

Na Cho Z*... “That It Is!”

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CNHRDC: Internship for Further Education on Hybrid Rice **by Hannah Cox**

Abstract

Hybrid rice (HR) technology is one of the most important innovations of all time. Since over 50% of all rice produced in China alone is HR, developing new techniques to create higher yielding and more disease resistant rice is vital. Currently many experiments are being undertaken to achieve this goal.

The purpose of HR experiments are to: (1) determine if a new transgenic variety of HR is resistant to bacterial blight using a DNA extraction method, PCR, and electrophoresis, (2) determine the purpose of the PC1301 gene in HR using the culturing of e-coli to quickly multiply DNA, and agro bacterium to transfer the PC1301 gene into rice, (3) determine what proteins are expressed during different stages of embryogenesis in rice by isolating embryos from rice and extracting proteins from the embryos at different stages, (4) determine if the C4 gene has been transferred into rice without the hyp gene using DNA extraction, PCR, and electrophoresis, and (5) determine if there is a genetic difference between three varieties of HR using DNA extraction, PCR, SSR, and electrophoresis.

The hypotheses were: (1) the transgenic variety of HR is resistant to bacterial blight, (2) the PC1301 gene creates some resistance to common rice diseases, (3) the proteins are expressed differently at stages of embryogenesis, (4) the C4 gene can be transferred into rice without the hyp gene, and (5) that there would be a genetic difference between the three types of HR.

The reasons for these experiments are as follows: (1) bacterial blight is a common rice disease that has been reported to have reduced Asia's annual production of rice by up to 60%. Transgenic rice resistant to bacterial blight is the most effective way to manage this disease. (2) Knowing the purpose of a gene in rice is the first step in utilizing the gene to its full potential. For this reason, many genes in rice over the past few years have been identified. (3) Embryogenesis is a plant reproductive process that results into a mature embryo. Finding out, which proteins are expressed during embryogenesis, is important to understand, which proteins are key to embryogenesis. (4) The C4 gene in plants improves photosynthetic abilities, transferring this gene into rice will help further increase yield as well as improve the health of rice. The hyp gene is also normally transferred along with the C4 gene, but the hyp gene is contributed to having negative effects to rice and humans, so a new process was developed to only transfer the C4 gene. (5) There must be a genetic difference between varieties of HR or the types of HR can not be commercialized.

So far, the first experiment was not successful. The new transgenic DNA was too degraded to determine if the gene was truly transferred. In the second experiment, the e-coli were cultured and the DNA was transferred to agrobacterium. The rice plants were infected with the e-coli. Over time, the effects of the PC1301 gene on rice will be determined. In the third experiment, the initial steps have been started: recording of flowering (pollination) and embryo extraction on specific days. In the near future, the proteins will be extracted and two-dimensional electrophoresis will be conducted. Then

the samples of proteins will be identified through mass spectrometry. 300 samples of transgenic rice leaves are sampled and tested daily for the hyp and C4 gene, but conclusions cannot be drawn yet from the fourth experiment. Therefore, more testing must be done. The fourth experiment has confirmed a genetic difference between two of the varieties. Further testing must be conducted to confirm these results and test if there is a difference between the third varieties.

The anticipated conclusions of these studies concur with the hypotheses, but time will show, if the hypotheses were correct. Because rice is the staple food for Asia, HR technology is constantly being improved and revised. The CNHRRDC (China National Hybrid Rice Research and Development Center) has a mission to improve world food security by providing knowledge for China, Asia, and the world to grow rice to the best of its abilities.

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Lastly, I would like to thank to my family for their support and for not protecting me so much that I would miss the best experience of my life.

Introduction

In the summer of 2006, I went on a mission's trip with my church to Honduras. This was the beginning of the unusual journey that led me to the World Food Prize Youth Institute.

When I went on the trip to Honduras, my whole worldview changed from a small town girl with small town views to a small town girl with global views. I told my Agriculture teacher that I was interested in learning about agriculture in other countries and their agricultural security issues. My Agriculture teacher later attended a seminar that had a session taught by Mrs. Micki Zartman. Mrs. Zartman told many Agriculture teachers across the state about the World Food Prize (WFP) Youth Institute Program and how Ohio was connected. I was deeply interested in this program, but I had to complete a research paper in less than two weeks from when my Agriculture teacher informed me about it! The paper was time consuming but, came together and was totally worth the effort, because I got to express my worldview ideas in a form, where others with similar interests would read it. I completed my paper on the educational issues in Honduras. I went on to compete at the Ohio State University for a space as one of the six delegates from Ohio to represent Ohio, my home state, and present my paper in Des Moines, Iowa to a panel of scientists interested in my point of view!

