Adventures in China

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2011

Table of Contents

Acknowledgments

To start, there are many people to whom I owe enormous thanks for their tireless efforts in making this internship experience amazing. The World Food Prize Organization, specifically Lisa Fleming, has established a wonderful program that I hope students continue to participate in and learn and grow just as much as I have this summer. At Peking University, I want to thank Dr. Hongya Gu and Dr. Dingming Kang for hosting me this summer and for showing me many of the wonderful sites in China. I want to thank my teacher and mentor for the Global Youth Institute, Mrs. Barb Nealon, for being one of the best teachers I have ever had and for her true commitment to education. To Hou Yingnan, I greatly enjoyed working on your research this summer and I appreciate the time you took out of your busy schedule to explain each step and process to me, and for trusting me to handle the different procedures.

Introduction

My name is Nicole Ferraro and I spent my summer in a plant genetics lab at Peking University in Beijing, China. I am from New Freedom, PA and I graduated high school 9 days before departing the U.S. for China. I attended the World Food Prize Global Youth Institute in October of my senior year, 2010. I had heard about the program as a junior from another student who had participated the year before. While I thought her experience sounded interesting, I did not give the program too much serious thought, as agriculture has never been one of my primary interests. The summer after junior year, I spent 11 days touring the country of Peru on a Spanish trip. We visited the coastal, mountainous, and rainforest regions of the country. We saw many of the farms in the Andes and we visited a farm in the rainforest. I was struck by how isolated these farms were, and by the lack of any sort of modern technologies. At the beginning of senior year, I remembered the World Food Prize program, and had a first period study hall, so I began to look into the Youth Institute. When I saw the essay topic for the year- Solutions for the World's Smallholders- and then saw Peru as a possible country, and then saw that barriers to market access was a possible topic, I knew that I had to write this essay. What started as a simple vacation with friends in South America turned into the chance of a lifetime, after I attended the Institute and learned about the Borlaug-Ruan Internship.

When I first arrived at the Institute, it was nearly midnight. I was exhausted after my two plane rides. I walked into the hotel and was immediately greeted by the four China interns from 2010. Their enthusiasm for the program was enormous. One began telling me about his project involving GM rice. Right then, I knew that this program was for me. One, my time in Peru convinced me that I wanted to travel and see the world.

Two, genetics has always been a passion and particular interest of mine, and the idea that, at 18 years old, I could work in a lab on one of the topics that I found so amazing and planned to pursue as a career was incredible. I really thank those four interns for their enthusiasm that night, as it really inspired me to seriously think about this opportunity. I had not intended on applying for the internship before attending the Institute. However, I recognized the opportunity to work on plant genetics and learn about the different procedures that goes into such research as useful to my future studies in biomedical engineering. Also, I, as many of my fellow interns would likely agree, possess a desire to have some effect on the world. Maybe my desired impact was more along the lines of saving lives through the field of medicine rather than increasing food security, but I wanted to change someone's life nonetheless. I believe medical research has a role to play in increasing food security, and I was interested to learn how my future studies could help the fight against world hunger. For me, this internship opportunity seemed like the perfect storm.

I spent my 60 days this summer at The National Laboratory for Protein Engineering and Plant Genetic Engineering at the Peking University in Beijing, China. As the throngs of hopeful middle/high school students visiting the campus every day can attest, PKU (or Beida as it is know locally) is arguably the most prestigious university in China. Located in the Haidian District of Beijing, the school was founded in 1898 and is currently ranked 47th on the international scale as of 2010. It was originally known as the Imperial University of Peking and it was the first national comprehensive university in China. The present name was adopted in 1912. The school includes 30 colleges (including my summer home- the College of Life Sciences), 12 departments, and 216 research

centers/institutes.

My first day in the lab, I was shown to Dr. Gu's office, where I was graciously welcomed before being introduced to a graduate student, Hou Yingnan, whose project I was assigned to work on all summer. I lucked out in my assignment. Yingnan is a 27 year old newlywed who is currently working towards his PhD. Yingnan majored in biotechnology at Heilong Jiang University in Harbin, China. He then went on to pursue a graduate degree at PKU. His project centers around the model plant Arabidopsis and the proteins that regulate pollen tube growth. He hypothesizes that a certain group of kinase proteins interact with attractant proteins to control the length and direction of pollen tube growth. So, in short, our goal was to prove this relationship. While I am not going to pretend that I am particularly thrilled by pollen tube growth, I found the scientific process fascinating, and it gave me the chance to do some experiments that were interesting and new. There were several ways in which we attempted to prove this relationship, and while no definite positive results were obtained during my internship, the project is still ongoing.

