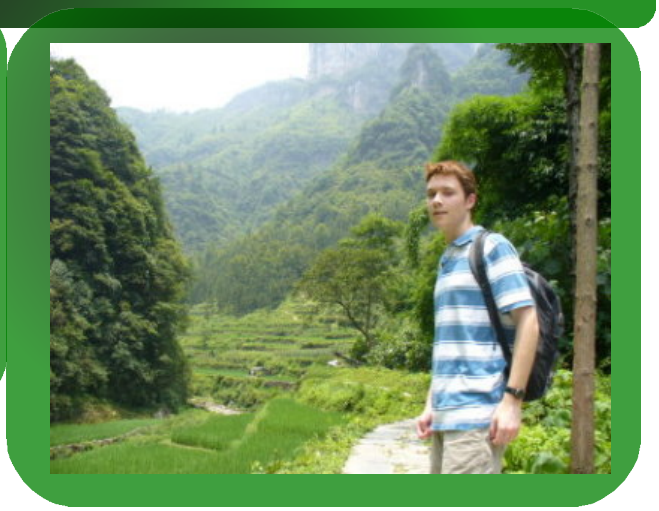
Rice on the Other Side of the World



My Journey to Changsha, China and the CNHRRDC

By Philip Day

Borlaug-Ruan Intern 2008

Introduction

My name is Philip Day. I am a citizen of Mitchell South Dakota, and I have lived there my entire life. Midwestern America is a great place, but there's more to the Earth than just it. This paper explains how I discovered that first hand by traveling to China for two months. I was a Borlaug-Ruan intern spending the summer of 2008 at the China National Hybrid Rice Research and Development Center in Changsha, Hunan. During my Journey I learned a lot, made great friends, and experienced an amazing culture.

In 2007 I attended the World Food Prize Youth Institute. I was compelled to participate by my interest in biological science and my desire to see how it could be utilized to combat the problems facing humanity. That year the paper topic was “Biofuels: Promises and Implications for Food Security in Developing Countries.” I chose to focus on the growth of the oil palm in Indonesia. Since then I have learned that the knowledge gained from researching a country is starkly different from that gained by actually visiting it.

I found it very exciting to be surrounded by information regarding new developments in agriculture and food security. The atmosphere of the World Food Prize symposium made my interest in food security and other cultures grow. Consequently, the international internship that everyone was talking about began to catch my attention. I had known about the Borlaug-Ruan internship before I attended the World Food Prize Youth Institute, but I left determined to apply.

To begin with, I wanted to experience something new. The B-R internship offered that in many ways. Firstly, I would be traveling to another country on the other side of the world. That alone would make it worthwhile. Secondly, I would be away from home for the longest amount of time I ever had.Lastly, I would be working in a lab where important and interesting work was being done. I hoped that the internship would add to the variety of the time line of my life.

I knew that spending a summer working in a biology lab at a major research center would help me when I moved on to college as I had decided I wanted to pursue a career in biology. The experience I could gain during the internship would give me a great head start. All branches of science interest me, so it was difficult for me to decide which one I would follow. The WFP Youth institute helped me see that, through biology, my interest in science could be used to improve the human condition. I saw the internship as a way for me to begin to gather the skills

and knowledge I would need to make an impact on the state of world food security.

Above all, I hoped that by becoming a Borlaug-Ruan Intern and traveling to another country would enhance my understanding of the world. I do a lot of thinking, and I decided that the new understanding I could gain from a traveling would expand my mind and make my thinking more rewarding. I wanted to see the world as it truly is; a change in perspective could help me do that. As this paper will explain, the two months I spent in China satisfied all of the above desires. My Borlaug-Ruan internship has given me an incredible life experience, and allowed me to grow as a human being.

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CNHRRDC

The China National Hybrid Rice Research and Development Center was founded in 1984, but at that time it was called the Hunan Hybrid Rice Research Center. In 1995 the CNHRRDC was established, based on the HHRRC. Over it's existence, the center has made over eighty scientific achievements including more than 40 hybrid rice strains that have gone into commercial production. The center has also received numerous awards for it's work (About the CNHRRDC).

The main building of the CNHRRDC is the scientific research building. I didn't spend much time in this building, but I know that there were many offices. The research that goes on in this building deals with the breading of new hybrid systems. This is also where the center publishes its journal.

The building that I spent most of my time in was the molecular breeding building. This is where experiments dealing directly with DNA and biotechnology took place. The molecular breeding building is fairly new, as it was built only a few years ago.

