Expression of the BIN2 Gene in Maize

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The World Food Prize-2014 Borlaug Ruan International Intern at the China Agriculture University, Beijing China

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Acknowledgements

As a preface to this report, I would like to extend my sincerest appreciation for all of the individuals who helped make my journey possible.

First and foremost the World Food Prize Foundation, Norman Borlaug, John Ruan III, and Ambassador Quinn deserve the greatest praise for their funding, as well as their vision and innovation for this internship program.

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I would also like to thank my parents for fully supporting my goals and passions during my formative years, and for allowing me to live for two months across the world.

Everyone who helped me feel completely at home in China, I would like to say *xie xie* (thank you). Every single lab member went above and beyond, from taking me to the Great Wall, to Yuanmingyuan Park, and ordering meatless food for me at the dining hall three times a day. It was through their generosity that this internship experience was so rewarding. I hope one day to go back and see everyone again!

I-Personal Motivation:

My first introduction to the world of agriculture came as soon as I was taken home from the hospital. My mother came from a generation of Jewish farmers in New Jersey, and she continued that tradition when she started Rosie's Organic Farm in North Central Florida. Some of my earliest memories are of the farm, viewed from my stroller as my mother single-handedly harvested the entire ten acres of vegetables, fruits, and flowers. Growing up on a farm in Florida set me apart from most of my classmates and friends, as the biggest employer in Gainesville is first and foremost the University of Florida, and then the hospital. My contact with other individuals my age who shared a similar farming experience was practically nonexistent until I attended the Global Youth Institute in October of 2012. While healthy, fresh food was always available to my family and I, through my high school education and the World Food Prize I came to realize that not everyone in the world was as lucky. It was during those three days when I realized that there were other individuals my age who were interested in the world of agriculture, international development, and food security.

There are few opportunities for high school students to have an internship that will send them to another country to gain experience in cutting edge scientific research. While at the GYI in 2012 I was blown away by the experiences that the interns of 2012 had during their summer. The interns that I met in 2012 were so excited about their internship and I was inspired to apply. After applying that December and getting to the semi-finalist stage to not be selected, I was devastated. Yet, it did not stop me from applying again in the winter of 2013. I believe that failing to earn the internship in 2013 made me more driven and honored to receive it in 2014. While I was originally aiming to be placed in India or Turkey, I feel that China was an extremely good fit, and I immensely enjoyed the perks of living in a big city for two months.

I am currently a first-year student at the University of Florida with a double major in Biochemistry and History, and a minor in French Language. My experience in China was a lesson in both of these areas, as it is a country with one of the oldest still standing civilizations as well as a country with goals of improving food security worldwide through biotechnology. As I further my studies at the University of Florida, I will look to return to China as I find the culture and language fascinating.

My primary motivation for pursuing this internship was to gain a well-rounded cultural perspective, as well as an appreciation for the cutting edge research that is being done in the field of agriculture. I came into this internship hoping to gain more insight into the world of genetic research, and I have not been disappointed with the outcome.

II-CAU History:

The roots of China Agriculture University go far back to 1905 when the College of Agriculture was founded while China was still under Imperial Rule (China Agriculture University). In 1949, corresponding with the beginning of the People's Republic of China, Beijing Agriculture University (BAU) was founded through the merging of agriculture colleges from Peking, Tsinghua, and North China Universities (China Agriculture University). In 1995 the name of the University was changed to China Agriculture University when BAU and the Beijing College of Agriculture Mechanization were joined (China Agriculture University). Today the University has

two campuses, East and West, with the older East campus being located closer to the center of the city, and the newer West Campus located in the Haidian District. The University has degree programs for undergraduate, graduate, and doctorate students in all areas of agriculture including soil and water sciences, as well as engineering and food science. Throughout its over 100 years of existence the University has been awarded many accolades and Norman Borlaug even visited the University (China Agriculture University).

I spent my internship time in the Center of Life Sciences (CLS) building, however the lab that I was working in was actually a part of the College of Agronomy and Biotechnology (CAB), but the school allowed Dr. Li's lab to be in the CLS because of the newer facilities and better equipment. Dr. Li received his PhD from Auburn University and along with his laboratory responsibilities he is one of the Vice-Presidents of China Agriculture University. In the lab there was an assistant Professor, Professor Zhang, who was instrumental in designing my research project and coordinating excursions to nearby a nearby field station. There were also ten to twelve other graduate students working on experimental projects; the number often differed, as the summer is the more flexible time period in the school year. Although Dr. Li oversaw my stay in China, I had the most day-to-day interactions with my two roommates and lab mentor.

Chinese History:

China is home to the largest number of people on the planet, roughly 1.3 billion, or about 20% of the world's population. China is also a leading country in terms of land area, coming in behind Canada, Russia, and (depending on the method of measurement) the United States. For the past four thousand years China has retained the same cultural values and traditions making it one of the oldest continuous civilizations. While the Egyptians rose and fell, China's dynastic rule carried on into the 20th century. Today China is often looked upon as a new player on the world stage, a country that has risen through much struggle to compete with the United States as the dominant economic and political force on the world stage.

