# FLOWERING FOOD:

## USING ARABIDOPSIS THALIANA TO UNDERSTAND GENE EXPRESSION AND MUTATIONS

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Thank you to **Dr. Norman Borlaug** for starting the organization that allows interns to journey across the world and learn how to better understand and solve issues of food security, **John Ruan** for his support of the organization and internship program, **Ambassador Kenneth Quinn** for speaking at the University of Minnesota and giving me my first exposure to the World Food Prize Organization, and **Lisa Fleming**, for meticulously organizing and caring for all of the interns to ensure that we had the best experience of our lives. Thank you to **Dr. Ronald Phillips** for helping me with my symposium paper and for encouraging me to pursue my interests in molecular biology and food security by applying to attend the Symposium and for the Internship.

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Thank you to **Sainan Liu** for teasing me in a mixture of Chinese and English, teaching me with her incredible stocks of knowledge, and trusting me enough to pipette $3000 of a bacterial vector on my third day on her project. Thank you to **Tren Gu** for translating…everything, getting mosquito bites so I didn't, explaining of biological processes in-depth, and for educating me on everything from microscopy to the greatest movies of our time. Thank you to **Jenny Hui** for talking through college stress, teaching me how to barter in Chinese, and for learning to ride on the back of my bike.

**Before Beijing**

It all began with a half sheet of paper, handed to me by my physics teacher, Mr. Sullivan, on a hot May afternoon. The paper had the time and date of a speech that Ambassador Kenneth Quinn was giving at University of Minnesota. I went home and left the advertisement on my kitchen table without thinking twice about it. After she came home, my mom commented that it sounded like an interesting speech, and that she could take a couple of hours off of work to take me. So, my mom picked me up from school at lunchtime and we were off to an event that neither of us knew anything about. It was a completely arbitrary decision that we made; we thought that the speech sounded intriguing, and was worth missing a few hours of school. To this day, my mom and I cannot remember what made us curious enough to attend, but whatever it was, those few hours were the start of my journey with the World Food Prize Organization.

I was offered a spot at the World Food Prize Youth Institute after applying through the University of Minnesota and I was absolutely thrilled. My research on necessary biotechnological advancements to bolster India's infrastructure and crop yields had proven fascinating while I was writing my YI paper, but now, I was given the opportunity to discuss this issue with other students and world-renowned specialists in politics and in agronomy.

I was certain that I would be applying for a Borlaug-Ruan International Internship even before hearing the 2008 Interns speak about their experiences, but the presentations just solidified my commitment to perfecting my application even though I doubted my chances of selection. I wrote my application essay and meticulously revised it, then checked my mail every day to see if anything had come. On April 9$^{th}$, 2009, I received my letter inviting me to be an Intern at Peking University in Beijing, China. I would be traveling across the world just a day after my summer vacation began. May and the first week of June flew by, with AP exams, visa applications, and reading up on Arabidopsis thaliana.

**Foreign Landing**

Once we made it through customs, the three of us traveling together (Colin Weaver, Linda Geiger, and I) were split; Colin went off to the Chinese Academy of Agricultural Sciences

(CAAS) while Linda and I were whisked away to a hotel for a short quarantine. When I stepped out of the airport, I immediately felt as if I had come home. I've been blessed with the opportunity to travel to India multiple times, and before leaving for Beijing, my favorite place in the world was my parents' hometown of Chennai, India. The number of people, cars, and bicycles on the streets of Beijing and the smell made me feel as if I was back in India. However, once we were situated in the taxi, I started to notice things like the Bird's Nest, and a dragon shaped building that were uniquely Chinese.

During our quarantine (the purpose was to keep us off of our campuses in case we virus transfer), Linda and I were given an opportunity to see many of the historical sites of China immediately upon arrival. A graduate student from the lab that I was working in, Yanbing Wang, stayed with us and was our liaison to the city of Beijing. Even though I was physically in China, it took time for me to realize that I was actually across the world. Upon seeing the larger-than-life portrait of Chairman Mao Zedong at Tian'anmen Square, I became conscious of the fact that I was over 7,000 miles from home.

**Learning about the Lab**

After our quarantine period was over, Dr. Kang came to meet me as Yanbing dropped me off at the Shao Yuan dormitories. A small and unassuming man, Dr. Kang is a professor of Plant Molecular Biology at Peking University ***and*** at China Agricultural University (CAU). I later found out that Dr. Kang begins his days at CAU by 6:00 AM, then bikes the 8 kilometers to Peking University by about 11:00 AM, where he stays for the remainder of his day. Even with his busy schedule, Dr. Kang still managed to find some time to show me the ropes in the lab and around the city. After helping me take my luggage up to my room on my first day at the University, Dr. Kang told me to get some good rest for the next few days, since I would need my energy once the lab work began.