The 2006 WFP Youth Institute was one of the best highlights of my life. I used this experience to become actively involved in giving briefings to other area schools with an emphasis to inform them about the WFP, the organization, and to change the world overall. Everywhere I traveled; I met new people, and made new friends with people that live far away from my rural home. Also, I saw how important it is to help other countries achieve their respective food security. After my participation in the youth institute, I had the opportunity to apply for a Borlaug-Ruan internship. There are thirteen internships awarded annually. I had dreamed of opportunity to go somewhere unique and exotic to study. Although this internship sounded surreal, there was nothing to lose except my hard work and effort. A few months after I had applied, I was informed that I was selected as one of the thirteen 2007 interns to go to other countries.

I was chosen to go to the China National Hybrid Rice Research and Development Center (CNHRRDC) in Changsha, Hunan Province, China.

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The CNHRRDC

I spent my internship at the China National Hybrid Rice Research and Development Center in Mapoling, Furong District, Changsha, Hunan Province, China. The center was founded in 1984 and is the first research institute to specialize in hybrid rice in the world. The goal of distribution of HR throughout the world is set in motion. Professor Yuan Longping is the Chief Director of the center and is commonly known as the “Father of Hybrid Rice.” The center’s 220 staff members focus on many divisions of hybrid rice production: scientific research, technology development, international cooperation and development, administration, personnel and finance, and human resources. Scientists research and develop new varieties of HR and perform a wide variety of research including: hybrid rice breeding, molecular breeding, agronomy, lab testing, field management, and observational journaling. The CNHRRDC is involved in the molecular breeding of hybrid rice. They are currently developing new combinations of three-line Super HR, crossbreeding between two line varieties and subspecies and exploring the applications of exotic QTLs (Quantitative Trait Loci found in wild specie of rice) in the modification and improvement of HR varieties. Also, the CNHRRDC is engaged in sharing information, research, and technical training of HR with both domestic and foreign institutions.

Goals for HR were set by Professor Yuan in 1996 called the Super Hybrid Rice Research Project: First stage Target - by the year 2000, the average production should be at or above 700 kg per mu (4200 kg per acre). Second Stage Target - by the year 2005, the average production should be at or above 800 kg per mu (4800 kg per hectare), and the Third stage target - by 2010, the average production should be at 100 kg per hectare for each day in the ground. The CNHRRDC is an important research, training, and development base for HR in the world.



Figure 1: The CNHRRDC office building

Programs I Worked With

I was given the opportunity to work on 5 different experiments after an introductory period of learning about HR.

These experiments were: (1) determining if a new transgenic variety of HR is resistant to bacterial blight, (2) determining the purpose of the PC1301 gene in HR, (3) determining what proteins are expressed during different stages of embryogenesis in rice, (4) determining if the C4 gene can be transferred into rice without the hyp gene, and (5) determining if there is a genetic difference between three varieties of HR.

Bacterial blight is a common rice disease that has been reported to have reduced Asia's annual production of rice by up to 60%. Transgenic rice, resistant to bacterial blight, is the most effective way to manage this disease.

Bacterial blight is one of the most serious diseases of rice and is known worldwide. It is common in both tropical and temperate countries. Types in tropical areas are more severe than that of in the temperate region. Yield loss due to this disease depends on the plant growth stages at which the rice plants become infected. The earlier the disease occurs, the higher the yield loss. Infection at booting stages (final stages) does not affect yield, but results in poor quality and a high proportion of broken kernels. Bacterial blight is reported to have reduced Asia's annual rice production by as much as 60%. For example, in Japan, about 300,000 to 400,000 hectares of rice were affected by the disease in recent years. There was 20% to 50% yield losses reported in severely infected fields. In Indonesia, losses were higher than those reported in Japan. In India, millions of hectares were severely infected; causing yield losses from 6% to 60%. The use of blight resistant varieties is the most effective and the most common management practices adopted by farmers in the fastest growing countries in Asia. When different strains of bacteria are present, it is recommended to grow resistant varieties possessing field resistant genes.

My mentor, LiLi, had developed a variety of HR resistant to bacterial blight. My job was to find whether this variety was truly resistant using DNA extraction and electrophoresis.

Protocol for DNA Extraction and Electrophoresis:

1. Harvest leaf tissue from rice plants by cutting several leaves into pieces and placing them into a mortar.
2. Add liquid nitrogen to the mortar to freeze the tissue.
3. Cut the leaves into small pieces and crush the leaf tissue by grinding the pestle into the mortar in a circular motion. Continue grinding for a minute or two, so that the tissue becomes a powder.
4. Transfer the powder into a 50 ml plastic tube.
5. Add 7 ml DNA extraction buffer (heated to 65 C).
6. Incubate tube in the 65 C water bath to shake gently for 15-20 min.
7. Add 7 ml of chloroform; shake very gently at RT for 15 min.
8. Centrifuge at 7500 rpm for 25 min. at 4C.