The center runs a training program that trains people from many different countries to breed and grow hybrid rice. The trainees are housed at the training center, and this is also where I stayed.

There were many other buildings within the center complex, such as apartments, an exhibition hall, and a restaurant. The center also runs many test fields near the center and around the Changsha area. In the winter months the researchers and workers go to Hainan Provence where the center maintains winter facilities.

Yuan Longping and Hybrid Rice

The father of hybrid rice and the director of the CNHRRDC is Yuan Longping. He discovered how to use hybridization of rice and began research in 1964. He developed a three line system in 1973 and it went into commercial production in 1976 (Peng). Since then, the area of hybrid rice cultivated in China has grown to 50% of the total rice area, and production has moved to different countries (Yuan 1). Yuan Longping is a national hero in China and has received numerous national and international awards for his advancements in agriculture (Peng). One of these, of course, was the World Food Prize, which he received in 2004.

The first system of hybrid rice in the three line system. This system uses a male sterile, or cms, line, a maintainer line, and a restorer line (Peng 3). The male sterile line does not produce pollen, so it uses the pollen of another line. New cms seeds are produced when the cms line crosses with the maintainer line (Peng 61). Hybrid seeds are produced by crossing the cms line with the restorer line (Peng 65). The three line systems have a yield that is on average 20% higher than inbred varieties (Yuan 1).

The next development in hybrid rice was the two line system. This was made possible by the discover of PTGMS which stands for Photoperiod-and-thermo -sensitive genetic male sterile line (Lei 25). This means that the male sterile line is only sterile in certain conditions, so it can maintain itself. Two line systems have 5% - 10% higher yields than the three line systems. In 2002 the area of two line hybrid rice was only about 18% of the hybrid rice area in China (Yuan 1). A recent development in hybrid rice is super hybrid rice which began in 1996-1997 (Lei 26). Super hybrid rice lines have a very high yield and grain quality (Yuan 1).

This is a perfect example of what hybrid rice should look like.. It has long thick sword leaves and large panicles.

Towards the end of my internship Yuan Longping gave presentation for the participants in the training program that I also attended. In the presentation he talked about the future developments of hybrid rice and how it would be integral in combating hunger and feeding the Earth's growing population. Yuan said that heterosis (hybrid vigor), biotechnology, and cultivation techniques will have to be used in conjunction to produce the rice yields necessary in the coming years.

My Mentor

My mentor throughout my internship was Xing Junjie. He is a researcher at the CNHRRDC in the molecular breeding lab. He is from a coastal province in the north of China and he came to Changsha to go to school at South Central University. He is now working on his doctorate degree at the center. During my internship I mostly worked as Xing's lab assistant. Xing also introduced me to many aspects of Chinese culture and places of interest in Hunan.

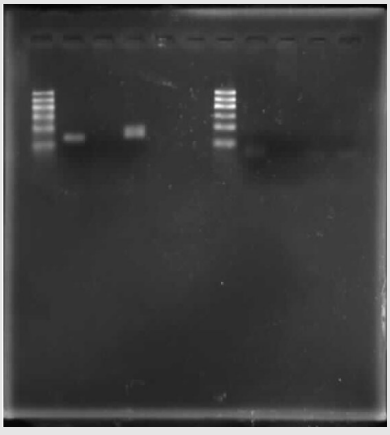
Common Lab procedures

Through out my internship at the CNHRRDC I worked as Xing Junjie's lab assistant as he did his research. During that time I aided Xing and other researchers in common lab procedures. Below I list explain these procedures, and I will refer to them when I explain the research in greater detail.

Electrophoresis

Electrophoresis was probably the most common procedure done in the lab. It was generally used to provide information about the DNA being tested such as it's length, or if it was cut properly. Electrophoresis was also used to separate different strands of DNA. Before electrophoresis could be done, an electrophoresis gel had to be made. This was a task I was assigned many times. To make a gel, I first used an electronic scale to measure out an certain amount of agrose powder. The amount depended on the size of the gel and the desired concentration of agrose. I would then mix a liquid called TAE with the agrose. To get the powder to mix with the TAE it had to be heated in a microwave. I let it cool and then poured it into the casting tray with a comb to form the pours. It would take about an hour to solidify. After it solidified it went into the electrophoresis tray with more TAE. I mixed the DNA samples with a blue loading buffer and pipetted them into the gel pours. I turned the electrophoresis machine on and it runs an electric current through the gel.