China has a deep and complex history with Western powers beginning with the first contact around the 15th century and continuing to the present day. During the time of maritime trade, China was the most coveted trading partner, along with India. The allure of spices, fine china, and silks was enough to whet the appetite of any ship captain. In the past, and even to this day, the Chinese people believe themselves to be a part of the Middle Kingdom, in a place between the Earth and the Heavens, with an emperor who is given his power through divine right and the Mandate of Heaven. China was extremely isolationist and disliked the presence of foreigners. This outlook would be extremely detrimental in the 19th century as Western powers began to advance in technology and military prowess (Schiavenza).

As the Age of Imperialism dawned, powers like Great Britain, France, Germany, and the United States sought to expand their trading empires into China. With the Open Door policy created by the United States, China was essentially exposed to the abuse of foreigners as the weak Qing Dynasty allowed other countries to exert their own laws in spheres of influence (Schiavenza). In the subsequent Opium Wars in each half of the 19th century the West humiliated China, with many cultural relics destroyed by the British and French as they looted the area of Beijing (Schiavenza).

In 1911 the yoke of the Imperial Qing Dynasty was thrown off, and China was plunged into roughly 30 years of Civil War as the KMT and the CCP wrestled for power with each other, as well as the expanding Japanese Empire. In 1949 the current government of China was established as a single party state under Mao Zedong, who is still regarded as the great leader of the country (Schiavenza). Today while the country still retains the designation of Communist state, it has become, as Mao would call it, "Communism with a Chinese face". In part this is what has helped China succeed where other communist countries have failed, as the Chinese economy today is extremely strong.
Now China looks to unite with other BRIC countries (India, Brazil and Russia) to form trade agreements. What these countries have in common is an extreme focus on agriculture. Unlike the present day United States, China has retained its small farms, and a majority of the rural population is involved in farm work. Contrary to popular belief, rice is not the only grain regularly consumed in by the people of China. In fact wheat and corn are used in cooking in the north to an equal extent as rice, because it was often difficult to grow rice in the north where the climate is not as favorable to the warm weather crop.
While China does produce an immense amount of food, it is still not enough to satisfy 1.3 billion mouths; thusly a lot of food is imported from other countries, especially meats and grains (Talbot). Food insecurity is a grave problem in the world, and one that is not aided by the rapidly growing population. In the past 20 years China has striven to not only become a strong political force but a strong scientific one as well. BGI, formerly known as the Beijing Genomics Institute, is the leading force in the field of genetics, especially genome sequencing. Surpassing the United States, this private company in China has the ability to be the new face of transgenic crops in China.
Corn, rice, cotton, soybean, and wheat are all field crops grown extensively in China (Munro). Individual farm plots are extremely small in China, compared to those in the US and Brazil, as all land is owned by the government, and the farmers can only rent it. Therefore many of the farming techniques that are popularized by the United States will not be applicable in China due to the small size of the farms, as well as the lack of mechanization on a majority of the farms. Therefore I believe that the solution to China's agricultural problems must come from within the country. From my summer in the country, I have found the Chinese people to be extremely proud of their long history, and how far they have come in the past sixty years.

III-My Project

Introduction

In 2011 China imported 2.1 billion bushels of corn, with 895 million bushels coming from the United States alone (Munro). While these numbers are extremely favorable to American agriculture interests, China desires to becomes 95% self sufficient in essential commodities (items such as corn and pork) (Munro). However with 20% of the world's population the idea of agriculture self-sufficiency is not attainable when only a seventh of the available land is used for food production (Qi). The lack of sufficient arable land to feed its extremely large population is an issue that many countries face, but none to as great of an extent as China. While one solution to alleviate this problem would be to engage better management practices, resource management, intensive cropping, and improved mechanization, the nature of agriculture in China is not the same as it is in the

United States, or Brazil. The farms in China are extremely small plots of land where the seeds are sowed by hand, and any type of machinery is rendered useless due to the size of the farmed land (Munro). Many individuals believe that transgenic, or genetically modified crops, are the way to circumvent the growing gap between the production and consumption of food in the world. In 1999 when the Chinese market was opened to U.S. agricultural products, soybean consumption drove the market and still does to this day (Munro). However all of these imports go to livestock feed, as the Chinese government still does not allow GMO products for direct consumption by humans (Qi). Earlier this year when traces of a gene from a transgenic corn species made by Syngenta were discovered in a shipment of corn from the United States, corn importation was halted, even for animal feed (Qi). This ban has resulted in a nearly three billion-dollar loss for American farmers and traders. Yet as the Chinese people and government are still wary about transgenic crops, research on the topic has continued at a rapid pace (Talbot).

Corn, or maize, was first domesticated in the area of Southern Mexico. It was staple crop of the Native Americans along with beans and squash. In 1492, with the discovery of the New World, corn was brought into Europe, and by 1575 corn could be found in areas of Western China (Bensen). By the 18th century corn was being regularly grown in China, with it then spreading to Korea and Japan. Today the United States and China lead the world in the production of corn. The type of corn that is commonly grown in both of these countries is Dent corn, or field corn, which is used for animal feed and industrial purposes (Bensen).