9. Transfer off supernatant carefully into new tube.
10. Dispose of remaining pellet into waste.
11. Add 6 ul of isopropanol alcohol to the supernatant and shake gently. You should be able to see the DNA.
12. Let the tube stand for 20 min. at RT.
13. Centrifuge at 5000 rpm for 15 min at 4C.
14. Pour off supernatant carefully into beaker and let the tube air dry.
15. Add 2 ml of 70% ethanol to wash pellet, be careful not to dissolve the pellet.
16. Centrifuge at 5000 rpm for 5 min at 4C.
17. Pour off ethanol carefully and tip tube upside down to dry.
18. Add approximately 100 ul of TE and dissolve pellet.
19. Prepare gel with 0.2 g of gel agar powder and 45 ml CTAB microwaved for 2 min.
20. Pour gel into form with comb prepared and let it set for 15 min.
21. While gel is setting, prepare DNA by adding approximately 3ul of blue dye.
22. When gel is set, remove gel from form and comb.
23. Add approximately 5 ul of DNA to each hole made by comb.
24. Add primer to one side to compare results.
25. Run electrophoresis at 100v.
26. After 30 min, remove gel from electrophoresis.
27. Then set in EB solution for 10 min.
28. Remove from EB solution, and place under special ultraviolet camera, and examine results.

I worked with this experiment for one and one-half weeks. Each day, upon examination, the DNA was too degraded to draw conclusions. Other variables affecting the quality of DNA could have had an effect on the results.

Knowing the purpose of a gene in rice is the first step in utilizing the gene to its full potential. A gene is any given segment in DNA that encodes instructions that allow a cell to produce a specific product - typically, a protein such as an enzyme that initiates one specific action. To know the purpose of a gene is to know whether it is truly needed for HR cultivation. Genes contain regulatory regions dictating under what conditions this product is made, transcribed regions dictating the sequence of the RNA product and/or

other functional sequence regions. For this reason, many genes in rice over the past few years have been identified. If the PC1301 gene is positive to rice, such as creating resistance to common diseases, there is good reason for the gene, but if there are negative attributes to the health of HR, then it would be beneficial to remove this gene. My job in this experiment was to prepare the DNA, not containing the PC1301 gene, by large scale boiling lysis with e-coli. This multiplies the DNA quickly, so that it can be transferred to agrobacterium. The agrobacterium contains the Ti plasmid that allows the DNA in the agrobacterium to transfer to rice.



Figure 2: Centrifuging DNA

Preparation of DNA by Large-Scale Boiling Lysis

Plasmid DNA is isolated from large scale (500 ml) bacterial cultures for treatment with Triton X-100 and lysozyme followed by heating. This method is not recommended for preparing plasmid DNA from strains of e-coli that express endonuclease A.

1. Inoculate 30 ml of rich medium containing the appropriate antibiotic either with a single colony of transformed bacteria, or with 0.1-1.0 ml of a small scale liquid culture grown from a single colony.
2. Incubate the culture at the appropriate T with vigorous shaking (250 cycles a minute on a rotary shaker) until the bacteria reach the late log phase of growth.
3. Inoculate 500 ml of medium (prewarmed to 37C) containing the appropriate antibiotic in a 2l flask with 25 ml of the late log phase culture. Incubate the culture for 2.5h at 37C with vigorous shaking.
4. Add 2.5 ml of 34 mg/ml chloramphenicolin. The final concentration of chloramphenicolin in the culture should be 170 ug/ml. Incubate the culture for 12-16h at 37C with vigorous shaking.
5. Remove an aliquot (1-2 ml) of bacterial culture to a fresh microfuge tube and store at 4C. Harvest remainder of the bacterial cells from the 500ml culture by centrifugation at 4100rpm for 15 min at 4C. Discard the supernatant. Stand the open centrifuge bottle in an inverted position to allow all of the supernatant drain away.
6. Resuspend the bacterial pellet in 200 ml of ice cold STE. Collect the bacterial cells by centrifugation as described in step 5. Store the pellet of bacteria in the centrifuge bottle at -20C.
7. Prepare the plasmid DNA from the 1-2 ml aliquot of bacteria set aside by the mini preparation protocol. Analyze the mini preparation plasmid DNA by digestion with the appropriate restriction enzyme to ensure that the correct plasmid has been propagated in the large scale culture.
8. Allow the frozen bacterial cell pellet from step 6 to thaw for 5-10 min at RT. Resuspend the pellet in 10 ml Erlenmeyer flask.
9. Add 1 ml of a freshly prepared solution of 10 mg/ml lysozyme.
10. Use a clamp to hold the Erlenmeyer flask over the open flame of a Bunsen burner until the liquid just starts to boil. Shake the flask constantly during the heating procedure.
11. Immediately immerse the bottom half of the flask in a large beaker of boiling water. Hold the flask in the boiling water for exactly 40 sec.
12. Cool the flask in ice-cold water for 5 min.
13. Transfer the viscous contents of the flask to an ultra centrifuge tube. Centrifuge the lysate at 30000 rpm for 30 min at 4C.
14. Transfer as much of the supernatant as possible to a new tube. Discard the viscous liquid remaining in the centrifuge tube.
15. If the supernatant contains visible strings of genomic chromatin or flocculent precipitate of proteins, filter through 4 ply gauze before proceeding.
16. Measure the volume of the supernatant. Transfer the supernatant, together with the 0.6 volume of isopropanol alcohol, to a fresh centrifuge tube. Store the tube for 10 min at RT after mixing.
17. Recover the precipitated nucleic acids by centrifugation at 10000 rpm for 15 min at RT.