Before I started in the lab, I was able to explore Peking University. I realized why the university had the reputation that it did. Peking University, established during the 100 Days Reform, is generally known as the "Harvard" of China. Peking University is the university of

dreams for children in China. Thousands of people flock to Peking University every year in hopes that their visit will help their children or family members get accepted in the future.

My first ten days in the lab were spent learning about the protocols, and everyone was willing to have me to work with them, even though I knew very little about the technical aspects of biological experimentation. Even as I mixed up steps of DNA extraction or mixed water with agarose instead of TAE buffer, my mistakes were brushed off and I was given opportunities to try again.

On my first day in the lab, Yanbing picked me up at my doorstep and walked me over to the lab building. I was introduced to about thirty PhD students, but had great trouble remembering their Chinese names. Most had English names, and told me to call them "Cecelia" or "Alex", but I made it my goal to learn and properly pronounce each person's Chinese name by the end of my internship. Yanbing was a phenomenal help at all times during my internship, especially because she was always willing to let me help with her experiments or take me sightseeing on the weekends.

After meeting the students in the East and West Halls (the lab is so large that there are two lab spaces, each accommodating about 25 students and researchers), Yanbing took me on a tour of the building. From the AutoClave machines (used to sterilize tools with boiling water) to the chemical stock room (I became fast friends with the manager of the stockroom, since I was often sent to get more agarose powder), Yanbing taught me where to find necessary supplies and showed me how to use the essential machines for common lab procedures. Dr. Kang came to the lab, and then took me through some potential project ideas, and I was touched by the amount of caring with which he asked me about my stay thus far. He made sure that my dorm was adequate and that I was getting enough vegetarian food.

As is tradition in many workplaces in China, after lunch is naptime. Yanbing showed me the way back to the dorm, and I was convinced that I could find my way back to the lab two hours later. I started my trek back to the lab, but instead of being at the end of a path near two parking lots (where the lab building was situated), I was on the other side of campus, circling a beautiful lake. Convinced that I had managed to maneuver my way off campus, I called

Yanbing, asking her where I was. She laughed, found me, and showed me how to get back to the lab from Weiming Lake.

**Making Mistakes**

After Yanbing showed me the basic ropes of the lab, different students showed me how to conduct basic experiments so that I would have a solid foundation when I began working on a project. I started with the one of the most ubiquitous procedures in the lab—extracting DNA from Arabidopsis thaliana plants. DNA extraction begins with cutting leaf samples, which are frozen, ground, heated, and centrifuged. During my first experience with DNA extraction, I made a mistake of epic proportions. Instead of using CTAB, a buffer that causes the DNA to release from the leaf in a hot environment, I accidentally put ethanol in each of the microtubes containing ground leaves. As a result, all 96 of the samples that we had prepared were ruined. However, even though I had made a major mistake, Dr. Kang and the PhD student who I was attempting to help, Ying Wang, had just one thing to say: "make more mistakes!" Their rationale was that if I made mistakes now, when there were no major consequences, I would be less likely to make mistakes during more important experimentation. I practiced DNA extractions for three full days, until I was able to get usable DNA out of every sample started.

After mastering DNA extraction, I worked with a student named Chris, who taught me the ropes of polymerase chain reaction (PCR) and gel electrophoresis. Because the amount of DNA extracted from plant leaves often isn't sufficient to conduct further experimentation, PCR is a technique that amplifies the amount of DNA available. In this situation, we used PCR to increase the DNA available for gel electrophoresis, a way to map the lengths of the DNA segments.

Making gels for gel electrophoresis has careful regulations in the lab, because of the use of Ethidium bromide. Ethidium bromide (Eb) inserts itself between DNA base pairs and causes fluorescence under UV light, making it possible to view the DNA segments. However, Eb is a toxic chemical that can cause mutations in human DNA through skin contact. As a result, areas of the lab are specified as "clean" or "contaminated" depending on contact with Eb.

Since DNA is negative, gel electrophoreses use a positive charge to attract DNA to one end of the container, and the segments move towards the charge. Once a gel has been run, it is placed in a UV light container, allowing researchers to view bands of DNA. In each gel, a marker is used as a way to accurately describe the length of each DNA fragment. The smaller the segment of DNA, the faster it can move.