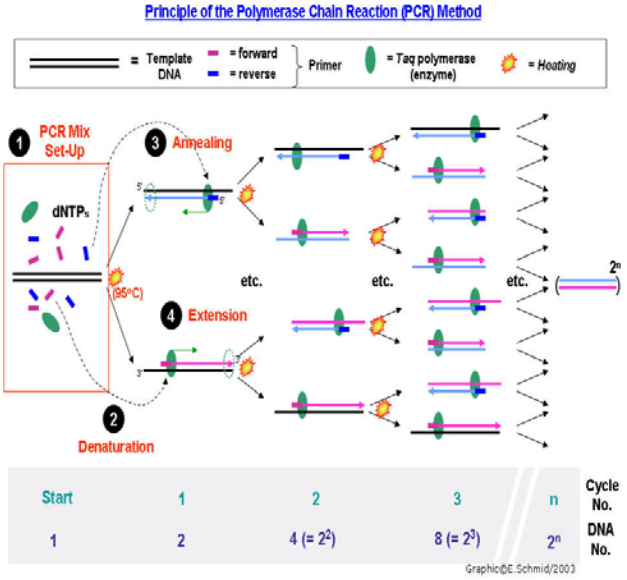
The growth of corn is impacted primarily by three factors: temperature, moisture, and solar radiation, with temperature and moisture being the most important (Wiatrek). The optimum growing temperature is between 68 and 73 degrees Farenheight, though corn plants can survive for short periods of time at extreme temperatures of 32 and 114 degrees (Wiatrek). Corn plants require ample amounts of moisture, with about 0.25 inches of water per day needed during growing periods (Wiatrek). Transgenic corn possesses traits that were not present in the original plant, but now are part of the transgenic corn's genetic material. These added traits can span from growth or yield potential to plant resistance to pests. In my research project I looked at genes that may make plants more resilient to environmental stresses.

Just as in the human body, plant hormones, or phytohormones, regulate the growth, development, and functioning of plants, by responding to signals from the plant and the environment (Baxamusa). The five types of plant hormones are auxin, cytokinin, gibberellin, abscisic acid, and ethylene (Baxamusa). However about fifteen years ago scientists confirmed that there was another hormones that was just as essential for normal plant growth as the five previously stated (Yan). Currently research on brassinosteriods is divided into three areas of analysis. In the microchemical section BR synthesis and metabolism is studied, also BR mutant plants, or BR insensitive plants are studied to ascertain information about the physiological effects of the hormone (Yan).

This photo gives a representation of what BR-insensitive plants look like. On the right is the mutant that does not possess the proteins to synthesize the hormone. Due to its smaller size, it is obvious the effects of Brassinosteriods on the growth of plants.

Throughout the research process I carried out several Polymerase Chain Reactions (PCR), this PCR procedure has become ubiquitous in the world of genetic research. It was first developed in the 1980s and resulted from the discovery that DNA polymerase could synthesize a new strand of DNA that was complementary to the a desired sample of DNA (Polymerase Chain Reaction). At the beginning of the reaction the high temperature of 94℃ separates the two strands of DNA, to then allow the DNA polymerase to begin synthesizing a new strand of DNA from the dNTPs (ATCG bases) that are placed in the PCR solution (Polymerase Chain Reaction). Each time a PCR solution is constructed it contains 10uL of total solution, divided between two primers, one forward and one reward, as well as distilled water, sample DNA, dNTPs and the PCR mixture with Taq. During the final stages of the PCR millions of copies of the same strand of DNA are made, it is through this process that the gel electrophoresis and DNA concentration can be determined.

The graphic below lends a visual understanding of the process of a PCR (http://classroom.sdmesa.edu/eschmid/Lab8-Biol210.htm).



Methodology-

DNA Extraction from Corn Leaves-

The second week that I was at the CAU Hao Ling and I planted transgenic corn seeds in the greenhouse adjacent to the lab for my project (and also for her long term experiment). From each plant of corn (there were 27 in total) I tore off a small section of a newer leaf (about 2 centimeters in length) and placed it in a 1.5mL eppendorf tube labeled corresponding to the tag on the plant. As each tube was filled I placed it in the cooler with the liquid nitrogen and closed the top quickly to avoid loss. After all of the leaves were harvested, back in the lab I used small blue sticks with a tapered end to grind the corn leaves. As opposed to the *Arbibidopsis* leaves that I had done previously the leaves of corn were significantly more fibrous and required more force and time to grind them completely into a powder. The leaf section inside of the tube needed to be completely brittle from contact with the liquid nitrogen to be ground easily.

One tube required an unused blue pestle, and after the samples were ground they were placed on ice. Before the next steps are to begin two separate water baths must be set at 37℃and 65℃for later use in the experiment. Using a 1000uL repeater pipette I added 350uL of Lysis Solution to each eppendorf tube. The tip may be reused as long as it does not touch the tube containing the plant tissue (to avoid contamination of the samples). Once each tube was closed tightly, I vortexed the liquid in short 1-second intervals to ensure that the contents of the tube were evenly mixed. After the tubes were placed in a porous flotation device I inserted them into the 65-℃ water bath for 10 minutes. After placing the samples tubes back in the hood, I used a repeater pipette place 150uL of precipitation solution into each tube, and then vortexed the tube until the solution was uniformly distributed (~1 minute). Then 500uL of chloroform (using a 1000uL repeater pipette) was added to each of the tubes, and vortexed until the liquid traveled freely in the tube.