18. Decant the supernatant carefully, and invert the open tube on a paper towel to allow the last drops of supernatant to drain away. Rinse the pellet and the walls of the tube with 70% ethanol at RT. Drain off the ethanol and use a Pasteur pipette attached to a vacuum line to remove any beads of liquid that adhere to the walls of the tube. Place the inverted, open tube on a pad of paper towels for a few min. at RT. The pellet should still be damp.
19. Dissolve the pellet of nucleic acid in TE.
20. Purify the crude plasmid DNA either by chromatography on commercial resins, precipitation with polyethylene glycol, or equilibrium centrifugation in CsCl-ethidium bromide gradients.
21. Check the structure of the plasmid by restriction enzyme digestion followed by gel electrophoresis.

Embryogenesis is a plant reproductive process that results into a mature embryo. Finding out which proteins are expressed during embryogenesis is important to understand what proteins are key to embryogenesis.

We had recorded the daily progression of maturation of the seedlings. When the seeds had pollinated, that meant they would soon be ready for embryo extraction. Five days after pollination, we began to extract the embryos. Soon we found it was not feasible to extract the DNA and proteins from something so small, because 100 embryos at that stage were not even 1ug. Also there were variables unaccounted for: there are three maturity types in rice - early maturity, normal maturity, and late maturity. There is also a different maturity date, when ratooning rice. These variables were found too late in the season, so the scientist will have to begin anew in the fall.

The C4 gene in plants improves its photosynthetic abilities. Transferring this gene into rice will help further increase yield as well as improve the health of rice. The hyp gene is also normally transferred along with the C4 gene, but the hyp gene is known to contribute to the negative effects to rice and humans, so a new process was developed to only transfer the C4 gene.

There must be a genetic difference between varieties of HR, or the types of HR cannot be commercialized due to patenting laws.



Figure 3: Examining and extracting embryos under a microscope

Goals or Missions of the Program

The goals of my trip were to introduce me to the Chinese culture, the Hunan food, and to gain an extensive knowledge of one of the most important crops in the world, HR.

Whom I Worked With

Miss Li Li was my mentor and companion throughout my time at the CNHRRDC. She graduated from the Hunan Agricultural College with her Masters degree in 2005. She now is an employee of the CNHRRDC studying in the lab. Lili, my mentor, took the time to teach me how to extract DNA from the rice plant, and on weekends took me on trips to show me about the Hunan, and Chinese culture.

Xin helped me adjust positively to Chinese culture, along with Li Li. He also let me help him on some of his experiments. Xin is employed by the CNHRRDC as well, but he is also a student at the Hunan Agriculture College for his doctorate degree.

Tan graduated from the Hunan Agricultural College with his Masters degree in 2006. He is an employee at the CNHRRDC, where he is studying the C4 gene in rice.

Professor Yuan is an extremely famous man in China. The three-line hybrid rice system was invented in 1973 and then production of hybrid rice began in 1974. He founded the Hunan Hybrid Rice Research Center (HHRRC) in 1984 in the Mapoling, Furong District of the city of Changsha. He has led the HHRRC to 83 scientific prizes, 12 which are state-level prizes and more than 40 are provincial or ministerial prizes. Hybrid rice has become the fifth invention after the “four great inventions” in China and is a monument in the history of science around the world. Professor Yuan’s expertise in rice production has not only brought China into the next generation with food security, but he has the desire to help the world in a similar manner. He speaks fluent English and Russian. He loves to play volleyball.

Professor Peng is not only an employee of the CNHRRDC, but he is also a resource person for the International Rice Research Institute (IRRI). He loves to joke while playing volleyball. He and Professor Yuan are very good friends.