Once I had mastered these three basic protocols in the lab, I helped students with their research whenever they needed assistance with DNA extraction, preparing for PCR, or running gels. I worked primarily with Ying and Yanbing in my first few weeks, as they often worked with about a hundred samples at a time, meaning that there was much preparation work to be done.

**Lecture Series and Experiment Training**

At the end of June, each of the students in their third year or above gave a lecture on one of the unique techniques that they were using or a synopsis of their research thus far. While the lectures were in Chinese, Dr. Qu Li-Jia(the head of the lab) asked the students to make their PowerPoint presentations in English so that I would be able to follow along. While learning about the techniques during the three-day series, I thought that the procedures were well above my abilities. However, along with the lecture series, the lab also had advanced experiment training. I was provided with the opportunity to learn methods including *in situ* hybridization and Southern blotting.

Dr. Qu himself gave one of the most useful lectures during the series. His speech, "Data Arrangement and Writing", provided tips on the most effective ways to relay experimental results in a scientific paper. Even though his lecture wasn't about the lab's research, Dr. Qu explained how to utilize each part of a research paper and offered guidelines. I was able to apply his information later in my internship, when I worked with a student to prepare a paper for publication in the *Journal of Integrative Plant Biology*.

**Playing Badminton**

As I learned in my first few days, students at Peking University (Beida) live differently than students in the US. Most students come to the lab around 9:00 AM, and don't leave until 10:00 PM. During the day, there are games of Ultimate Frisbee and badminton, but students stay in the lab until the work gets done. I became very fond of this way of life during my time at Beida. While we were waiting for PCR products, we would play a pick-up game of Ultimate Frisbee in the parking lot or grab popsicles, making it enjoyable to stay at the lab for long hours.

The day after the lecture series concluded, the lab schedule reverted back to normal—including the weekly badminton games. Each Wednesday, the lab reserves several badminton courts across campus for students and teachers to go and play in the afternoon. Even though I didn't have a racquet or skills at the game, I was always invited to play. Most teams didn't keep score, and playing was simply a fun way to relax in the middle of the week. While I was sitting out for a while (there were usually more students than available courts), I met a Berkeley student, Tren Gu, who was interning at the lab for the summer. He told me that if I wanted to work on a project, he would find something for me to do. Tren was working with second-year PhD candidate Sainan Liu, and I worked with them for the remainder of my internship.

**The Database of Arabidopsis Transcription Factors**

Since Tren had been working with Sainan for more than a month before I joined them, he knew the procedures and protocols inside and out. Therefore, he started explaining the purpose of the research, and broke it down so that I would be able to understand. Because Sainan was planning to leave Beida to study at the University of California—San Diego in November 2009, she was trying to get as many results as possible before moving. The same day that I began working on the project, a student from Hong Kong, Jenny Hui, also came to assist Sainan.

Arabidopsis thaliana is the primary plant studied at the research center, because Arabidopsis is known as a model plant for 17,000 flowering plants, including rice. Since rice is such an important crop in China (and across the world), developing new strategies to produce stronger hybrids is absolutely vital. Understanding gene expression, regulation, and ways of mutation is key to this production. As a result, over the years, the Peking-Yale Joint Research

Center has developed the Database of Arabidopsis Transcription Factors (DATF). Transcription factors regulate the expression of genes and control the transfer (transcription) of DNA to mRNA. The DATF is a library of all of the transcription factors of Arabidopsis known to exist. There are a total of 1,922 unique transcription factors in 64 families, and the goal of Sainan's research was to produce transcription factor clones in order to certify that the particular transcription factor does exist. Moreover, in order to find the function of each specific transcription factor, the full-length transcription factor needs to be produced. A comprehensive library of transcription factors allows for a complete understanding of the interactions between different transcription factors and the DNA targets of each unique transcription factor. Two methods that have been developed to understand the interactions between transcription factors are the Yeast-1-Hybrid and Yeast-2-Hybrid, but in order to use these, the complete DATF is first necessary. Thus, during my work with Sainan, our goal was to produce matching clones to transcription factors that are coded in the DATF.