DNA is negatively charged in the TAE so it moves toward the positive end. The longer strands move slower than the shorter, due to the agrose particles in the gel. The electrophoresis generally ran for twenty minutes to an hour. Then I put the gel in EB for about 10 minutes. EB bonds with the DNA and makes it visable under ultraviolet light. To see the DNA we used a machine to shine UV light on the gel and take a picture. The result is separate bands of DNA. The bands furthest from the pores are the shortest strands of DNA, and the closest are the longer bands.



This is an electrophoresis gel. The florescent bands contain DNA.

PCR

Another Lab procedure that is closely involved with electrophoresis is PCR. PCR is a method of cloning specific segments of DNA. PCR stands for Polymerase Chain Reaction. To begin PCR, the ends of the desired DNA segment must be known to make primers. A primer is a very short segment of DNA. Another thing that is needed is dNTP's, or deoxyribonucleic triphosphates. During PCR these will become the base pairs in the newly formed DNA strand. A template strand of DNA is also added along with sterilized water and 10x PCR buffer. All components are added in small precise amounts depending on the DNA being cloned.

After the components are mixed in a small PCR test tube, the tube is placed in a PCR machine. The PCR machine subjects the tube to changes in temperature based on a program decided by the operator. DNA is denatured at high temperatures, which means that the two sides of the double helix separate. This allows the primers to attach to the template DNA strands when the temperature goes down, and the dNTP's fill in between the back and front primers. The new DNA is a copy of the opposite side of the DNA. The same thing happens to the other side of the template DNA. The copy separates from the template when the temperature goes up again. When the process continues, both the template and the clone are copied. When the PCR program is completed, the solution will be filled with copies of the desired segment of DNA.

PCR can be used to test an organism's genome for a specific gene. To do this, a sample of extracted DNA is mixed with PCR solution that contains primers for the gene being looked for. If the gene is present in the DNA, the primers with attach and the polymerase chain reaction will begin. Electrophoresis is used to test if copies were made. If a bar appears in the correct place, that indicates that the gene is present.

Electroporation

In order to multiply a gene in large quantities, it has to be spliced onto a plasmid vector placed in E coli. The process by which we moved the vector into the cell membrane of the E coli was Electroporation. Electroporation uses an electric pulse to create a temporary hole in the cell membrane, and the plasmid vectors flow in when the hole is open. We referred the the machine that provided the pulse as the electropore. The general procedure was to pipette 1uL of DNA and 20uL of E coli solution into a special capsule. The rectangular capsule has two metal electrodes

on either side and fits into the electropore. The electropore machine has preset programs depending on the capsule size. The programs run a series of pulses after a big green button is pushed. After the process, which takes a few seconds, is complete, the solution is pipetted into a test tube along with growth solution. Electroporation can also be used to insert a gene into Agrobacteria which can later be used in rice tissue culture.

E coli and Agrobacteria Growth

After the bacteria have had plasmid DNA injected into them, the solution is spread on a selective medium in a petri dish. The selective medium will only allow the bacteria with the plasmid DNA to grow. Once bacterial colonies appeared, they would be washed from the medium with LB. LB solution is a special solution for the growth of bacteria containing distiled water, tryptone, yeast extract, and NaCl. The LB solution, now filled with bacteria, would be put in a flask with more LB to grow at 37 Degrees Celsius. As the bacteria multiplied, so would the plasmid DNA. If the bacteria was E coli, then the plasmid DNA would be extracted. If it was agrobacteria, then it would be used in rice tissue culture. E coli is used to multiply DNA for extraction because it reproduces quickly, resulting in many copies of the plasmid DNA. Agrobacteria is used rice tissue culture because it is able to inject the vector DNA into plant cells.

Rice Tissue Culture

The rice tissue culture was the method used at the lab to add genes to rice plants. To begin, the callus, or embryo has to be removed from a rice kernel. This is a tedious and meticulous task because the callus is very small and hard to distinguish from other parts of the kernel. After removal, the callus is placed on an NB medium in a petri dish to grow. After about 2 days they develop into clumps resembling honey bunches of oats. The bunches are cut into smaller pieces and soaked in a solution of agrobacteria. Before hand, the gene was added to the agrobacteria with an electropore. The agrobacteria will transfer the gene into the rice tissue. The rice tissue is then removed from the solution and placed on a co-culture medium where both the rice tissue and agrobacteria can grow. The agrobacteria is washed off with water and antibacterial. The rice tissue is placed on a selection medium which will only allow the tissue

with the new gene to grow. All the remains is the transgenic tissue, and that's placed on a regeneration medium, so it can grow into rice plants.