There are many reasons why *arabidopsis thaliana* was the chosen plant for this research. It is a small flowering plant and a member of the Brassicaceae family. Arabidopsis itself is not useful in ending food insecurity; it is more of a weed than a crop. However, it is important because of its small, sequenced genome, rapid life cycle (6 weeks from germination to maturation), high quantity of seed production, easy cultivation, large number of available mutant lines, and the large community of researchers using the plant in studies.

Coming into a project that has already been in progress for nearly 2 years, it is

difficult to grasp how the individual experiments relate to the overall goal. However, there were several procedures that I learned thoroughly that contributed to the overall goal of the project.

Cloning Genes (PCR):

Cloning genes through a polymerase chain reaction (PCR) is an important component of this research, as well as many other projects. PCR is used to increase the quantity of DNA for further experiments and study, such as gel electrophoresis. The DNA product is combined with distilled water, buffer, dntp, forward and reverse primers, a template (cDNA, in our case), and an enzyme (Fast PFU or taq polymerase, in my experiments) and then the solution is run in the PCR machine, for a set amount of time, at certain temperatures (this varies based on the preference of the researcher and the specific experiment), progressing from hotter to cooler (starting with 95/94 degrees Celsius, progressing to 60 to 72 degrees Celsius). PCR consists of three stages- denaturing, annealing, and elongation. Denaturation involves the breaking of the hydrogen bonds between the strands of the DNA. During the annealing stage, the two primers in the solution bind to the beginning and the end of the strands that are to be copied. The primers are short, specifically designed DNA strands. In elongation, the enzyme adds new nucleotides to the DNA strand in a 5' to 3' direction.

PCR Mix (amounts depend on the enzyme used, these are for Fast PFU):

-30 microliters ddH2O

-10 microliters buffer

-4 microliters dntp

-1.5 microliters of each Forward and Reverse primers

-1.5 microliters template

-1 microliter enzyme (add last)

-1 drop of oil to prevent evaporation

The amplified DNA could then be used in a variety of experiments. Often, we would extract the DNA of certain lines of Arabidopsis, run PCR, and then run gel electrophoresis in order to identify what genes are present in that plant. In this way, we can test if our DNA insertions were successful. PCR is also useful in amplifying the genes that we can later insert into the DNA of Arabidopsis.

Bacteria PCR:

Bacteria PCR is similar to DNA PCR in that the end goal is to amplify a product. For this procedure, we first need to transport the bacteria colonies from the plates to the tubes. To do this, take a pipette tip and carefully set it on the desired colony in the plate. Make a record of the bacteria colony used on a new plate by tracing two lines over the correct label with the tip. Then set the tip into the PCR tube. Repeat for however many bacteria colonies are needed. Once all the tips are in the tubes, shake the tubes carefully so that the bacteria leaves the tip and settles at the bottom of the tube. Discard the tubes. Prepare enough mix so that 10 microliters can be added to each tube. The bacteria serves as the template.

-5 microliters taq enzyme mix

-4 microliters ddH2O

-0.5 microliters of each Forward and Reverse primers

Run the tubes in the PCR machine for 32 cycles. The bacteria produced in this procedure can be used in other experiments. For instance, the bacteria can be used as a vector to

infect plants with a specific plasmid.

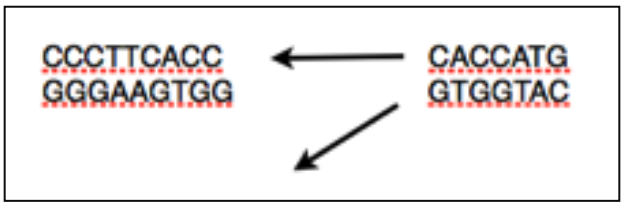
TOPO Cloning:

One way to insert a plasmid into a DNA strand is using restriction enzymes to cut open the strand, then inserting the plasmid and using ligase to seal the strand back up, which is what I was taught in high school. In the lab, Yingnan introduced me to another method, referred to as TOPO cloning. TOPO cloning allows a PCR product to bind to a blunt or sticky end cut in a DNA fragment. PCR is the first step in a TOPO clone. In PCR, chose a correctly designed PCR primer in order to obtain blunt-end PCR products. Our primer used ATG as the start codon, followed by CACC in the sequence. Use gel electrophoresis to verify the results after this step. There should be a single, discrete band of a specific size. Then, complete the reaction by mixing together the PCR product and the TOPO vector (from Invitrogen) and incubate for 5 minutes at room temperature. For maximum efficiency, use a molar ration of 0.5:1 to 2:1 for PCR product: TOPO vector. The reaction includes:

- 0.5 to 4 microliters PCR product
- 1 microliter salt solution
- 1.7 microliters ddH2O
- -0.8 microliters TOPO vector plus enzyme

Allow this reaction to incubate for 5 minutes at room temperature. Then transform to e.coli competent cells. Cells should be stored at -86 degrees Celsius. Add the vector solution and wait 15 minutes. Immerse cells in a water bath at 42 degrees Celsius for heat stimulation for 90 seconds. Keep cells in ice for 2 minutes to keep the cell membrane closed. Then add the culture medium (40 microliters). Put the tubes in the

shaker at 200 rpm at 37 degrees Celsius for one hour. Spread about 50 to 200 microliters of the product onto plates and culture overnight at 37 degrees Celsius.



At left- TOPO cloning works by using a primer (sequence on right) to replace part of the original strand. The CACC sequence in the primer will replace the CACC in the original strand and then the nucleotides following the CACC in the primer will also be attached. The bottom part of the primer will be removed in the reaction and will not be part of the finished product.

To check for positive results, test ability of bacteria to grow on a kanamyacin plate (as they should have antibiotic resistance) and has the correct anticipated bands in a gel electrophoresis analysis.

Bacteria DNA Extractions:

Extracting DNA from bacteria can be important to the overall project, as we can use those plasmids and insert them into Arabidopsis to force the plant to produce new proteins that may inhibit the target kinase proteins from performing their usual function. In this way, we can see what the function of the kinase proteins is, and see if it does in fact affect pollen tube growth.

The process for DNA extraction from bacteria is as follows:

1. Pour the cultured bacteria and medium from the large test tube into the smaller centrifuge tubes. Pour until tube is nearly full.
2. Centrifuge for 1 minute, twice, removing the supernatant. Be sure the centrifuge is balanced.
3. Add 250 microliters of S1 (TE buffer) and vortex until thoroughly mixed.
4. Add 250 microliters S2 (contains NaOH, SDS-soap like substance that allows cell

membrane to break apart, enzyme, and water). Gently shake the tube to mix. Wait 90 seconds.

5. Add 350 microliters S3 to neutralize the solution. Gently mix again.
6. Centrifuge for 12 minutes.
7. Move liquid to new tube (containing a filter) and centrifuge for 1 minute.
8. Add 600 microliters washing buffer and centrifuge for 1 minute. Repeat.
9. Centrifuge the tubes empty for 1 minute.
10. Move the smaller filter part of the tube and set in a new tube.
11. Heat tubes at 55 degrees Celsius for 5 minutes to eliminate ethanol.
12. Add 50 microliters distilled water because DNA is soluble in water and allow it to sit for 1 minute for DNA to dissolve.
13. Centrifuge again for 3 minutes. Take liquid in the bottom of the tube and put back on top of the filter. Centrifuge again. The DNA should be dissolved in the solution that is left.

Plant DNA Extraction:

Plant DNA extractions are important to the project because they can be used to observe whether DNA insertions were successful by producing plant DNA that can be amplified through PCR and then run through gel electrophoresis. The procedure is as follows:

1. Cut leaves from the plant and put in labeled tubes.
2. Add 100 microliters CTAB buffer (contains detergent to break down membrane) to each tube.
3. Attach each tube to a machine that uses a motorized blue stick to smash and break apart the leaves. Wash stick between each tube. Keep tube on machine until the

solution appears completely liquid and light green in color.

4. Add 300 microliters buffer.
5. Heat at 65 degrees Celsius for 1 hour.
6. Add 400 microliters CHCl3 pentanol (helps decrease surface tension) to each tube at a ratio of 24:1 (20 mL pentanol to 480 mL CHCl3). Wear face mask and gloves for the rest of the procedure (ChCl3 is chloroform).
7. Mix for 2-3 minutes and centrifuge for 15 minutes.
8. Add 700 microliters ethanol to new tubes.
9. Pipette out the top layer of liquid (contains DNA) from the centrifuged tubes into the new tubes.
10. Store at -20 degrees Celsius for 1 hour.
11. Centrifuge at 4 degrees Celsius for 15 minutes.
12. Add 700 microliters 75% CH3CH2 plus 25% H2O.
13. Centrifuge again for 5 minutes and remove supernatant.
14. Heat at 65 degrees Celsius for 5 to 10 minutes.
15. Add 25 microliters H2O.