**The Cloning Procedure**

Our procedure began by using BLAST computer program to find the DNA base-sequences for the transcription factors that we wanted to clone. Often, this took place on a Sunday afternoon, when one of us would sit at a computer copying and pasting rows upon rows of A, T, C, and G bases from one program into another. Once we had the desired sequence, we designed primers to cut the cDNA (one stranded DNA transcribed from mRNA) to the desired sequence. The primers were also designed through a computer program. In order to properly slice the DNA, we had to have a forward primer (5' to 3' end on DNA) and a reverse primer (3' to 5') so that both strands would be cut. We usually designed primers in batches of 200 (we would then work with 100 sequences). After we had enough primers, we sent the primer sequences to a company who produced the primers and brought them to the lab.

Once the primers arrived from the company, the cloning procedure took between five and six days. The primers arrived in a dried powder form, and the first step each time we received a new shipment was to add $ddH_2O$ to make all of the primers the same initial concentration. The next step was to label a new set of microtubes with the transcription

factor's family code and the primer number as "f" (forward) or "r" (reverse). We then took 10 μL of each concentrated primer, placed it in the matching microtube, then added 90 μL of $ddH_2O$ to the concentrate. By doing this, we had 100 μL of 0.1M of each primer prepared and organized.

After the primers were prepared, we performed first round polymerase chain reaction (PCR). With each of the transcription factors that we were cloning, we placed a mixture of enzymes, forward and reverse primers, cDNA, and $ddH_2O$. Because different transcription factors have different numbers of base pairs, the transcription factors have to be sorted depending on these lengths for PCR. The annealing phase of PCR takes 2 minutes per thousand base pairs, so we generally grouped transcription factors into three groups: 小 (small), 中 (medium), and 大 (large), depending on the range of sizes in each particular batch. Once the first round of PCR was finished, we took 1 μL of the PCR product from each unique tube, placed it on top of a piece of Parafilm (Parafilm keeps liquid from running). With the PCR product, we added loading buffer and $ddH_2O$, then loaded it into a gel to check if the primers cut the cDNA to separate the transcription factors from the rest of the cDNA. If the bands on the first round PCR products matched the theoretical band numbers (according to the standardized marker), the accurate PCR products moved onto second round PCR.

Second round PCR has a nearly identical procedure when compared with first round PCR, with the major difference being the amount of enzyme mixture, $ddH_2O$, forward and reverse primers, and the remainder of the first round PCR product. In order to ensure that the primers were completely successful, second round PCR cleaned up any loose ends that may have been left over from the first round. Instead of the 25 μL total product in first round PCR, second round PCR had 120 μL in order to be successful in the gel extractions. When running the second round PCR through the gels, we use extra-large wells so that all 120 μL is run. As a result, the bands are extra large and easy to read. With the bands that were accurate with theoretical lengths, we performed gel extractions. Gel extractions are a technique used to cut the accurate bands out of the gel, and then several buffers are used to remove the DNA from the gels.

After gel extractions, a TOPO reaction is performed. The TOPO vector is the backbone of a plasmid into which the transcription factor is inserted. The TOPO vector is then transformed into E. coli. The transformation occurs with a heat shock that occurs at 42°C, which allows the TOPO vector to enter the E. coli bacteria. The reason for this process is to multiply the number of TOPO vectors because the E. coli replicates the plasmid as if the plasmid is the E. coli's own DNA. As a result, after this process, we have much more of the desired transcription factor to work with. A portion of the E. coli bacterial broth is then mixed with a glycerol solution to maintain a stock in case of mistakes after the next few steps. The TOPO vector is an extremely expensive product; redoing the entire procedure would be inefficient and far too expensive.

Following the stocking of the glycerol solution is the most time-consuming task of the cloning procedure. The Mini-prep procedure occurs in order to extract the plasmid DNA from the E. coli. Mini-prep is essentially consists of using different buffers to break the E. coli membrane and allow the TOPO vector to be separated. Subsequently, we perform bacterial PCR to check the transcription factors using M13 primers. The M13 primers are unique to the TOPO vector, because the transcription factor inserts occur in the same place on the TOPO. As a result, the forward and reverse M13 primers, if the transcription factor is still intact, will cut the transcription factor out of the TOPO vector. The gels run after the bacterial PCR should show bands of the transcription factor size plus 300 base pair length, because teach M13 site (forward and reverse) is 150 base pairs away from the insert site. This is a quick way to use PCR to check if the backbone had the correct insert in it. Once the bacterial PCR and gel electrophoresis have been completed, the correct inserts are sent to a sequencing company. After the company sends back sequences of the inserts, we check the sequences against the theoretical transcription factor to see if they are accurate or not.

