BORLAUG-RUAN INTERNATIONAL INTERNSHIP

China National Hybrid Rice Research and Development Center

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CHANGSHA, CHINA

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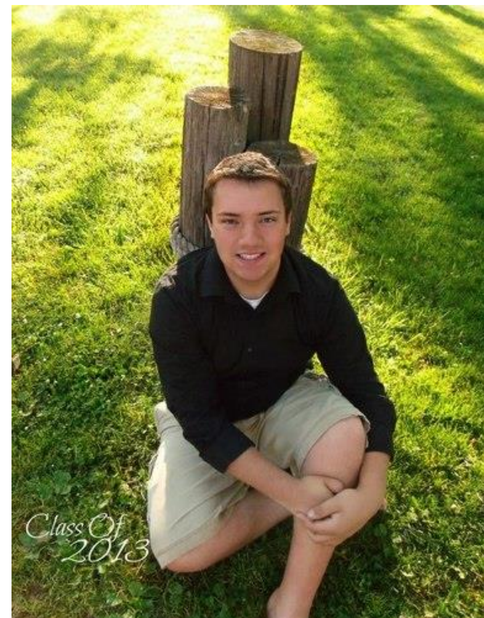
INTRODUCTION
A LITTLE BIT ABOUT ME

As a student coming directly out of high school, thinking about going to China for the summer is probably one of the last thoughts that you could have on your mind. However, for me it was the first. I attended the 2012 Minnesota Youth Institute and then was able to continue on to the 2012 Global Youth Institute. The first research that I conducted for the WFP was on a small country near the Philippians, called Palau. Although the research paper was long, I learned so much about food security and what life is like in small developing countries.

I am from a very small town in Minnesota called Medford. It is in Medford where I attended the small K-12 school that also hosted a Borlaug-Ruan Intern in 2010. In my school life I participated in many organizations such as FFA, SADD, Choir, Band, Yearbook, Student Council, BPA, NHS and some others. With academics and extracurricular activities, I kept very busy.



The organization that I would like to thank so much is FFA. I am so glad that I was able to be a part of such a warm and welcoming organization. I was fortunate enough to attend the National Convention in Indianapolis in 2012, competed at state in team events, and win state proficiencies and participate in other FFA events. It was with the help of my AG teachers (see acknowledgements) that I became interested in the WFP.

When I am not busy with school and other activities, I work an organic dairy farm. I have worked at the dairy farm for 6 years; it has been one of the most rewarding experiences of my life as I have learned so much about agriculture and farming. A rather interesting hobby that I like to do with my family is go scuba diving! Everyone but my mother also is a certified scuba diver. I am so blessed to have such a loving family. The five of us - mother, father and two older sisters - love to go camping, boating and other family things. We live in the country and raise some chickens, so this internship was nothing that I was familiar with, which made for a better learning experience.

The learning experiences and community activities that I participated in while abroad has driven a desire in me to pursue a career in public health. I am now currently enrolled at the University of Minnesota and plan on majoring in public health with the goal of one day working in the global health care field.

CHANGSHA CHINA

The home of my research center was in Changsha, China. Changsha, a famous historical and cultural city, is located at the northeast of Hunan Province, serving as the capital of the Hunan Province (*see figure 1*). The population of Changsha was over 6.5 million permanent residents at the end of 2007. Changsha covers 11,819 square kilometers (7343.99 miles). With a 13 hour time difference to the Central Time Zone, Changsha sits in the China Standard Time Zone (China Tour Online, 2013).

Under the Ming (1368–1644) and Qing (1644–1911) dynasties Changsha was made a superior region and prospered as one of China's chief rice markets. Changsha's population nearly tripled between the start of its rebuilding in 1949 and the early 1980s (Hays, 2008). The city is now a major port, handling rice, cotton, timber, and livestock. It is a center of rice milling and also has oil-extraction, tea- and tobacco-curing, and meat-processing plants. While I was in Changsha, I was present for some holidays such as the CPC Foundation day (equivalent to the Fourth of July), Maritime day (alike to Columbus Day) and the Double Seven Festival (alike to Valentine's Day). While Changsha still focuses a lot on Chinese Agriculture, it has developed into an entertainment hub and has attracted a growing number of foreigners, including Borlaug-Ruan Interns.

