so I did not fall on my face. The view was well worth it though. We went to the highest point on the wall, and I could see for miles around. It was very striking. It took a lot of determination to finally make it to the top and climb the whole length of the wall just as it took determination to not get discouraged as all my experiments failed. There were points when I was climbing that I wanted to call it quits because I was so tired and sore. I did not think I could do it, but I just kept thinking that if I did stop, I would regret it because this may be my only chance in life to climb one of the new Seven Wonders of the World. As my experiments failed one by one, I doubted myself just as I did on the wall, but I kept going until I finally succeeded. I knew that if I gave up, I would regret it for the rest of my life. It was well worth the pain and frustration for both situations. It all worked out in the end.

On our way back from the Great Wall, we stopped at the Ming Tombs. I had just read a book about the Ming Dynasty and the descriptions of the great tombs each emperor built for himself, his empress, and concubines. It was great to see them and make the connection from book description to real life. Everything I had read about suddenly fell into place and made sense. We walked through the big stone archway and along the Spirit Way. There were huge stone statues of animals and warriors on either side of us. It was believed that this was the way the spirits would take to get to their final resting place. It was also the path that noblemen took to see the emperor and pay their respects. It was humbling to think that royalty had walked along this path seven centuries before me.

Just as seeing the Ming Tombs in person helped me understand what I was reading, performing the experiments I had read about had the same effect. It was very difficult for me to understand the scholarly papers I was reading for background on my project. There were so many new terms that I had to look up in either the dictionary or within another article to understand what I was reading. It was very hard for me to put it all together, so I had to reread every article several times. I read many papers on the methods for PCR, DNA extraction, transformations, insect bioassay, and ELISA, but I did not fully understand them until I actually did the experiment and wrote the steps down myself in my lab book as we did them. Then, when I went back to look at the papers I had read previously, it all made sense. As time went on, I could read and understand what I was reading much more efficiently.

Lily and Alex also took me to Olympic Park one night when we were free during the week. It was great to be able to see it at night because the Bird's Nest, broadcast tower, and Water Cube were all lit up with colored lights. The lake had lighted fountains in it that danced to music being played through a sound system. The only downside was that it was late enough that we could not tour inside the buildings, and it also rained quite heavily for most of the time we were there. It was still a great experience though. I ended up going back to see it with Colin and Meera one weekend when we got together because they had not seen it yet. It was nice to see it during the day, but we still did not have the chance to tour it because it was a little too late. We were also there when the lights were turned on. The Bird's Nest really impressed me because it is both simple and complex. All the facilities built for the 2008 Olympics were built with the intention of being able to be reused. Lily took me back once more the morning before I left, so I could tour inside the complexes.

China wanted to be able to reuse the equipment used for the Summer Olympics, so money was not wasted on them. They were also built with the intention of protecting the environment in the modern green world. Similarly, this is what Bt cotton helps to enable. Since farmers do not have to spray as many pesticides on their crops, the water supply is better protected. It also has minimal effect on non target pests that benefit cotton crops, such as the bees and butterflies that pollinate it.

On my second to last weekend there, one of the graduate students, Shen, took me to see the aquarium and military museum in Beijing. It was the first time that it actually hit me how big the city really is. I realized that almost every time I went out somewhere, I rarely saw the same neighborhood twice. It hit me because we accidentally took the wrong subway, and it took almost two hours to navigate ourselves to the aquarium in the new surroundings. The aquarium was not the same one that is located next to the Beijing Zoo. It is in the opposite part of town and is named the Blue Zoo. It was quite small. The actual aquarium was built under a lake. The salt water tanks are hidden below murky water, so they cannot be seen from outside. It had a wide variety of sea creatures and even a large underwater tunnel.

We went to the military museum located near Tiananmen Square the same day. I love history, so I was excited to go. However, my hopes sunk some as we were walking through the first exhibit hall and everything was written in Chinese. They rose significantly, though, when we walked up to the fourth floor to begin the real tour. Nearly everything had an English translation on it. The first hall was devoted to the dynasty period in China. I walked through the history of China reading the historical plaques about each significant event. We went through the museum in historical order: the feuding dynasty period, the demise of the Qing Dynasty, the Agrarian revolt, the Japanese aggression, and the Chinese civil war. We also went through the hall of weapons where there were real tanks, howitzers, airplanes, and naval ships.