Due to the number of steps in the procedure of cloning, the majority of transcription factors don't make it to the final sequencing stage. Therefore, the number of accurate clones out of 100 primer sets at the beginning of the procedure, we are lucky if 40 or 50 inserts come out as accurate clones. With the clones that were accurate, we perform one last reaction—the LR reaction, which is used to stock the clones for use by other companies, universities, or

databases that wish to use the new clones to study transcription factors or Arabidopsis thaliana.

**Making an Impact**

During the summer, Sainan, Tren, Jennifer, and I cloned almost 200 transcription factors. The DATF can now be used with greater accuracy, since there are more accurate clones that can be cross-referenced. The Yeast-1-Hybrid and Yeast-2-Hybrid systems are based off of the accuracy of the DATF, which is steadily improving. The gene expression of plants is so imperative to understanding the life cycle and mutation occurrences that completing the DATF is the first step to create a rice (or other key grain) plant that can be produced consistently without mutations.

A particular impact that I made was in the bHLH family of transcription factors. The bHLH family is made up of 127 transcription factors, and I worked with a group of bHLH transcription factors that had failed cloning several times. While working with the transcription factors of the group, the 100 or so each week, I also tried again and again to clone the group of bHLH factors. The clones of bHLH loci AT5G54680,AT1G74500,and AT2G31210 were all clones that I personally took through the procedure, and received perfect clones that matched the original sequence.

**Venturing to Qu Zhou and Handan**

As the majority of my time in Beijing was spent working in the lab, it was sometimes hard to understand the practical application of the research we conducted on a microscopic scale. When Dr. Kang and I spoke about my impressions of Beijing, one of my biggest surprises was the incredible industrialization. I was stunned how, within the city, farming was nowhere to be seen. Dr. Kang therefore decided that we would take a trip to the outskirts of town, so that I would be able to see more of China outside of the city proper. Tren also wanted to see more of the countryside, so he came along as well. Dr. Kang picked up Tren and I from the lab one day, and we were off. The train took about eight hours, and as the train zoomed through the city, I saw the statistics that I had read and heard about for so long. The farming

communities were ubiquitous starting just a few kilometers from the center of the city. Once we exited the Handan train station, we took a taxi 75 kilometers further to the village of Qu Zhou. In Qu Zhou, there is a branch of the China Agricultural University, where we stayed during our four-day trip.

After our long day of travel, Dr. Kang, Tren, and I all fell right asleep, and began our day early the next morning. Dr. Kang showed us the experimental plots of the university, and we met with a student of China Agricultural University (CAU) who showed us the extensive corn research being applied on the plots. Most of the lab work occurs on the Beijing campus of CAU, but the experimental application is clearly successful on the farms. The five-year ongoing experiments were different rotational procedures of spring maize, summer wheat, and certain soybean plants. Seeing the research in the form of thriving plants was an incredible motivation to continue lab work at Beida.

**Biking around Beijing**

Throughout my time in Beijing, my bike was my constant companion. Dr. Kang handed me a key to a bike lock on my third day in the lab, and told me that this bike would be mine for the summer. He took me biking around the city a few times, and after the first time, he told me that, "you bike like a little boy!" Dr. Kang was surprised at how quickly I rode my bike, and told me that I could ride around the city as long as I told him where I was.

A common mode of biking is double-teaming—one person riding on the back and the other pedaling extra hard to haul two people. Jenny, another student working with Sainan, Tren, and I, didn't have a bike of her own. As a result, she became a common fixture on the back of my bike. In the evenings, because Jenny didn't live in the dorm, I would often ride her back to her dorm. This experience was one of my favorites during my stay. Riding out of the university, Jenny and I would talk about everything under the sun—applying for college in the US versus China, political and social differences between China and the US, and the difference between Southern and Northern China. Moreover, Jenny often taught me bits of Chinese, like numbers and how to barter while shopping. It was this time of day that helped me better understand the United States in the global setting.

I also found joy in the ability of my bike to take me anywhere in the city. There was a subway station just about two kilometers from the Beida campus, from where I could go anywhere in Beijing. Most often, I traveled with Colin Weaver, the BR intern at China Academy of Agricultural Sciences. The subway station was right between our campuses, so we would meet there on Sundays and explore parts of the city. We were able to visit some of the most famous sites in Beijing, like Olympic Park and the Forbidden City. Colin and I also took the time to visit less popular sites like a tea and flower market, and the electronics district.

**Saying Goodbye**

During the last week of my internship, I couldn't believe how quickly my time breezed by. Since Jenny and I were leaving within three days of each other, and Tren was leaving just two weeks after, we all worked to wrap up most of the loose ends in the experimentation. We completed our last LR reaction, and stocked the as many clones as possible for Sainan to transport to the University of California—San Diego, so that she will be able to continue her research.