In the large centrifuge, I centrifuged all of the tubes at 12000rpm at 4 ℃for about 15 minutes. When the tubes were removed from the centrifuge a white film separated the liquid contents of the tube. Using a 1000mL repeater pipette I removed the liquid that was above the film, being extremely careful as to not irritate the film and absorb the liquid from the bottom. I then dispensed all of the liquid into a fresh 1.5mL eppendorf tube that is labeled with the same number as the tube it was withdrawn from. Using a new tip each time, I repeated this process until each of the new tubes has the upper phase liquid in it. I then centrifuged this liquid for 2 minutes at 12,000rpm and placed the tubes on ice. The goal of this procedure is to extract the DNA, inside this liquid supernatant is the DNA, usually looking like a tiny opaque spec in the liquid. The liquid should be poured out, and the DNA dried in the tubes for 30 minutes. Then distilled water can be added to the tubes to preserve the DNA in cold conditions. As DNA is very hardy compared to RNA it can be stored in a freezer for an extended period of time.

Plasmid Purification-

The plasmids in this case will be purified from bacteria DH52. There are 4 tubes labeled 1-1, 1-2, 2-1, and 2-2. These are also the labels that go on the 1.5mL eppendorf tubes. When the contents of the vial are transferred to the tube they are then centrifuged at 12,000rpm for 1 minute. The liquid supernatant at the top is poured into the waste, and

the solid at the bottom of the tube is kept. In each of the 4 tubes I used a 1000mL repeater pipette to add 250uL of RNAse 1, and then I vortexed the tubes until the solid completely dissolved into solution. I added 250uL of Buffer P2 (from the TIANGEN MiniPlasmid Kit) and inverted each tube ten times, or until the solution was clear. Then I added 350uL of Buffer P3 and mixed each tube eight times immediately after the solution was added. The solution in each of the four tubes was then cloudy. I placed the 4 tubes in the centrifuge for 10 minutes at 12,000rpm. I ensured that the tubes were arranged symmetrically in the centrifuge so that the spinning occurred uniformly. While the tubes were spinning in the centrifuge 4 more tubes were labeled with the same numbers. These tubes were CP3 tubes, which have a detachable top and bottom; the top section has a cap and a filter that empties liquid into the bottom section. Each of the CP3 tubes were filled with 500uL of the Loading Buffer Solution and centrifuged at 12,000 rpm for 1 minute. The liquid in the bottom section was then poured out into a waste container. The four-eppendorf tubes were removed from the centrifuge after the 10 minutes, and the liquid present in them was transferred into the CP3 tubes.

Creating Primer Concentrations:

1. Once the primer mixtures were received from the company located in Beijing (BGI) there are 8 tubes present in the package, with 2 copies of each of the primers.
2. Place the tubes in the centrifuge for 1 minute at 12,000 rpm.
3. The tubes are all labeled with the names BIN2 2129F2, BIN2200F1, BIN21541R1, and BIN21457R2.
4. Inside the tube is a fine white powder in an extremely small quantity, to make the primer a liquid, a certain amount of distilled water is needed for the correct concentration.

5. After the water has been added vortex the tubes for approximately one minute each.

Longer Sequence PCR

1. In this PCR the primers used changed, while the other components remained the same. All of the solutions were on ice in a cooler until they were needed. I ensured that there is no frozen liquid in any of the tubes before I withdrew solution.
2. I labeled 8 small tubes 1-8, with the 1 and 2 tubes corresponding to the forward and reward primers (BIN2200F1, BIN21541R1) 3 and 4 (BIN2200F1 and BIN21457R2), 5 and 6 (BIN2 2129F2 and BIN21541R1), and 7 and 8 (BIN2 2129F2 and BIN21457R2).
3. In a 1.5mL eppendorf tube I prepared the mixture that will be added to each of the smaller tubes. I multiplied the base value of the PCR mixture by 10 to achieve the total volume of each of the components needed. (The multiple of ten is used instead of eight to reduce the loss in transfer and to ensure that there will be enough of the mixture for each tube).
4. I used a 100uL repeater pipette withdraw 50uL of the *Mix* solution, and deposited it in the 1.5mL tube.
5. Using the same repeater pipette I withdrew 41uL of water from the tube labeled H_2O and deposited it in the 1.5mL tubes.
6. Using the 5uL repeater pipette I withdrew 5uL of DNA, and examined the tip to ensure that the liquid was absorbed, and then holding the 1.5mL tube I deposited the DNA into the now blue solution. (The blue solution is because the Mix solution has a dye that is used so that is easier for the researcher to detect where

the PCR mixture is on a plate. The dye has no effect whatsoever on the outcome of the electrophoresis.