Plating Seeds:

One important task in the overall project was collecting seeds from the different plant lines and then growing them on plates, before transferring them back to the soil. These plants have purposeful mutations incorporated into their genomes. The mutation results in a change in amino acid production, and this change affects the kinase proteins that are suspected to control pollen tube growth.

Above: Yingnan and I in the greenhouse, preparing to collect siliques from the Arabidopsis samples.

This causes a dominant negative situation- the protein exists, but cannot function. In this way, we can see how the loss of the kinase proteins function affects the growth of the pollen tube in the plants. The inserted gene was tagged with GFP fluorescence, and so when collecting seeds, we observe them under the microscope and chose the lines with the strongest fluorescence, indicating that the mutation was successful. Then, we plate the seeds and eventually transport them to the soil and observe their growth, and how the lack of functional kinase proteins affects the eventual pollen tube growth. The procedure for collecting seeds and germinating them on plates is as follows:

1. Collect the yellow siliques from the plants in small tubes.
2. Add beads to the tubes for storage to keep the environment dry and prevent germination.

3. Filter the siliques, shaking the filter so that the seeds fall onto the paper below, and leave the covering behind.
4. Put the remaining seeds in new tube, making sure to properly label.
5. Wash in 1 mL of 75% ethanol and put in shaker for 15 minutes.
6. Extract ethanol, and then add 1 mL of 100% ethanol and shake for 8 minutes.
7. Extract ethanol and wash with 1 mL of distilled water twice.
8. Pipette mixed water and seeds onto the plates.
9. Remove excess water from plates and try and spread seeds out as much as possible.
10. Let dry and then store 2 days at 4 degrees Celsius.
11. Move to 22 degrees Celsius for 7 days, with 16 hours light and 8 hours dark.
12. Transfer to soil, making sure the radicle is in the soil, and the plants are spread out properly.

Transform Vectors into Agrobacteria:

This procedure is useful in the process of infecting the plants in order to transfer a specific plasmid into their genome. The gene is in the vector and then the plant is literally dipped into the bacteria, and the plant takes in the bacteria through the flowers and it is incorporated into its genome, and hopefully replicated for the next generation. In this way, we can see how the mutations affect the function of the kinase proteins and pollen tube growth. The procedure for transforming the vectors into the bacteria medium is as follows:

1. Centrifuge the bacteria at 500 rpm for 5 minutes.
2. Add 100 ng of vector per 1 microliter of bacteria into the competent cell (in our case it was 500 ng), and keep the cell on ice for 5 minutes.

3. Move competent cell into liquid nitrogen for 5 minutes as the vectors attach to the membrane.

4. Move to a water bath at 37 degrees Celsius for 5 minutes.

5. Add LB medium and culture for 3 hours at 28 degrees Celsius.

Preparing LB Medium:

The LB medium is used to culture bacteria for a variety of experiments. To prepare 1 L of the medium:

-10g Tryptone

-5g yeast extract

-5g NaCl

-1 L ddH2O

It was often my responsibility to prepare the LB medium when the experiments we were doing that day required culturing bacteria in a liquid for storage.

Infecting Plants with Bacteria:

Infecting the plants with bacteria was an important step in incorporating the mutations into the plants genome in order to observe the role of kinase proteins in pollen tube growth. In my time at Beida, the results of this experiment were not what we expected. That is, we expected there to be problems with the male gametophyte and thus we predicted that the double fertilization would not occur. This has not happened, but this is a complicated procedure with many steps, and so the experiment will be repeated after I leave, hopefully with more favorable results. The procedure for infecting the plants is as follows:

1. Culture bacteria in a small bottle for 24 hours.

2. Transfer 2 mL of the bacteria into a 300 mL bottle with medium.

3. Transfer 300 mL into a 1000 mL bottle.

4. Cut off all existing siliques on the plants so that any future seeds will be affected by the bacteria infection.

5. Infect flowers without siliques by pouring the bacteria/medium mix into a plate and carefully setting the planter upside down so that all flowers are submersed in the mix for 5 minutes.