Figure 4: Professor Yuan and me with my provided certification of achievement

Professor Ma is one of the centers' most experienced HR field scientists. He works often with government officials concerning economic issues and new varieties of HR. He also has a very sweet daughter.

Professor Zhao is a prominent geneticist with emphasis on plants. He showed me how to create transgenic rice. He likes to swim often and works at another molecular genetics lab.

Experimental Responsibilities and Contributions

I had a 7 hour workday each weekday, where I either learned about HR or was involved in experiments with HR. I normally worked from 8 -12 a.m., then from 3-6 p.m. The time in between was to eat lunch and have a nap, if desired. Sometimes, I worked longer hours, if there was an experiment in progress.

Presentations from Professors

The first two weeks consisted of an informational training course, where professors, who specialize in specific areas, taught me about their research and developments. This was the most I had ever learned about HR.

I first learned from Professor Peng about the cultivation of HR outside of China. He showed me how China's government helps other countries learn how to cultivate their own HR through the sharing of technologies and knowledge. Currently, in Vietnam, Myanmar, the Philippines, Bangladesh, and Thailand, these countries have benefited economically from China's hybrid rice varieties. Also, China is now sharing experience with many African nations. Professor Peng showed me his work in African Guinea, where he educated the native people how to grow their own hybrid rice. It was so interesting and exciting that the CNHRRDC tries to help all nations, not just China.

Professor Ma gave me the opportunity to accompany him to the Super Hybrid rice test plot. This is where the seeds developed to have super high yields are tested to be sure that they are marketable. He also taught me about hybrid rice cultivation and technology. Prior to his teachings, I did not know how three line HR and two line HR were developed. Professor Ma showed me the effects of bacterial blight on rice plants, and he also taught me the differences in rice nurseries. There are: wet nurseries, upland nurseries, and tray nurseries, which are the most common. About 15



Figure 5: Transplanting Hybrid Rice

days after germination, the rice plants should be transplanted into a new area. The optimum area for rice growth is a 10x10 cm area. The rice is divided into scheme (as pictured above).

Professor Liao taught me about the economical benefits of HR inside of China. Before HR, there was sparse to no food security. China, also, was not self sufficient. Because of the higher yields of HR, China became self sufficient, and was also able to export surplus rice. For the first time in many years, China was self sufficient, and so they now have food security.

Professor Zhao taught me about transgenic hybrid rice. He demonstrated two different methods of transformation. The first method is a transformation using agrobacterium with calli and the second method is the spike-stalk injection. The first method is more tedious, but has more immediate results. The second method is very easy, but only 1/1000 seedlings actually carry the transgene.

Also, visiting professors gave interesting and inspiring presentations that I was able to attend. I was invited to attend decision making meetings at the center that are altering the course of CNHRRDC research.

Professor Tanksley from Cornell University visited to explain about exotic QTLs. I also attended a lecture from another professor from the IRRI, who came to talk about how to correctly compose a research paper about HR.

Extraction of DNA

Many times my job for the day included extracting DNA for experiments. DNA extraction must be used for the Polymerase Chain Reaction (PCR) program and also to use Simple Sequence Repeat (SSR) markers. Many times I would help Professor Tan to extract DNA for his experiment, if I had any free time. He was trying to find if the C4 gene could be successfully inserted into HR without the hyp gene. He performed this process for 300-400 samples a day.

DNA extraction is a relatively easy way to get real results quickly, but it seemed very difficult to get a perfectly banded gel. Many experiments we worked on did not get satisfactory results, while I was in China, because the degraded DNA caused a blurry gel.

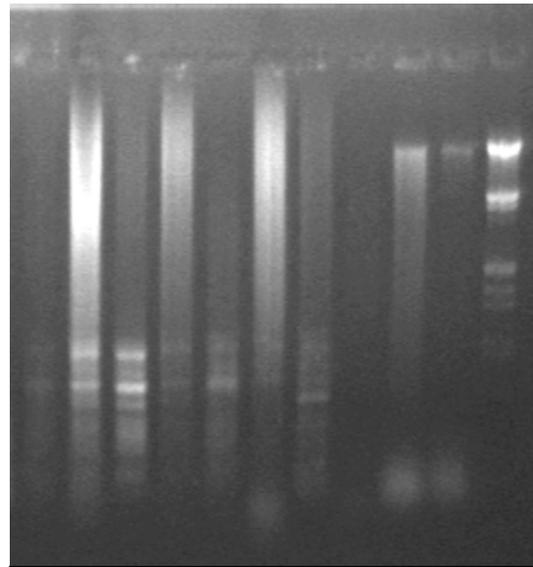


Figure 6: Extracted DNA with smeared results - inconclusive

PCR Program

PCR stands for Polymerase Chain Reaction is essentially a tool to amplify DNA. This is used in genetic research. What we call the “PCR machine” is actually a thermocycler that pulls DNA sequences apart and back together many times by rapidly heating and cooling the DNA in a tube.