7. Using a 100uL repeater pipette I mixed the solution in the 1.5mL tube by setting the uL volume at 70 and pumping the solution 5-10 times until it was evenly mixed.
8. Using a 10uL repeater pipette I withdrew 9.6uL of the solution and placed it in tube 1. Dispose of the tip. I repeated this step until the remaining 7 tubes had the PCR solution in them.
9. Using the 2.5uL repeater pipette I withdrew 0.2uL of each of the primers (forward and reward) corresponding to the list of primers and tube numbers in step 2. I ensured that when the 0.2uL was withdrawn that it was visible in the pipette, and that it had been dispensed into the solution. I placed the tip in the bottom of the small tube to make sure that the primer was inserted into the liquid and not the wall of the tube.
10. I then ran a PCR with the machine by using the standard settings for a tube amount of 10uL.

Making an Agarose Gel:

1. For the above PCR a 10 well gel will need to be made. The extra well will be used for the marker.
2. On an electronic balance measure out 0.5 grams of the agarose powder on a square of wax paper.
3. Pour the powder into a 50mL Erlenmeyer Flask. Put on the plastic gloves while working at the lab station.
4. In a graduated cylinder measure out 50mL of TAE solution and pour it into the Erlenmeyer flask.
5. Swirl the contents and place it in the microwave for about 2 minutes.
6. Prepare the medium 50mL mold for the gel with the 10 tine comb.
7. Remove the flask from the microwave while wearing a heat tolerant mitt, swirl the solution and look for any residual particles, if there are still some remaining it needs to be heated longer.
8. Take 2uL of the bromophol red using a repeater pipette and insert it into the flask. Dispose of the tip immediately in the proper container.
9. Swirl the solution; it should now have a slight red tinge.
10. Carefully pour the liquid into the mold, ensure that there are no bubbles and that there is liquid in between all of the tines of the comb.
11. Let the mold sit until it is solid and ready to use.

Running the Gel Electrophoresis: After the PCR is finished running (approximately 6 hours)

12. I took the comb out of the gel, and then placed the mold and the gel into an electrophoresis tank filled with the TAE (Tris-acetate-EDTA) buffer solution that was connected to a power supply. The buffer assists in the transport of an electric current across the gel. I made sure that the TAE solution covered the entire gel and filled the wells made by the comb.

13. In the first well I placed 6uL of the marker solution using a 10uL repeater pipette. I placed the tip of the pipette inside the well, not too far below the

surface of the buffer, but still inside of the well, and dispensed all of the liquid.

14. In the second well I placed 8uL of the DNA from the tube labeled 2. At the end nine of the wells should be full with a blue solution.
15. Place the cover on top of the tank and turn the power source on, leave the current running for about 10-15 minutes.
16. After the required time has elapsed turn off the power source and remove the gel from the solution and place it inside of the UV Light Machine using the computer program GeneSnap the marker and the DNA will be highlighted on a black background. A strong white line indicates a positive signal as to if the primer has sufficiently amplified the gene.

KOD PCR

1.

Creating Cell Culture:

1. After the vector has been created, and the 2 identical test tubes inserted into the incubator at 30 degrees Celsius for 12-16 hours, remove them.
2. Using a bunsen burner, two glass rods, two amp and kan treated plates (antibiotics), I placed them under the sterilized hood with the two plastic tubes. I opened the two plates and let them air dry in the hood for 10 minutes.
3. Using alcohol in a small beaker, I sterilized each of the glass rods by alternating between immersion in the alcohol and immersion in the flame. When the bubbles ceased to rise from the rod, I then moved the rod over the flame for about 1 minute.
4. I repeated step 3 three times for each of the glass rods (the last step should end in the flame not the alcohol). I waited for the rods to cool back to room temperature.
5. Using an eppendorf exact measurement extractor I withdrew 300*u*L from the tube labeled 1. I removed all of the liquid from the tip onto a plate labeled 1, and using one of the glass rods, spread the liquid around the plate.
6. I continued to spread the liquid until the plate was dry, and then I placed the lid back on and secured it with a strip of parafilm.
7. I repeated this step with the next tube.
8. I disinfected the glass rods by repeating step 3.
9. I put the plates in the incubator with the lid facing down overnight (12-16 hours).
10. The next day I removed the plate, and there was evidence of small off white dots in the gel at the bottom of the plate.
11. Using the sterilization hood again, I labeled 24 eppendorf tubes (1.5mL) 1-24, in two sets of 12. I took a kan and amp solution from the fridge and use a eppendorf repeater pipette to measure out 1mL of the solution into each of the 24 tubes.
12. Using a 10uL repeater pipette, I affixed a tip to it and picked up one of the off-white cell cultures from the plate, and release the tip from the pipette into the tube. I repeated this process until there were 24 tubes with cells inside them, with 1-12 being from plate 1 and 13-24 being from plate 2.
13. I placed the tube inside the same incubator for approximately 4 hours.

PCR with 24 tubes

1. In the time that the tubes are being incubated, the rest of the PCR mixture can be prepared. With 24 tubes of bacteria DNA there are also two additional ones for the negative and positive indicator strip that must be also run by the PCR.