Extracting and Isolating DNA

In order to test or use the DNA of an organism, it has to be extracted from the tissue and isolated. The details of the extraction process varied depending on what DNA was being extracted and who I was working with. For extraction of rice DNA, plant tissue had to be ground up. In most cases it was the leaves. Many times we used liquid nitrogen to make the grinding easier. An extraction solution was added before or after grinding. The extraction solution generally contained hydrochloric acid, EDTA, and salt. The solution sometimes had to be heated. A centrifuge was used to separate the liquid from the remaining pieces of leaf. The liquid, or supernatant, would be removed and mixed with cooled isopropanol. The solution was centrifuged again, this time bringing the DNA to the bottom. The rest of the solution was poured out and the DNA dried. The DNA was generally redissolved in water or another solution.

Here I'm using a a centrifuge to separate DNA from the solution it is suspended in.

The process for extracting DNA from Bacteria was very similar. The bacteria cell had to first be removed from the solution they were growing in with a centrifuge. We usually used a special kit to extract and purify the plasmid DNA from the bacteria. The kit came with buffers and instructions. The process included a lot of centrifuging and filtering.

DNA Cutting and Ligation

In most cases, a desired piece of DNA was located on a plasmid vector when it was multiplied. In order to use it in further experiments, it would have to be cut off. To do this, the correct cutting enzymes have to be found. The type of enzyme used depends on the sequence of DNA at the ends of the gene. The AVR-PTO and BD vectors were both grown on different vectors, and after cutting had to be spliced together. This is called ligation. The AVR-PTO DNA and BD vectors are mixed in a one to three ratio along with 1uL of buffer and 1uL of DNA

ligase. The solution is left over night, and the two segments of DNA are connected.

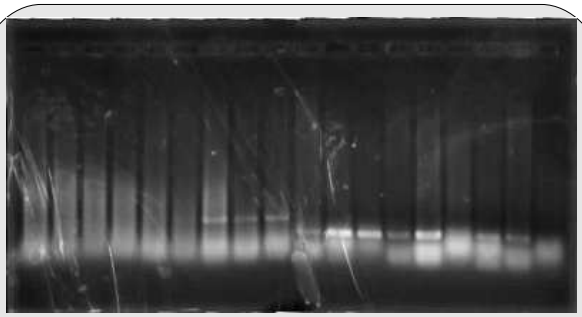
Adding the C4 Gene to Rice Research with Tan Yanning

Abstract

I was only able to participate in Tan Yanning's work for a week, so I did not witness most of it first hand. Tan had a graduate student named Gou assisting him. Tan's project involved the photosynthesis pathways in rice. Rice's pathway is called C3, while maize's is C4. The C4 pathway is more efficient than the C3, so it would be beneficial for the rice to also have it. The portion of the project that I was involved with dealt with testing trangenic rice for the C4 gene.

Background

Prior to the segment of the project that I witnessed, the C4 gene from maize was inserted into rice tissue using a rice tissue culture. The gene was inserted along with a h gene on a super-binary vector. The h gene makes the rice tissue resistant to an antibiotic, and allows it to grow on a selective medium. This insures that only the transgenic rice will survive. Before the rice tissue is transgenic, it is the T_0 generation. Once the new genes are added, it is the T_1 generation. The super-binary vector allows the two genes, C4 and h, to separate onto different sides of the chromosomes in the T_1 generation. The seeds produced by the T_1 generation were planted and grown into the T_2 generation. Each plant in this generation has the potential to have the C4 or h gene on both sides of the chromosome or a combination. The desired plants have a C4 gene on each side of the Chromosome and no h gene. This is what Tan was testing for when I worked with him.



The first 9 lines are individual samples corresponding to the last 9. The thin bands indicate that The C4 gene is present in the first 9 and that the H gene is present in the second 9.

Procedures

To begin with, we had to collect samples from the T_2 plants. They were growing in a small field near the lab. Each plant was labeled, and we walked through the mud cutting leaves and putting them in the test tube with the same label as the plant. We extracted the DNA by

grinding the leaves in individual mortars with extraction buffer. After the DNA was extracted, each sample was placed in two PCR test tubes. One tube had primers for the C4 gene, another for the h gene. In this way, if either the h gene or C4 gene were present in one sample's DNA, it would be multiplied during PCR. To test if the gene was multiplied, the PCR solutions were run through electrophoresis. If a band appeared in the correct location, that indicated that either the C4 or h gene existed in the sample's genome.