The starting material for PCR, the 'target sequence,' is a gene or segment of DNA. In a matter of hours, this target sequence can be amplified a million fold. The complementary strands of a double-stranded molecule of DNA are separated by heating. Two small pieces of synthetic DNA, each complementing a specific sequence at one end of the target sequence, serve as primers. Each primer binds to its complementary sequence. Polymerases start at each primer and copy the sequence of that strand. Within a short time, exact replicas of the target sequence have been produced. In subsequent cycles, double-stranded molecules of both the original DNA and the replicated copies are separated; primers bind again to complementary sequences and the polymerase replicates them. At the end of many cycles, the pool is greatly enriched in the small pieces of DNA matter that have the target sequences



Figure7: Running the PCR machine

and this amplified genetic information is then available for further analysis. I was responsible for creating a PCR program for finding genetic differences between HR and for determining whether the C4 gene was successfully transferred without the hyp gene.

SSR Markers

SSR, stands for simple sequence repeat, markers is a set of DNA primers used for identifying common sets of simple sequences in short strands of DNA. I learned how to make the SSR markers and how to use it to find a genetic difference between three varieties of HR.



Figure 8: Preparing SSR Markers

Embryo Extraction

I was requested to find an efficient way to extract embryos. Three methods were found with differing levels of success. I was able to quickly extract embryos by holding the seedling in one pair of tweezers and squeezing with another pair of tweezers. This method was not very accurate and was time consuming. The second method consisted of extracting the embryo from under a microscope; this method was accurate, but again was not quick. The third method was performed by cutting the embryo out; this method was neither fast nor accurate.

DNA Transformation with Calli

The calli, used for DNA transformation, consisted of broken rice embryos (with maturity of about 15 days after pollination). These are grown in agar Petri dish molds for 5 days, and then cut again. The dark pieces of callus are then thrown away, while the white-yellow pieces are kept to be regrown. These saved samples are then transferred to new agar Petri dish molds to be grown for another length of time. When the calli are approximately 5mm in diameter, the calli are transferred to a flask, where they are soaked in agrobacterium. This creates a DNA transformation. I was responsible for each of these steps over a two week period of time.



Figure 9: Calli prepared in a Petri dish

Agrobacterium

Agrobacterium is a type of bacterium that contains the Ti plasmid used in DNA transformation to infect the rice plants. My job was to multiply the DNA quickly using e-coli and then infect the agrobacterium with that DNA for transgenic rice.

I was responsible for multiplying the DNA transferred to the Agrobacterium with e-coli. Also, I was responsible for transferring the DNA from the agrobacterium to the rice plant by cutting rice plant leaves about forty days after germination. These were important duties; if I made a mistake, it could set the scientist behind on his experiment for days.



Figure10: Agrobacterium being prepared with transformed DNA

How My Research Contributed to World Food Security

My research in each of these areas contributed directly to world food security issues in varying degrees. Working on creating a transgenic variety of HR resistant to bacterial blight is extremely important to the yield of rice in Asia. Bacterial blight is reported to have reduced Asia's annual rice production by as much as 60%. For example, in Japan, about 300,000 to 400,000 hectares of rice were affected by the bacterial blight disease in recent years. There was 20% to 50% yield losses reported in severely infected fields. In Indonesia, losses were higher than those reported in Japan. In India, millions of hectares were severely infected causing yield losses from 6% to 60%. This rice lost alone could have fed thousands of people. Resistance to this disease provides some control over the loss of rice to bacterial blight.

Determining the purpose of a gene does not initially stand out as something that improves world food security. Finding the purpose of the PC1301 gene will help the world to fully understand why this gene is in plants and how we can best utilize this gene to its fullest potential. This purpose may directly impact food security in the future.

Knowing what proteins are involved in embryogenesis is also very important to understanding rice. Finding out what proteins are involved could prove useful to those involved in proteomics with rice and for academic interest in the short term. In the future, knowledge of proteins could prove useful to help people to improve embryogenesis.

The C4 Gene in plants helps to increase photosynthetic abilities in plants. Rice plants naturally have the C3 gene, which scientists believe does not help photosynthetic abilities to the extent that the C4 gene has an effect. Scientists are able to transfer the C4 gene successfully, but another, not so welcome gene, comes with this transfer, the hyp gene. Scientist must dispose of this hyp gene. The hyp gene has contributed to making problems in the health of the plant and could be detrimental to the consumer as well. If the C4 gene can be transferred successfully and the hyp gene can be deleted, the implications could be very promising. The photosynthetic ability of the C4 gene contributes directly to the healthiness and higher yield of the rice plants. This can definitely improve the rice as a food staple and contribute to world food security.