(Figure 1: Hunan providence (yellow))

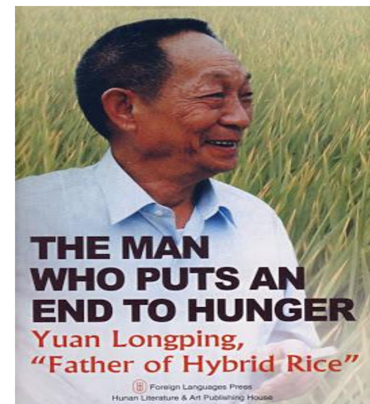
The hot climate for growing rice in Changsha is very helpful. "We can plant hybrid rice year-round," says Dr. Yuan Longping, 2013. Changsha experiences a humid subtropical climate with annual average temperature of 17.0 °C (62.6 °F) in January and 29.0 °C (84.2 °F) in July. Average annual precipitation is 1,330 millimeters (52 in). The summers are long and hot, with heavy rainfall, and autumn is comfortable with abundant sunlight. Winter is comparatively dry and rather brief, but cold can occur with temperatures occasionally dropping below freezing. When I was staying in Changsha, I found those statistics to be very low. On some occasions, the temperature would reach 102°F or more and what seemed like 80% humidity.

While in Changsha, everyone showed immense hospitality and enthusiasm towards me. I was able to visit many places in Changsha, such as the river were I went drifting for the first time, mountains that we could hike, sightseeing in the city along with watching the fireworks. A fact that I found very interesting is that the London and Beijing Olympic fireworks were all made near Changsha.

(Figure 2: National Hybrid Rice Research and Development Center)

Established in 1984, the China National Hybrid Rice Research and Development Center's (CNHRRDC) purpose is to eradicate malnutrition and poverty through the development and distribution of new strains of hybrid rice (友情链接, 2003). Another purpose of the CNHRRDC is to carry out research and development of hybrid rice, including a new type of rice that was recently created, super rice.

At the CNHRRDC (*figure 2*) I would work alongside international researchers in research labs and in the field. Most of the time it was with my mentor Yu Dong, but everyone at the lab was an international researcher that helped me learn more about hybrid rice. The purpose of my mentor was to show me the molecular biology experiments and to teach me more about genetics. Some of those researchers were Dr. Yuan Longping *(figure 3)* and Dr. Xin Yeyun. Dr. Yuan has inspired many in this world; he created the Hunan Yuan Longping Agricultural Science and Technology Award Foundation to comrade those who have made outstanding contributions in the field of hybrid rice promotion and application. Yuan was also awarded UNESCO awarded the Science Award (友情链接, 2003).



(Figure 3: Dr. Longping)

Yuan Longping started the research of indicia hybrid in the 1960s.
Early on, he discovered male-sterile rice. It was Yuan's breakthrough in 1973 that revolutionized rice. He was the first person to develop indicia hybrid rice. With Dr. Yuan, China became the first country that is capable of producing hybrid rice. This earned him the title "Father of Hybrid Rice." His accomplishments made possible the feeding of 22% of the world's population on only 7% of the world's total arable land. "Yuan Longping selfless dedication has been highly praised by all walks of life, and agreed to further expand the sources of funds, increase the reward efforts and to expand the social impact surface." (友情链接, 2003)

A GRAIN AND A LIFE FOR CHINA

"Happiness does not fall out of the blue and dreams will not come true by themselves. We need to be down-to-earth and work hard." (Yuan, 1995)

When people think of Chinese agriculture, many think of rice. Rice is a crop that has come to mean so much to the Chinese people for thousands of years. Rice is used for food security and also holds great economic importance. Why is rice so important to Chinese civilization? The Chinese culture is based on numerous subcultures and its agriculture along with way of life is centered on rice. Along with food distribution, products of the rice plant are used for a number of different purposes, such as fuel, thatching, industrial starch, and artwork.

Growing, selling and eating rice is integral to the culture of many countries. In China, it has been suggested that rice has been cultivated for 3000 to 4000 years. This makes rice a longstanding staple of the Chinese culture. China's rural culture has developed around the practice of growing rice. On some national holidays they celebrate the start of rice planting. Festivals such as the Land Opening Festival and the Spring Festival mark the start of the rice cultivation season.