The museum was very modern. It was, honestly, the best museum I have ever been to. It had huge colorful, realistic murals that showed historical scenes and meticulous models of important events. It really put certain events into perspective and gave me insight. When we were walking through one of the halls, I read that the United States was one of eight countries to loot China in the early 1900's. Shen was reading the list of countries out loud and instead of saying "the United States" or "America", he said

"your country". I felt almost embarrassed to be associated with the United States after it bullied China with the help of other countries to make a profit because China, at the time, was weaker.

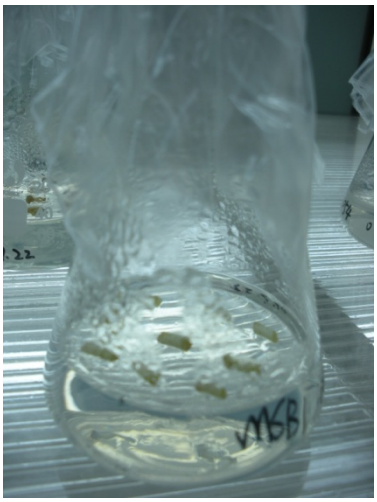
China has so much history and culture. It has obviously been influenced from the western world, but it has also managed to maintain its own customs. The two have blended well and made China much more unique. The clothing is a mixture of new and old. The women still wear dresses for the most part, but t-shirts and jeans have made their way into their closets. Silk shirts and dresses are also worn with new jeans or high heels. The men still practice chivalry by carrying items, opening doors for women, and giving up their seats on public transportation for them. Also integrated within this is the younger generation give up their seats, regardless of gender, to the elderly, disabled, and children. Respect for one's elders is still vehemently practiced here. It is a long way from the actions of the younger generation in America.

The public transportation is very efficient. Nearly everywhere in Beijing is accessible by bus or subway. It is also quite cheap, costing only 2 yuans for a single journey on the subway and 1 yuan for the bus. This equals approximately $.30 and $.15 US dollars, respectively. The taxis are also very clean and widely available. The taxis are able to navigate traffic quite well. Many people in Beijing do not own a vehicle and use bicycles instead. There are special lanes on the streets meant specifically for the bikers. They are easy to navigate in traffic because they can fit in between cars. Especially popular here are motorized bikes that require very little work and can cruise along at a relatively fast speed. Drivers constantly honk their horns, but it does not necessarily mean yelling at pedestrians as it does in the United States. I realized that drivers honk simply to announce their presence and intention to drive around the pedestrians.

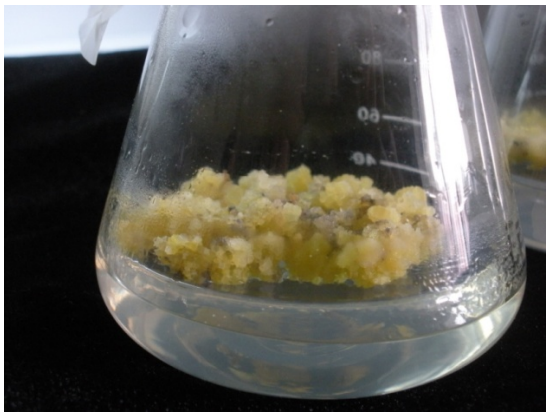
Watching the news in a different country helped me gain a new perspective. The United States is slowly losing power, and its economy will soon be surpassed by Asian countries, especially China and India. The world is realizing just how much it depends upon the US dollar and what that mistake is now costing them as America's economy continues to slip. The United States' approval is increasing worldwide with the recent election of Barack Obama and his efforts to raise the country's image in the world. International relations are imperative in a world that is becoming flatter. As an interesting side note, many of the people I talked to in China believed that the US has more people because the US has more power. They had associated power with population. In a few short years, however, this thought will correct itself.

I learned many things during my visit to China this summer. I learned much about the scientific process and Bt cotton, as well as transgenic plants in general. It was an honor to be chosen for this prestigious internship this summer. However, I feel I was very spoiled. I always had a full belly and a warm, comfortable place to sleep. I did not experience the woes of food insecurity. One of my Chinese acquaintances explained that rice is used as a "filler" in China. It is eaten after the meat and vegetables are gone to fill the void in one's stomach. From that moment on, I never looked at the food I was eating the same. I was very mindful of not being wasteful like many Americans are. I would not change anything about it because overcoming the challenges made the experience much more valuable to me. This internship helped me grasp how the United States and, more importantly, I fit into the world.