2. Preparing the PCR Mixture

In a 1.5mL eppendorf tube I prepared the mixture that will be added to each of the smaller tubes. I multiplied the base value of the PCR mixture by 30 to achieve the total volume of each of the components needed. (I used 30 instead of 26 to reduce the loss in transfer and to ensure that there would be enough of the mixture for each tube).

While tubes 1-24 are labeled accordingly, the two extra tubes were labeled P and N corresponding to the negative and positive control. The Negative tube had only the PCR mixture, with no DNA added, while the positive had cDNA as opposed to the bacteria.

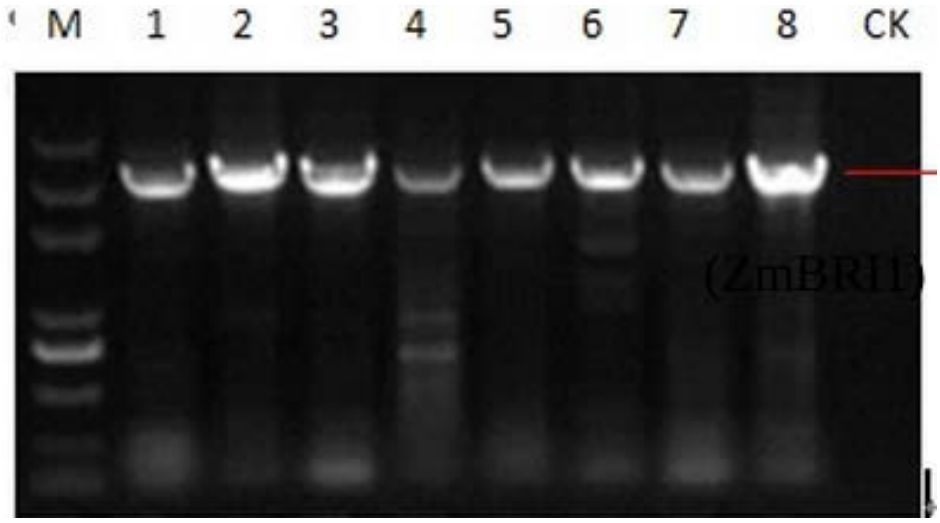
Using a 200uL repeater pipette I withdrew 150uL of the *Mix* solution, and deposited it in the 1.5mL tube. Using the same repeater pipette I withdrew 123uL of water from the tube labeled H2O and I deposited it in the 1.5mL tubes. Then using the 10uL repeater pipette I withdrew 6uL of the forward primer BIN2-129F2, examined the tip to ensure that the liquid was absorbed, and then holding the 1.5mL tube, I deposited the DNA into the now blue solution.

Using the 10uL repeater pipette I withdrew 6uL of the reward primer BIN2-1541R2, and examined the tip to ensure that the liquid was absorbed, and then holding the 1.5mL tube I deposited the DNA into the now blue solution. Then using a 200uL repeater pipette I mixed the solution in the 1.5mL tube by setting the uL volume at 200 and pumping the solution 5-10 times until it was evenly mixed.

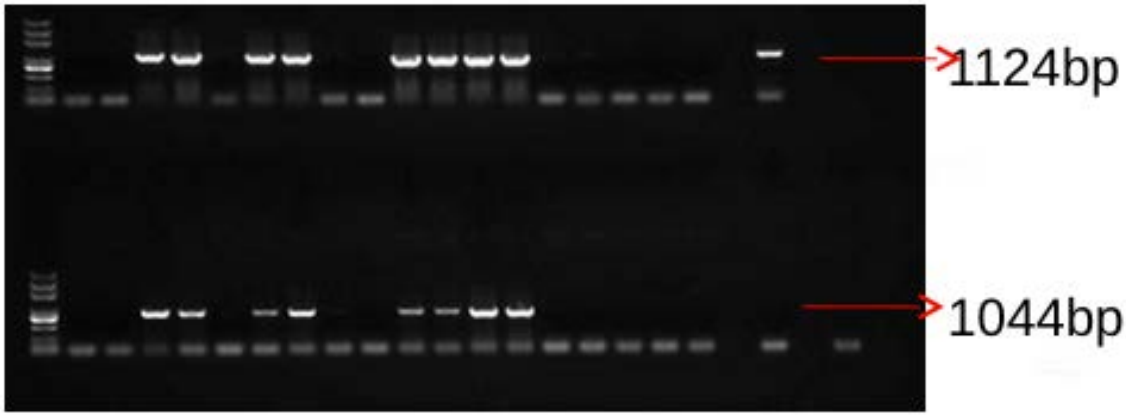
Using a 10uL repeater pipette I withdrew 9.0uL of the solution and placed it in tube 1. I repeated this step until the remaining 25 tubes had the PCR solution in them. In the tube labeled P I used the 2.5uL repeater pipette to place 0.5uL of cDNA in the bottom of the tube, ensuring that the liquid was transferred from the pipette to the small tube.