How this will affect food security

The C4 photosynthesis pathway of corn and other crops generates far more energy from sunlight than the C3 pathway of rice. Rice only needs a certain amount of energy for plant growth. Rice with the C4 gene could use the C4 pathway to produce a surplus of energy. That energy would be added to the rice kernels. The kernels would be much larger. This rice will have a much larger panicle weight and yield, meaning it will produce more food than other rice.

Finding the Gene for Resistance to Pseudomonas Syringae in Wild Rice Research with Xing Junjie

Abstract

For the majority of my internship I worked with Xing Junjie on his research. This research involved an effector protein called AVR-PTO which is used by a group of pathogenic bacteria (Pseudomonas Syringae) to enhance susceptibility in host plants. Some plants, such as tomatoes have resistance to the disease caused by these pathogenic bacteria. The gene in the tomato plant that provides resistance is named PTO. If wild rice is able to survive in the wild, it likely has resistance to the pathogenic bacteria that makes use of the AVR-PTO effector, Pseudomonas Syringae. And if this is true, then it must have a gene similar to PTO to convey this resistance. The purpose of this experiment is to find the resistance gene on the DNA of wild rice and confirm that wild rice has this gene. Domestic rice, and subsequently, hybrid rice does not have this resistance. Confirming that the resistance gene is in the genome of wild rice could someday allow that resistance to be transferred to the hybrid rice. I was only able to participate in a portion of this research, as it took longer than two months.

Lab Procedures

Among the first tasks I took part in was the mixing of solutions. These were LB solutions that would be used in the growth of E coli. We poured some of this with agar mixed in into petri dishes to make a solid growing medium. After the growing mediums were ready, we used electroporation to insert plasmid DNA connected to AVR-PTO genes into some E coli. We also did this with BD vectors, which would be spliced with the AVR-PTO later in the experiment. After electroporation we grew the E coli on and in the LB medium. An Antibiotic called kanamycin made the LB selective. Part of the DNA on the plasmids that the AVR-PTO and BD vectors were connected to allowed bacteria to grow in this selective medium. After the E coli had time to grow and the plasmids had time to multiply, we extracted the plasmid DNA from the E coli solutions.

At this point, the plasmids that the two genes were connected to had served their purposes, and needed to be removed. We used the enzymes Nde I and Xho I to cut the AVR-PTO from it's plasmid, and Nde I and Sal I for the BD vector. We used electrophoresis to confirm that the cutting worked. After that we did electrophoresis again, but this time with a larger amount of DNA. The electrophoresis separated the shorter segments of DNA (the AVR-PTO and BD vector) from the longer plasmid segments. After the electrophoresis was ran, we cut the sections of agrose gel that contained the bands formed by the AVR-PTO and BD vector from the rest of the gel. We extracted the DNA from the gel and ended up with test tubes containing AVR-PTO and BD vector. Now came the time when the AVR-PTO had to be spliced together. To do this we used ligation. This is where I stopped working with Xing.

Experiment Conclusion

All the things done so far were working towards a point where results and information could be collected. There was however still more to be done before that point was reached. The same processes used on the AVR-PTO and BD vector needed to be used to get segments of wild rice DNA connected to an AD vector. The wild rice DNA would be taken from a complimentary DNA library. Different segments of cDNA will be tested. The AVR-PTO spliced on the BD vector and the cDNA spliced on the AD vector will be inserted into yeast cells. If the cDNA segment being tested is a gene similar to PTO which is the gene that gives tomatoes resistance to

Pseudomonas Syringae, then the AD and BD will connect. The AD and BD vectors are designed to produce a protein when connected. This new protein will allow the yeast to grow on a special selective medium, so the appearance of yeast colonies will confirm that the cDNA being tested is a resistance gene.

How this will affect food security

The variations in the genome of domesticated rice in China is beginning to be used up, so a new source of genetic variation must be used. The genetic variation in wild rice is much larger than domestic rice, and wild rice has many traits that could be used to improve domesticated rice. Before the genome of wild rice can be utilized, it must first be studied. Xing's experiment is part of that study. He is finding the genetic basis of a trait of wild rice that would benefit domesticated rice. This experiment is adding to the pool of knowledge necessary for the continued improvement of hybrid rice in China.

My Experience

Geography and Nature

Earth is huge. I have known this most of my life, but until recently, I did not understand it completely. The earth is also a sphere, but for most of my life that was just an arbitrary fact with no bearing on my every day life. Before the summer of 2008, for all I knew, the earth could have been a small flat piece of rock under the sky. Spending the summer as a Borlaug-Ruan intern at the CNHRRDC in Changsha China allowed me to begin to comprehend the size and shape of this planet.