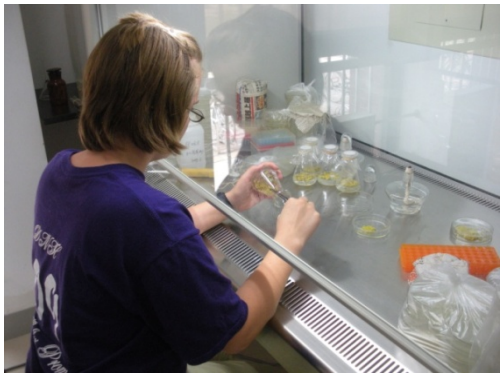
Appendix A



1. Explants

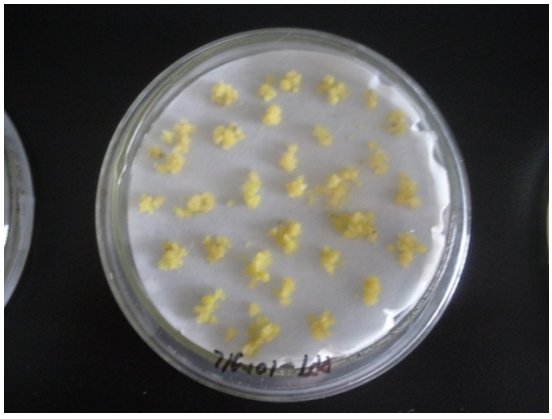


2. Embryogenic Calli

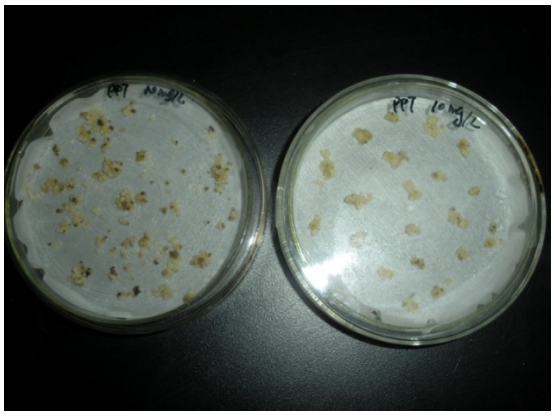


3. Separating embryogenic calli from dead calli

4. Embryogenic calli after *Agrobacterium*



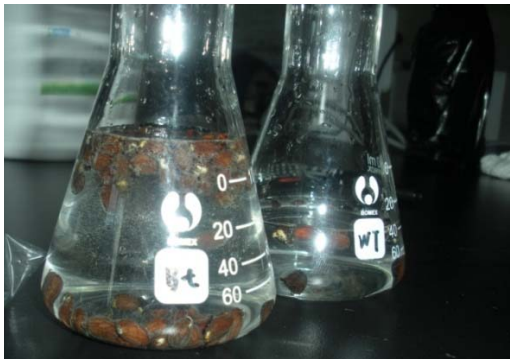
5. Calli in selection medium



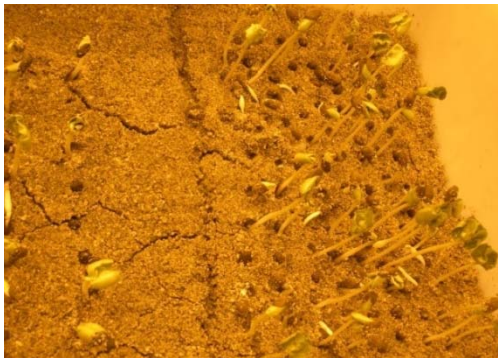
6. The left is wasted calli and the right is subcultured calli