3. I then ran a PCR with the machine by using the standard settings for a tube amount of 10uL.
4. I then made an agarose gel as outlined above in a previous section. The 50mL mold was used with 2-15 tine combs inserted into the gel.
5. In the first well of the first row I inserted 5uL of a marker and then in the subsequent wells (numbered 2-13) 8uL of PCR product from tubes 1-12 were dispensed into each. At the end of the row I placed 0.4uL of the positive marker and 0.8uL of the negative marker.
6. In the next row I placed a marker in the first well like the row above it, and then 8uL of each of the PCR products in tubes 13-24. At the end of the row I placed another 4uL of the positive marker.
7. After the required time had elapsed I turned off the power source and removed the gel from the solution and placed it inside of the UV Light Machine using the computer program GeneSnap. The marker and the DNA were highlighted on a black background. A strong white line indicates a positive signal as to if the primer has sufficiently amplified the gene.

All of the above processes were the crucial ones to the research project that I was assigned. Through the above steps I could determine if the BIN2 that I selected for was amplified in the plant tissue, and could then be imparted into bacteria. In this case the PCR component showed that the gene was expressed in a number of the samples.



In wells 1-8 it is evident that the gene is expressed through the solid white line that is brought out in the UV Light Machine. The M line stands for the marker.



This snapshot of the well plate after electrophoresis. This picture shows that the Gene is expressed in nine of the plasmids at 1124bp, and eight of the plasmids at 1044bp. From this part of the experiment one can determine that it is possible to incorporate this gene into plasmids. Due to the short amount of time that I had I could not fully examine the gene in the corn, as the growing cycle was too long for my 2-month stay in China. However with the use of plasmids I was able to witness on a smaller scale the methodology that is incorporated into transgenic corn.

IV. My Mentors

In my research project I was primarily working along side my lab mentor who was a graduate student at the University working on her research. My project was a small offshoot of hers, and she painstakingly taught me every lab procedure and made sure that every procedure that I preformed was as comprehensible as possible. When we were not in the lab she would often take me with her to the germination house where I would assist her with the seed collection of the *Arabidopsis* plants, as well as the germination count of the seeds in the petri dishes.

Aside from Hao Ling I spent a majority of my time with Yan and Lily. Both of them were my roommates in the apartment that I lived in off campus. They were the most accommodating people that I ever met and were always making sure that I felt

included in anything that they were doing. Lily and Yan had their own research projects, and I often came along when they went to the greenhouse, to lend another pair of hands. Lily is currently looking to come to the United States to go to school at Iowa State University to go further with her current research. After her last year in graduate school Yan is hoping to get a job, and also finish her research and acquire her degree.

V-Responsibility and Contributions

As I was working in a biotechnology lab, the equipment that I was working with on a daily basis was extremely expensive. While initially I needed supervision when using the PCR machine, or the agarose gels molds, by the end of the summer I was deemed fully capable to do almost all of the laboratory procedures that I learned by myself. While slightly intimidating at first, the knowledge that I had acquired from my mentor made this possible, as I was confident that I had the skills necessary to preform the standard laboratory procedures.

I went to China with limited experience in a lab regarding biological procedures such as Polymerase Chain Reactions and DNA extractions. As a result, the research that I could conduct was a small offshoot of a pre-existing project by a graduate student. The depth of knowledge that would be required of me to create my own research project would be extremely difficult with almost no background in the scientific reasoning behind processes such as DNA primers and plastids. I went to China, and my mind was essentially a *tabula rasa* and by the time I returned I could comfortably talk about procedures and biological facts that I had never been taught in a classroom. The hands-on learning aspect of the research laboratory was one of the most precious things that I took back with me.

The laboratory skills that I learned in China have already begun to pay off. Upon my return to the United States I began research in a microbiology laboratory at the University of Florida. I am working on an independent research project on an invasive grass found in the Eastern United States, and tracing its genetic origin back to China, ironically enough.

VI-Impact on Global Food Security

During my eight-week stay the reminder of one of the reasons why hunger is a global issue constantly surrounded me: population growth. Waking up every morning in a city of almost 22 million inhabitants I could see how such a densely populated area affected the natural environment. Many of the bodies of waters that I saw while in the city, as well as the canals in the countryside, were extremely polluted. However one of the most striking things for me to witness was shopping in the supermarket and the open markets of Beijing.

Upon my arrival into the supermarket my senses were flooded by the overload of stimuli that was occurring around me. Store employers were shouting out prices of goods, shoppers were wheeling carts through narrow aisles packed with twenty to thirty people, and the smell of the largest fresh produce section I had ever seen, greeted my eyes and ears. I saw mountains of fruits and vegetables that could not be restocked quick enough to meet the demand. Then when I was at the open market I saw sellers with their produce, much like the markets that I sold at back in Florida, I saw the immense demand that the Chinese diet requires for fresh vegetables and their growing demand for meat.

When I returned to my room in the apartment for the night I reflected on what I

had seen during the day and I came to realize just how much manpower goes into the process of growing enough food to feed a single family. When doing laboratory research it is easy to lose sight of the long-term goal of the project, due to the minute scale that the research is being done on. However time and time again science proves that research and questioning natural processes is what leads to new discoveries that eventually better the world, as well as the human race. The research that I did over the summer plays a small role in a graduate students research project into the role that plant hormones play in plant growth and development. Hopefully the further study of these brassinosteriods can lead to a deeper understanding of their role. If scientists can fully comprehend how plants synthesize proteins and grow, this information can be used to engineer plants that are better suited for changing environments. It is through exploration of the plant genome that we will discover a way to feed 9 billion people by the 2050. While not the solitary answer to the global issue of food security, I hope that the future study and application of biotechnology will be part of the solution.