My thirteen hour flight was about two thirds complete when people started to open the windows of the plane. I was tired, sick, and feeling a little claustrophobic. When I opened my window, I forgot all of that. The plane was somewhere over the Arctic, and as far as the eye could see there was ice. I had seen this ice plenty of times on television and the Internet, but seeing it first hand was far more exciting. It was then, when I was basking in the white glow of light coming off the endless sheets of ice, that I realized the magnitude of the journey I was taking.

Eventually the windows started to be closed, and I closed mine in courtesy to the other

passengers. They didn't open again until the plane was flying over China near Beijing. At this point I got my first glimpse of life on the other side of the globe. The plane landed more than an hour late, and I had some trouble navigating the Beijing airport terminal three, so I missed my flight to Changsha. I stayed in a hotel in Beijing and took a flight the next day. I met Xing Junjie and Li Li at the airport and took an interesting car ride to the center. There seemed to be very few traffic laws. I eventually got used to the driving, but this first ride was quite exciting. The center is a very beautiful place with scores of trees and plants, and ponds that had just been added during the past year.

Coming from South Dakota, the landscapes and environments I am used to are the flat prairies covered in cornfields or grass. This is in extreme contrast to the land of Hunan. Changsha itself is a valley surrounded by hills. The rest of the area is innumerable small hills covered in bamboo. The first time I was fully immersed in the Hunan Landscape was when I visited a small national park near Changsha. The park surrounded a small river running between the hills. The River started in the mountains and made its way down in many waterfalls. That trip was early on in my stay. Later on I visited a much larger national park with much higher hills. In the middle of this park was a stunning gigantic waterfall. These excursions familiarized me with the beautiful environment of Hunan, and helped me understand the variety of landscapes in the world.

Culture

My stay in China has also expanded my cultural horizons. I was fully immersed in the Chinese culture, and I also got glimpses of other cultures from the other people staying at the training center. This has caused me to see world events from a different perspective and understand that what happens in one country affects others. On one occasion I had a conversation with some people from Africa about the US presidential election. I had not realized that this election would concern people outside of America as much as it did, but it makes sense that it does. Knowing how far reaching the influence of the president is will certainly effect my voting decision in November.

Living in another culture gave me the chance to try new things. When it came to eating, this was definitely true. First of all, I had to learn how to use chop sticks. I knew the basic

technique, but the first meals were still very awkward. I ate most meals with the other foreigners in the training center, so there was silverware then, but whenever I got the chance I used chopsticks. By the end of my internship I had become quite proficient with them. As for the food itself, I know now that the food we call Chinese in America only slightly resembles real Chinese food (in Hunan at least).

My internship also gave a me a taste of he rich history of China. One of the first historical things I witnessed was the Dragon Boat Festival. This festival commemorates a famous poet and politician from before China was united, that walked into a river because he was unable to remove corrupt officials from his country's government. The people celebrate the festival by having dragon boat races and making a food called Zhongzi. Dragon boats are very long boats with dragon heads at the bow. Zhongzi is made of rice, meat, and peanuts wrapped in long leaves. They eat them and also throw them into the river out of respect for the politician.

From my visit to China I can see that just as China's history is rich, so will its future be. The economic growth was evident in all the construction. There were new buildings popping up and renovation on most city blocks in Changsha. Another thing that I saw on almost any city block was an Olympic reference. Everyone I knew in China was extremely excited about the Olympics, and rightfully so. As I am sure anyone who saw the opening and closing ceremonies would agree, the Beijing Olympics show that China will be taking an increasingly important role on the world stage.

My cultural perspective has shifted. I am no longer tethered to my Western point of view. This will probably be the most important and long lasting effect my Borlaug-Ruan internship has had on me. The experience has made my view of humanity clearer and has provoked me to take a greater interest in all cultures around the globe.

Science and World Food Security

Gaining a better understanding of geography and culture were great side effects of my internship, but the main focus was science and world food security. There were so many projects going on at the center, all of them working on improving rice. The researchers used various breeding methods such as conventional breeding in the development of hybrid parent lines and molecular breeding. Whatever the method, the CNHRRDC is continuing to find ways to increase

the value of rice as a food crop. Being around all of this innovation has directly shown me the potential of science for combating world hunger. Faced with a growing population, science is finding solution and improving world food security.

Acknoledgements

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