The lab heads, Dr. Gu and Dr. Qu also took our lab group, along with Dr. Kang, out to lunch. They surprised me by taking me to a local Indian restaurant, because they knew I missed my mom's cooking. During the lunch, we talked about how our research had the potential to impact significant change in Arabidopsis thaliana and rice research, and how my cultural experience had changed my personal perspective of food security and lab-based research. After the diner, I was even further saddened to leave from Beijing, because I had become so connected with the lab and the research center culture. Today, I miss more than the weekly watermelon and mealtimes with the Jennifer, Tren, and Sainan, but I also want to return to the peaceful but productive culture of the research center. On my last day, I couldn't help but realize that it would be a long while before I would be able to return.

**Finding Inspiration in Understanding**

I've learned that biology and social change go hand and hand. Without focusing on lab-work like the DATF, solving hunger at the roots is impossible. Even though it felt like the DATF

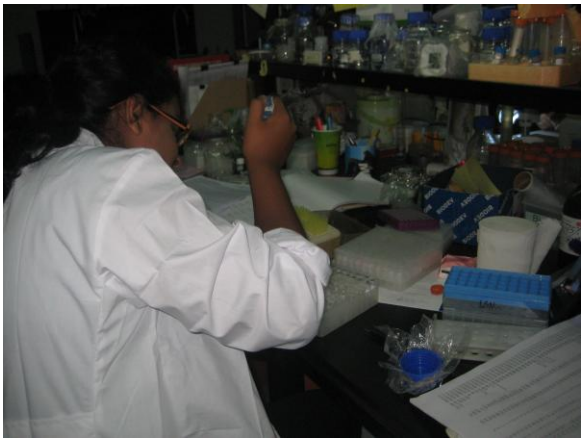
was far removed from the immediate impacts of growing crossed rice or corn plants, the DATF has real impacts in understanding and hopefully preventing gene expression mutations.

During my trip to Handan and Qu Zhou, I learned the most about a country that I knew so little about. Before learning that I was a Borlaug-Ruan International Intern at Beida, I didn't know anything outside of China's economic impact on the world, because of my debate and speech research. However, my knowledge before my time as a BR Intern was so superficial that I wasn't able to understand the impact of China's economic standing on the country itself. When I arrived in China and started learning from students in the lab, I was able to see that China's supreme workforce comes from the intense education system.

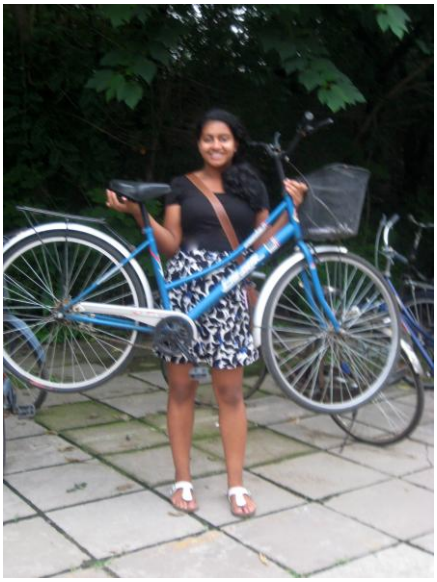
I was given the opportunity to work with some of the brightest plant biology students and professors in the world. With that opportunity, my time in China has motivated my future. I hope to combine biology and international relations in hopes of going into agricultural policymaking. China helped me realize that without both aspects of change—lab and implementation, fighting hunger will not be as effective of an effort as it can be.

The simplest way to describe the change I experienced is in my lab notebook. Every page is a combination of Chinese characters and lab graphs and charts. Learning about other cultures and working across borders is the only way that cultural understanding will be reached on a global level. Without attempting to work as one world, fighting hunger will not be a multilateral effort to enact true change. As a Borlaug-Ruan Intern, I've found that I do have the potential to stimulate change and bring it about in an individual effort, but understanding other cultures is the primary way to benefit those that need the most help in gaining food security.

**PICTURES**



Preparing primers for first PCR



My constant companion

Clockwise from top left—Dr. Kang, Sainan, Tren, Dr. Gu,

Dr. Gu and Dr. Qu's daughter, me, and Jenny

Bacterial colonies growing

Checking sequences for clone matches

Experimental cotton plots in Qu Zhou

Rural farms in Qu Zhou

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