6. Leave in dark for 24 hours.

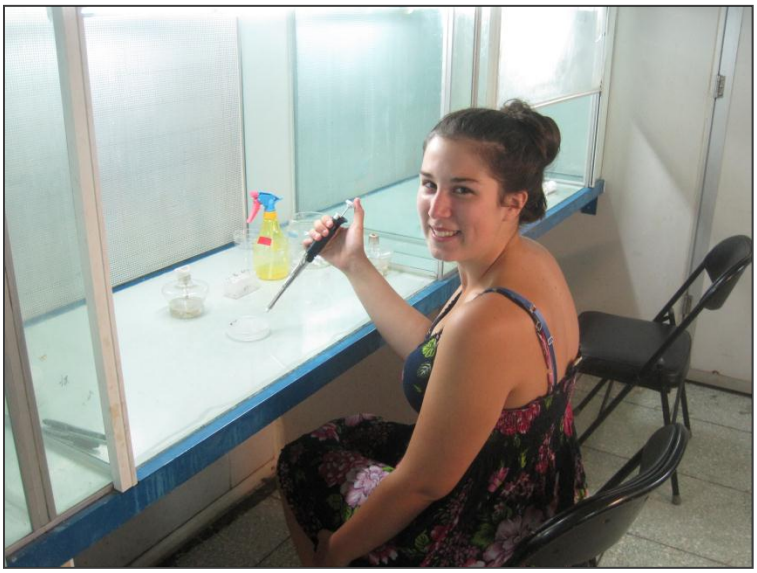
7. Collect seeds 2 weeks later and grow on plates using a previous procedure.

8. Transfer to soil and observe the phenotypes as the plants grow.

9. Look for problems with male gametophyte- the non occurrence of double fertilization.

Pollen Tube Germination:

To observe the location of the kinase proteins in the pollen tube, we can collect flowers from the plants and remove the pollen and germinate them on slides. We can then observe the pollen under the confocal microscope to see where the kinase proteins exist in the tube. Before collecting the flowers, the pollen tube germination medium must be prepared.



Above: Working in the sterilized hood, plating seeds in order to germinate them in a controlled environment.

This consists of 18% sucrose (18 g for every 100 mL), 1% boric acid, 1mM CaCl2, 1mM Ca(NO3)2, 1 mM MgSO4, and ddH2O. We needed 20 mL total, so we used:

- 3.6 g sucrose
- 0 .2 mL boric acid
- 200 microliters CaCl2
- 66 microliters Ca(NO3)2
- 20 microliters MgSO4
- 20 mL ddH2O

After preparing the medium, we collected the flowers and began the process of germinating the pollen tubes for observation, using this procedure:

1. Cut flowers from each plant (about 20) and put into tube nearly filled with the medium.
2. Vortex softly for 10 minutes to release the pollen.
3. Centrifuge at 800g for 2 minutes.
4. Remove flowers and most of supernatant, leaving about 50 microliters.
5. Drop medium onto a microscope slide and confirm pollen presence under the microscope and then keep pollen in a wet chamber.
6. Keep in 22 degrees Celsius for about 6 hours.
7. View slides under confocal microscope to see fluorescence.

The kinase proteins are tagged with a GFP fluorescence so that under the correct settings with the microscope, they will glow green on the picture, revealing their location. We expect to see the fluorescence concentrated at the tip of the tube, where the tube is experiencing growth, hypothetically controlled by these proteins. That was what was observed the first time the experiment was conducted. However, when attempting to repeat these results, we found that the fluorescence was present throughout the tube, thicker around the tubes edges- but not especially concentrated at the tip. Yingnan

proposed that there was a possible problem with the medium that caused the difference in the results. The experiment will have to be conducted again to try and replicate the original results, and find where the kinase proteins are typically located in the pollen tube.

Conclusion:

While the project I worked on at Beida did not have a direct effect on increasing global food security, the research was indirectly important in helping to create crops that can be used to alleviate hunger. The main goal of the project was to gain a deeper understanding of which proteins are involved in regulating growth of the pollen tube in Arabidopsis Thaliana. Arabidopsis itself is of no economic significance, but for reasons mentioned previously, it is useful as a model plant for many crops that are considered significant. Pollen tube growth is essential for successful fertilization. Successful fertilization is essential to high crop yields. High crop yields are essential to increasing food production and creating food security. The more scientists are able to understand about the proteins and mechanisms that control the rate of fertilization in plants, the better they are at discovering ways to increase the success rate of fertilization, and create higher yields. Should Yingnan's hypothesis be correct, and the group of kinase proteins is found to play a role in regulating pollen tube growth, scientists may be able to use this knowledge to improve the process of fertilization. The more information known about plant functions, the better off food security scientists are in their mission to create technologies that can feed the ever-increasing population.