Rice is a central part of the Spring Festival (equivalent to the U.S. New Year). On this occasion, Chinese families make New Year's cake and eat them in the hope of a better harvest and higher status in the New Year. Rice dumplings are made on the first day the full moon that can be seen each New Year. People eat rice dumplings hoping everything will turn out as they wish. *Zongzi* are eaten during the Dragon Boat Festival on the 5th day of the 5th lunar month and is also made of glutinous rice. This is a day to remember because of Qu Yuan, an official of the Chu State (340 BC – 278 BC), who committed suicide by jumping into the River. People throw *zongi* into the river to prevent fish eating Qu Yuan's body.

In a Chinese myth retold by Lorin Brown, she tells of rice being a gift from animals rather than from gods. "China had been visited by an especially serve period of floods. When the land had finally drained, people came down from the hills where they had been taken refuge, only to discover that all the plants had been destroyed and there was little to eat. They survived through hunting, but it was very difficult, because animals were scarce. One day the people saw a dog coming across a field, and hanging on the dog's tail were bunches of long, yellow seeds. The people planted these seeds, rice grew, and hunger disappeared." As it may just be a grain to some, it is a past, present and the future to others. (Brown, 2008)

INFORMATION

HYBRID RICE HISTORY

Hybrid rice is not a natural grown substance. It has to be grown specifically to create the quantities needed to feed a nation. Scientific advances such as gene isolation, free combination and the breeding of plants were first discovered by Gregor Mendel in the late 1800's. This technology has recreated and reused over many years, in domestic and foreign food production, to grow food such as hybrid sorghum, hybrid corn, watermelon and many other widely distributed food products (CAAS/HAAS, 1991). In 1960 Dr. Yuan learned that the same domestic and foreign production that could be brought to a staple food of the world; that food product being rice. He was in the experimental field of conventional rice varieties and found a distinctive rice plant. Yuan's practice, patience and precision "broke the shackles" of the traditional concept of self-pollination of plants without that hybrid plant in rice (CAAS/HAAS, 1991).

Dr. Yuan immediately then turned his attention to the cultivation of the artificial hybrid rice as he saw it as a plant of the future. Dr. Yuan conducted two observation tests of the spring and autumn. Six years, more than 1,000 varieties and more than 3,000 crosses and they still do not breed sterile plants. Dr. Yuan took those six years of experience and formed lessons about hybrid rice to create a sterile breeding plant.

American scholar Lester Brown, 1995, conducted the "China threat" theory, which theorizes that in 30 years, China's population will reach 1.6 billion. "Who will feed China, who will save the subsequent global food shortages and turbulent crisis?" It was at this time Dr. Yuan announced to the world: "China is fully capable to solve the problem of feeding themselves and will also be able to help the people of the world to solve the food problem". Dr. Yuan's techniques have revolutionized the rice production industry by increasing yields to feed the future.

Timeline

1964 - Research on three-line hybrid rice initiated
1970 - Wild abortive rice identified on Hainan Island in China
1974 - First sets of three lines (A, B and R lines) developed for three-line system hybrid rice
1976 - Hybrid rice commercialization started
1977 - Systematic hybrid rice seed production technique developed
1983 - Hybrid rice seed yield more than 1.2 ton/ha
1987 - Hybrid rice seed yield more than 2 ton/haHybrid rice acreage more than 10 million ha
1990 - Hybrid rice acreage more than 15 million ha
1995 - Two-line hybrid rice system developed
1983 - Hybrid rice seed yield more than 1.2 ton/ha
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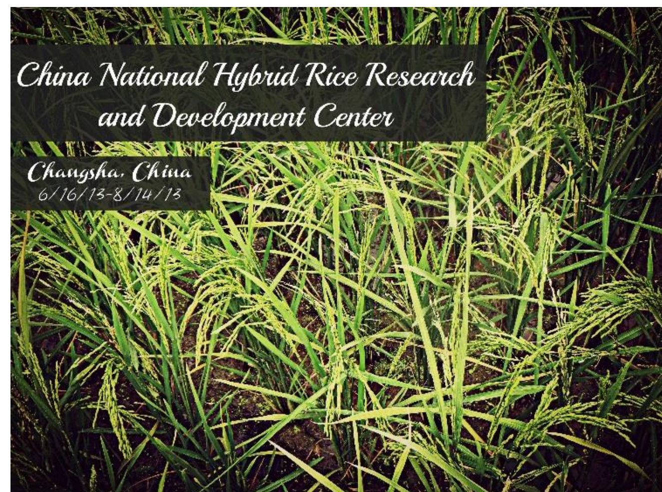
HYBRID RICE TECHNOLOGY IN CHINA

Hybrid rice breeding strategies is divided into three-line and two-line hybrid rice breeding methods and a series of three stages of development. The three stages of development of hybrid rice breeding takes advantage toward increasing strong direction for the plant (Yuan, 1995).