When determining the genetic differences between plants, it is important to remember the commercial marketing aspect of HR. Many of the genes mutated to be improved have been patented. If one producer has an identical genetic product as another producer, who has been marketing their own genetic product with a patent, the first producer can not market their product. If a genetic difference can be discovered, the other producer may be able to market their rice product.

How This Experience Changed Me

Personally, going to China was a continuous series of firsts for me. It was the first time that I had traveled alone on an airplane. It was the first time that I was away from home for more than two weeks at one time. It was the first time that I struggled with people understanding me. Overall, it was the best time of my life!



Figure 11: Changsha city from the top of the Yuele Mountain

Growing up in a fairly large family, I had been accustomed to very little privacy. In China, personal questions were not considered rude and being in close proximity or within close personal space to

someone else was considered an everyday occurrence. Boundaries, in China, were very different. In the first two weeks, I learned to rethink my cultural norms and manners in order to deal with these cultural differences. In China, it is not considered rude to stare at someone for indefinite periods of time. It is not considered rude to chew loudly with your mouth wide open. This took some time getting acclimated. In China, there were no such things as personal questions. Every Chinese's curiosity seemed insatiable.

Going to China was a wonderful learning experience and has contributed greatly to my personal growth. I have learned to deal with hardships and minor troubles by myself. I could not call my parents with my problems all the time; they were literally halfway across the world. All Chinese people were friendly to me. Even while I was on the airplane, Chinese adults would offer their assistance to me if I needed it.

My views of the world and China changed dramatically. In the western world, there is a common misconception that "Red" China is some type of evil nation bent on out pricing and out bargaining America, that every non-western country bears the US ill will. By traveling to China, I learned first hand that these ideas were simply not true.

While in China, I learned a lot about myself. I learned to be positive and deal with things with an upbeat attitude. I learned everyone makes mistakes, even 'nutty' professors. I learned how to be more confident about myself and the way that I am. Before this trip, I did not appreciate my qualities that made me unique or different from other seemingly normal sixteen year olds. But everyone is unique and has a different set of interests. I learned to appreciate this aspect more earnestly in China, where everyone is trying to break out of a mold. The change in attitude was most apparent in fashion. The clothing I normally wear is considered 'old-womanish' and simple as compared to the clothing that a typical Chinese teenager would wear. The focus is about being different for the typical Chinese girls' day to day outfits. When on summer break, I did not see one girl, who had the exact same outfit as another. To see someone with similar dress and point it out is actually considered an insult.

While I was in China, I met many new people. Typically the first two questions by students were: “Do you support George Bush?” and “Are you a Christian?” Both of these questions are not typical questions asked from one American to another. Most Americans have the similar values until proven otherwise. It is considered rude to inquire regarding one’s faith in America. The Chinese people have a very open curiosity for all religions. Christianity seems to be tolerated in China and many churches could be found. Contrary to popular belief, there are many Christians in China in addition to other religious beliefs. It was some relief to me to know that there were others who had the similar beliefs and values that I share; in a country, where I was not quite sure about many things.

There were other “foreigners” at the CNHRRDC with me. These folks were from Africa and Asia. At times, when I was with them, I felt like I was adjusting to two cultures simultaneously rather than just the Chinese culture. As I expressed near the beginning, this trip provided quite a few first time experiences for me. One of my unforgettable experiences was my first marriage proposal. One of the men from Madagascar offered to marry me, if I wanted. He gave me his e-mail address just in case I changed my mind and would accept his offer.

While I was in China, I had the opportunity to visit many famous places in the Hunan province that would not normally be on someone’s top travel destinations. I visited Chairman Mao’s hometown of Shaoshan. In this town, there is a huge copper statue of Chairman Mao in the center of the town plaza where people pay their respects. Many people come to Chairman Mao’s birthplace as a pilgrimage. One intriguing site that I saw was this ping pong table that had a sign saying, “Chairman Mao once played ping pong on this table”. The people of China simply deeply admire Chairman Mao. The Chinese people believe that Mao Tse Tung is responsible for their prosperity and that every sensible Chinese person must have a monument to him in their car, in their restaurants, or in their homes. These monuments or pictures are sometimes more like shrines, though some others are very simple.