VII-Personal Growth

During my senior year of high school I took a class that focused on Single-Party States during the 20th century. I came to China with a plethora of background knowledge about the political, social, and economic issues that had plagued China in the past. However nothing changed my perspective on the country as much as my time there did. The memories that I cherish the most were the conversations that my lab mentor and I would have when we were seed sorting in the greenhouse. I was able to gain insight into the life of a young woman, and the sacrifices that she and her family made to put her where she is today. My experience in the city was very isolated from many of the challenges that rural Chinese families face on a day-to-day basis. Compared to the city, the rural areas are composed of many small farms where the farmers do not own the land, and must live unemployed for a significant part of the year, with no source of income to sustain them and their families. My lab mentor came from a family such as this; her father was often not as home during certain seasons as he would travel to large cities to work as a temporary construction worker. She often asked me questions about my life, and I often had to correct many preconceptions that she held about life in the United States. During those hours in the greenhouse we shared so much about our respective cultures, and I believe that both of us developed a better understanding of how people live differently around the world. Her story is a constant reminder to me of how lucky I am to have the life that I do.

While much of my summer was spent within the walls of the University, I would go on weekly trips varying from the Great Wall of China, to the National Museum, and WuQiao (a famous city dedicated to the circus). When I look at the photos that hang on my wall, what strikes me the most is the riot of color. Compared to the white marble and granite of Washington D.C. all of the buildings in China are gorgeous mixes of deep reds, emerald greens, and azure blues. The colors that I saw on a daily basis are a reflection of the complexity of the society that I lived in for two months. Upon my arrival back in the United States I like to claim that while I was in China I never had the same meal twice, and apart from my daily morning *bao zi* (bread dumpling) this was completely true. I had vegetables that are never eaten in the United States like taro (a

fibrous root much like a potato) and at least ten different varieties of mushrooms.

Before I left for the summer, at the orientation when Ambassador Quinn told the group of interns that who we were when we left, and who we were when we returned would be two different people. While I never thought that such an extreme statement would be true for myself, I have found that my internship has truly changed me as a person. The moment that I stepped on my plane at six in the morning in early June I was essentially alone for the next two months. Relying completely on my own skills and sense of direction, and setting my own schedule made my transition to college in the fall completely nonexistent, as I had already made that transition two months prior. While on the outside I appear to be unchanged, I have developed a level of maturity and responsibility that I did not posses at the end of my senior year of high school. I am truly grateful for this experience and how it has changed my perspective on my own future as well as the future of the world.

Works Cited

Baxamusa, Batul. (25 June 2010) *Plant Hormones and Their Function.* http://www.buzzle.com/articles/plant-hormones-and-their-functions.html

Bensen, Garren and Gibson, Lance. (2002) Origin, History, and Uses of Corn (*Zea mays*). Iowa State University, Department of Agronomy. 26 October 2014. http://agron-www.agron.iastate.edu/Courses/agron212/Readings/Corn_history.htm

China Agriculture University. (10 Dec 2012) *Brief History-China Agriculture University.* (2011) http://english.cau.edu.cn/col/col5408/index.html

Munro, Edith. (Oct 1 2012) Transforming China -Will a Modernized Chinese Agriculture Undercut U.S. Corn, Soybean Sales? *Corn and Soybean Digest.* 29 July 2014. http://cornandsoybeandigest.com/marketing/transforming-china-will-modernized-chinese-agriculture-undercut-us-corn-soybean-sales

Polymerase Chain Reaction. *National Center for Biotechnology Information.* 26 Sept 2014. http://www.ncbi.nlm.nih.gov/probe/docs/techpcr/

Schiavenza, Matt. (25 Oct 2013) How Humiliation Drove Modern Chinese History. *The Atlantic.* 26 July 2014. http://www.theatlantic.com/china/archive/2013/10/how-humiliation-drove-modern-chinese-history/280878/

Talbot, David. (31 July 2014) Chinese GMO Research Outpaces Approvals. *MIT Technology Review.* 31 July 2014. http://www.technologyreview.com/news/529546/chinese-gmo-research-outpaces-approvals/

Wiatrek, Pawal.(2014). Environmental Conditions Affecting Corn Growth. Clemson University. http://www.clemson.edu/extension/rowcrops/corn/guide/environmental_conditions.html

Qi, Liyan. (7 Mar 2014) China Seeks Its Own GMO-Food Path. *The Wall Street Journal.* 25 July 2014. http://blogs.wsj.com/chinarealtime/2014/03/07/china-seeks-its-own-gmo-food-path/

Yan,Z. , Zhao, J., Peng P., Chihara R.K., & Li, J. BIN2 Functions Redundantly with OtherArabidopsis GSK3-Like Kinases to Regulate Brassinosteroid Signaling. *Plant Physiology.*Vol.150, pp.710-721, www.planthysiol.org.