In the two-line method, the two lines are involved in a cross for hybrid rice seed production. One line is genetically controlled by recessive genes, and the other is any inbred variety with a dominant gen (Virmani, S. S). The male sterile can be controlled by temperature and are known as thermo-sensitive genic male sterile (TGMS) lines. At CNHHRDC the male sterile line can be created in two different ways, bother in the field via hand pollination or using chemicals in the lab. I have practiced it both ways. However, my research dealt with more with lab research and development.

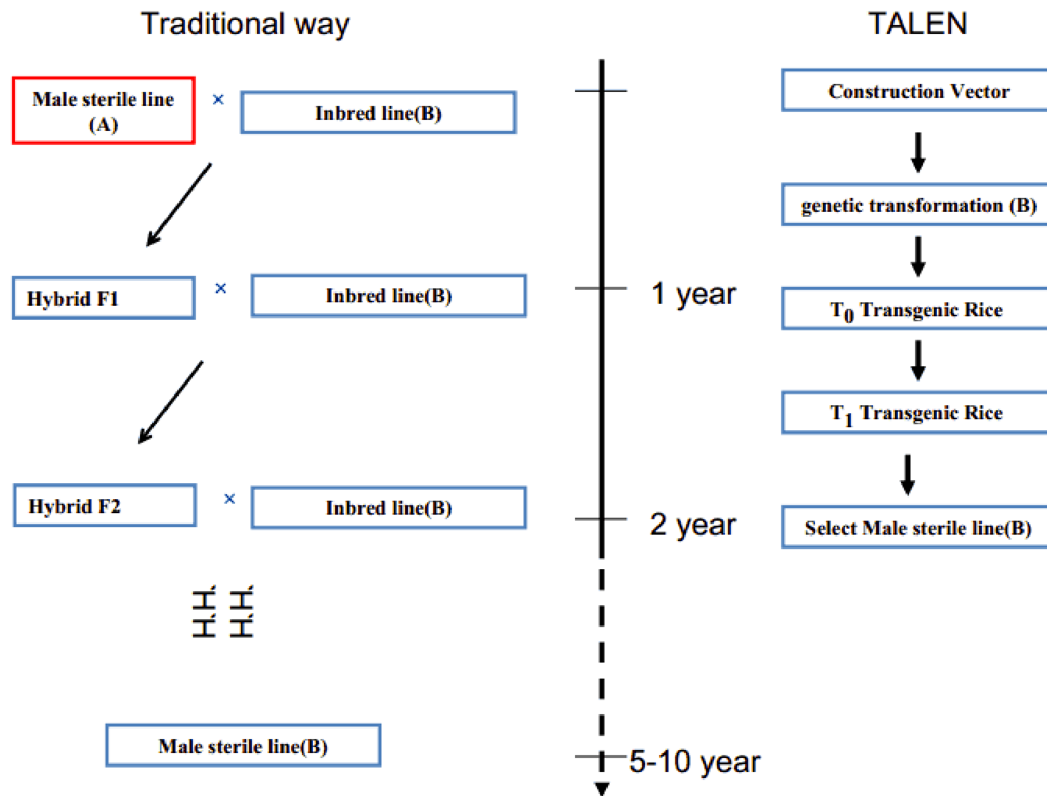
The use of the three-line hybrid rice breeding technique is very different than the two-line. To use three-line and two-line rice hybrids, farmers have to buy fresh seed every season. This seed is produced by a seed production agency in the public or private sector. Three-line rice production method was used at the research center. However, two line method is more favored.

Each of the breeding techniques involve a male sterile line. The traditional way does take a lot of more crossing of the rice plants, thus creating more time until a result of a male sterile line is produced. Another time factor that takes into play is the inbreed line (B). These rice plants are very specific and can take a long time to find.



(Edited picture by me (Glen Morris), took at the rice fields)

The male sterile line can be created using a TALEN (Transcription Activator Like Effector Nucleases) vector. This new technology, which will be mentioned later in this paper, will take less time and will hold better results. This process is created with a TALEN kit that holds all the proper sequencing chemicals that are needed to create the TALEN vector. .