7. Regenerated transgenic cotton plant

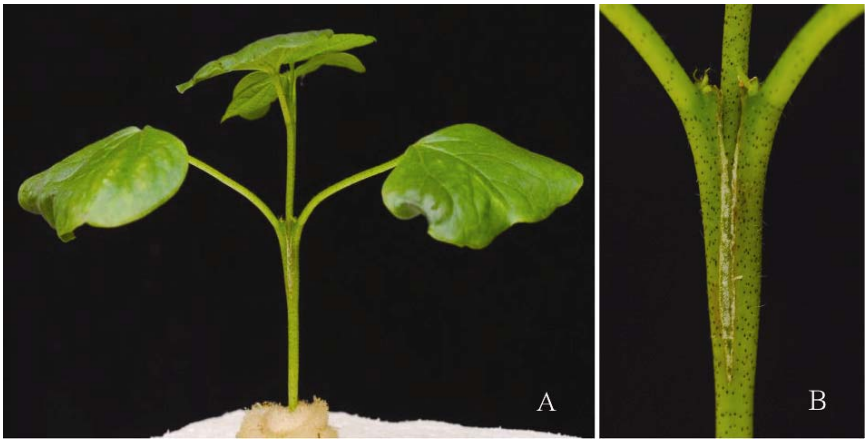
Appendix B



1. Surface sterilizing cotton seed

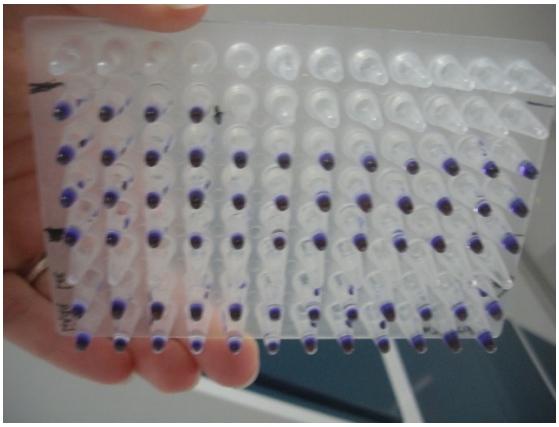


2. Cotton seedlings in sandy soil



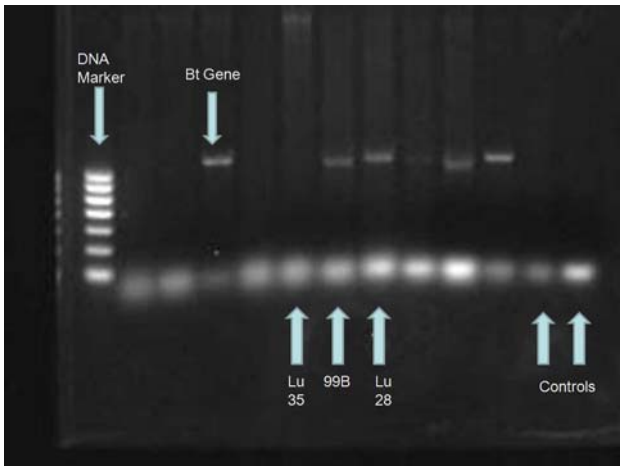
4. Cotton plant after grafting

Appendix C



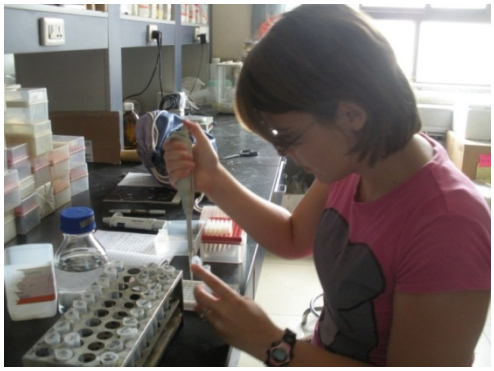
1. PCR results with loading buffer

2. Me performing gel electrophoresis

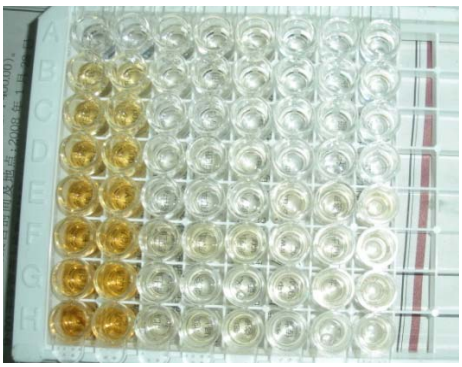


3. Gel electrophoresis results

Appendix D



1. Me performing ELISA



2. ELISA results before spectral-analysis

Line	Place of Procurement	Average Protein (ng/g)
DP99B	Monsanto	9.790
Lu 28	Shandong Province	9.737
Lu 35	Shandong Province	9.533
Shi Yuan 321	Control	9.595

3. Table of ELISA results

Appendix E

1. Examples of leaf damage from pests

2. Me inspecting the cotton plants

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