Also I visited Zhang Jie Jia, which can best be described as the grand canyon of China. It has a surreal beauty that I have not seen before nor can I adequately describe. We went on a 3 day trip to this location and this was to be my “big” off campus trip. The park was so big that we went to three different entrances, while we were there. We rode a huge sky lift. This experience was a slightly scary for me, since I’m not terribly fond of heights. Also, we took an elevator that extended from the bottom of one mountain to the top of another. This was the tallest elevator that was not in a building in Asia! I was able go swimming in a lake with my friends, while I was there and had a great time. I found that the quality of hotels in China is not quite like the quality of American hotels. Chinese hotels occasionally offer hot water and a TV, but usually, they do not have these conveniences. In China, all hotels offer a complimentary low quality restaurant meal.



Figure 12: A picture of my mentor and companion Li Li and me at Zhang Jie Jia and the elevator to the top of the mountain!

In addition to my visit to Zhang Jie Jia, I got to go to the Yuelu Mountain, which is considered one of the 5 most famous mountains in China. The mountain top offered an expansive cityscape of Changsha, which was so surprising to me. Changsha is as large as Chicago, Illinois. I had not realized this fact until this point. This location was where Chairman Mao penned a famous writing and it was very beautiful. At the bottom of the Yuelu Mountain, is where the Hunan Common College sits.



Figure13: Visiting and fishing at the Hunan Agricultural College

Colleges in China are much different from colleges in the US. Generally everyone goes to kindergarten. The senior year for a student is concentrated on studying for the college entrance exam that everyone must take, if they plan to enter a facility of higher learning. In China, there is a very high standard set to be accepted into a college. Acceptance is solely based on one's college entrance exam and on one's knowledge of a foreign language, particularly English for science majors. These students get one chance to impress the college and if they do not score well, they may never go to college. Students may apply to one college at a time and if they do not get accepted, they may not go to that college for the year. There are not enough colleges to support the potential student population; so many people do not attain a college education due to that problem. While at college, students live on campus throughout their entire educational career. There is no option to find alternative housing. This is quite different in America where most students are given the option of off campus housing. Most students do not find employment to pay for their education, but simply rely on parental support. In China, scholarships are extremely rare. In many instances, students rely on their parents until they have secured their first full-time employment. At times, the student could be thirty years old before they are independent of their parents. College in China is relatively inexpensive as compared to the US. It costs three thousand Yuan (\$ 390 dollars US) for one full year of college in China, versus approximately three thousand dollars for one quarter of a year in America.

At first, I had a lot of trouble with the language barrier between the Chinese and I eventually became accustomed to "Chinglish" as I like to call it, which is what I learned to communicate with these people. "Chinglish" is a cross between Chinese and English. Many of the Chinese would think I was Russian (which was quite amusing to me on more than one occasion) for some reason. Once someone spoke directly to me in Russian, which of course I did not understand!

One cultural barrier that was difficult for me to overcome was the government social support. In China, social security and government assistance for handicapped people and senior citizens do not exist. There are two sources of income for the handicapped: begging or singing. Some sing popular Chinese songs on bus rides and then hope everyone on-board will pay for the service. In America, most people learn to mistrust beggars. It was very different to see real beggars, who would not survive if it were not for the kindness of strangers. The sheer number of beggars was phenomenal; this made travelers slightly uncomfortable.

Another social difference between China and America involved the restrooms. The restrooms consist of a 'hole' and a place for two feet. There are no toilet seats or flushing mechanisms. I learned to deal with these somewhat comical hardships by myself and enjoyed China so much because of it.

Every day, after work, it was time for all of us to play! After work, I could choose to go swimming with Prof. Yuan, or play basketball with Xin, or some of the friends I made while I was at the center. After eating my supper, I got to play a form of volleyball using a rubber ball with the senior citizens who live nearby. Prof. Yuan would play volleyball with me also. This was one of the best experiences of my whole trip, because I was accepted, and got the chance to make friends with people of another generation. Also, I realized, when playing volleyball, there is a universal language: sports. I could communicate basic ideas while learning about the personalities of other who played with me.

This experience in Chinese culture has been changing my entire worldview. Much of what we see or hear of China is negative propaganda. While I was in China, I experienced first hand the positive aspects. I will never forget Professor Yuan's parting advice to me about hitting the volleyball or about swimming with ease. I will always remember the effort Li Li took to teach me about HR and the trips she accompanied me on.

Conclusion

When I left from the United States, I had no concept of living two months, by myself, in a foreign country where I do not know anyone. Out of this picture of what life in China was like for me, came the best experience of my life. This was a once in a lifetime opportunity that I took. I learned about many different processes and experimental procedures. It prepared me for real life, without the shelter of my parents, and society. It changed me from being a close-minded child, to an aware adult. I have made friendships I will keep forever. This internship changed me and my life significantly. It was truly the best experience of my life. I will always be grateful to the WFP organization for sponsoring this program and benefiting me personally.