My experience at Beida and the overall perspective I acquired on food security was different from some of the other interns. Unlike students placed in rural facilities, I

did not have the chance to see food insecurity firsthand. The people I encountered were university students who ate in the dining halls, which offered a decent array of options for very cheap prices (my average meal was about 7 yuan, barely more than a dollar!), three times a day. There are several different aspects of increasing food security, including social, political, and scientific. The social aspect would involve going out and talking to the people actually affected by a lack of nutritional food, seeing how this affects their society and ascertaining their immediate needs. Political would involve public policy and trying to create a coalition between government officials, farmers and scientists to work together to end the problem of global hunger. And then, there is the scientific aspect, where I was involved this summer. I, and most of the people in the lab, have never experienced true hunger. We may not know what it is like to harvest crops by hand, or to not have available clean water. But despite our lack of experience, we put in long hours to try and increase our collective knowledge of plant functions in order to help the people that do know what it is like to be hungry. It is my view of the scientists behind these technologies that really changed through this internship, rather than my view of food insecurity as a global issue. I still recognize the needs of millions of people worldwide to be fed and nourished, just as I did at the Global Youth Institute last year. Seeing these people firsthand may have increased my desire to help this cause, but seeing the determination of the researchers here in the lab had just as much of an impact, I believe,

Above: My labmates and me on the grassland in Inner Mongolia.

as observing the rampant under nutrition present in many rural areas around the world. The students here work seven days a week, more than 12 hours a day. That commitment to science and to increasing the global understanding of plants is admirable and downright inspiring. The World Food Prize has a large focus on increasing awareness. While my awareness of the specific problems facing those without food security may not have increased through this internship, my awareness of the sheer number of devoted people working to help those hungry people sure has, and I find that realization inspires me to devote myself to such a cause with just as much fervor as the people in the labs here in Beijing.

Above: Dr. Kang's graduate students and me on the final watchtower at the top of the Great Wall.

Not only did working in the lab have an impact on how I view my life and the world, but the experiences I had outside the lab also made an impression. I had amazing opportunities to travel around Beijing and Inner Mongolia during my internship. We traveled to Inner Mongolia on an overnight train, and we rode horses and slept in yurts in the grassland and went sand sliding in the desert. That experience showed me a different side to my lab mates and forced me to step outside my comfort zone. I was terrified of riding the horse, but I felt so accomplished afterwards. It was a good experience to see a completely different lifestyle from what I am used to, even more radically than what I experienced in Beijing. While visiting different sites in Beijing, I was shown pieces of history I never learned about in school. When climbing the Great Wall and visiting museums, I heard about the

violent history of the Chinese empires. In visiting the Lama temple, I was exposed to a different religion. In visiting the Olympic stadiums, I was able to actually set foot in the places I saw on television in 2008.

The conversations that I had with my different lab mates changed my perspective of how I view America and the personal liberties we enjoy here. Yingnan and I would often discuss politics, and I quickly realized that I had been conditioned throughout my schooling to hate communism and so I assumed that the average Chinese citizen would much rather have a democracy. However, I found that they had no problem with their government. I asked Yingnan if he votes, and he said that he is able to, but does not, because it does not really affect him. I asked if they minded not having access to Facebook or other sites, and he said no, that he understood how such sites could be used by people to "go against the country." In the United States, we often assume that everyone in the world wants what we have, and we forget to put ourselves in their position, and realize that many different systems exist. Living in China showed me how often I take my basic rights for granted. Some are rights that I don't even exercise, such as owning a gun. Someone asked me, very excitedly, if I owned a gun. He assumed all Americans did. When I replied that I had never even touched a gun, and there was not even one in my house, he was shocked. In China, the average citizen cannot legally own a gun. There are many rights that I do not even consider that many people do not have.

This entire experience has changed the way I view my own future, and the future of the world. Personally, this reaffirmed my desire to do lab research in the future, and work on discovering cures to the diseases that plague millions of people all over the world. As for the future of the world, and food security, this experience helped me

understand the importance of genetics, as the field I want to study, and how GM foods can really be a solution to feeding the world. However, agriculture alone cannot solve food insecurity. Meeting people in China showed me the consequence of local customs and beliefs. We cannot just go into a place and implement whatever solutions we come up with. We must be mindful of local traditions and cultures, because it is those unique qualities that really make a place beautiful and special. As Norman Borlaug once stated, "food is the moral right of all who are born into this world." We all must work together, whether we study biology, agriculture, engineering, or political science, to ensure that everyone has access to food. We should strive to recognize the unique differences in every culture, but remember that it is our similarities that bind us together, and we all deserve to know where our next meal is coming from.

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