**This image was given to me by my mentor (Yu Dong) to help me understand the old traditional way. I have a lot of literature and photographs that helped me get "up to speed" in the first two weeks of my internship.*

The traditional way does take a lot more crossing of rice plants, thus creating more time to create a male sterile line. Creating the male sterile line with the TALEN vector will take less time and will likely hold better results due to its specificity that researchers have in the lab. This process is created with a TALEN kit that holds all the proper sequencing chemicals that we need to create the TALEN vector. It is a very expensive kit and it is also very new technology. I was fortunate enough to have performed some of experiments by myself, however, I did have supervision on the experiments involving immense work the TALEN kit as one mistake can ruin a day or even a week's worth of work.

NUTRITION GAIN, PLANTING AND HARVESTING OF RICE

Size wise, rice may seem small and simple, but there are many important aspects to a simple looking grain of rice. This history of the importance to the people of China, along with all of the advances in technology to help mass produce the grain, are very important aspects of this crop. China has put forward great efforts into the future and present of the production of rice. Along with the significance of how the rice is planted and harvested, the biggest factor is the nutritional values that come from it.

Nutrition

Why is rice so good for us? Rice has been a staple food for the country of China and many other nations for many years past and many years to come. Rice is rich in energy and is a good source of protein. Table 1 shows that rice contains a reasonable amount of protein, thiamine, niacin, vitamin E and other nutrients. *(Note- These nutrition facts are based on USDA Data and is not a complete replica of the rice that is being grown at CNHRRDC.)*

RICE NUTRI

Nutrition Facts

Enriched White Rice

Serving Size 1/2 cup cooked rice

Calories 103

	% Daily Value*
Total Fat 0 g	0%
Saturated Fat 0 g	0%
Trans Fat 0 g	
Sodium 0 mg	0%
Total Carbohydrate 22 g	7%
Dietary Fiber 0.5 g	2%
Protein 2 g	4%
Iron 0.95 mg	5%
Thiamin 0.129 mg	8%
Niacin 1.166 mg	5.8%
Folate 46 mcg	11.5%

* Based on USDA Data

(Table 1- Rice Nutrition)
Household USDA Foods Fact Sheet, 2012

Research conducted last year from the University of Minnesota shows that a side dish of white and brown rice might add more than delicious flavor and nutrition to meals. Results show that white or brown rice provides a high level of satisfaction and fullness, which is important for people trying to control calories, while still enjoying their food (Klein, et al, 2012).

The University of Minnesota compared markers hunger among 20 normal weight adult men and women. Each subject ate three test breakfasts on different occasions, and the results showed that the test breakfasts increased satisfaction and fullness. "Our results show that white and brown rice provide fullness or satiety, which indicates that either choice can be a great addition to meals or as a snack, as satisfaction can make it easier to control calories,"(As quoted in Klein, et al, 2012).

Planting

The most known way of growing rice is in rice paddies. This is the most efficient way of growing large volumes of rice. Rice grows best in lowlands and on the slopes of hills and mountains. Most rice paddies and terraces are irrigated with water to help with growth. In most cases water from one paddy drains into another paddy.

At CNHRRDC, there are large cement tranches that one could personally control the volume of how much water is added into the fields. There are two ways that rice is planted. The time-consuming and tougher work is planting the rice by hand. This can ensure even rows and that all of the rice seedlings are planted. Another way that I was fortunate enough to participate in was growing the rice seeds in a long plastic bed with cups. First, rice seedlings are started in cups where the plant is given time to grow. Then, then rice plant is removed from the cup and thrown like a dart into the mud of the rice field. This saves time and energy that is consumed with the other way of planting (友情链接, 2003).

Harvesting

Rice has to be harvested when the soil is dry and consequently, the water must be emptied from the paddy before the harvest and filled up again when the new crop is ready to plant. In many places rice is harvested by a machine, yet some places still harvest the more traditional way, by hand.

The rice is placed on large sheets and left to dry on the ground for a couple of days before being taken to the mill to be processed. After the rice harvest the stubble is often burned along with waste products from the harvest and the ashes are plowed back into the field to fertilize it.

While demands for food increase, land used to grow rice is being lost to urbanization and industry and the demands of a growing population. This is what makes the work done at the CNHRRDC so much more valuable. All of the scientists there are providing for the growing population and are trying to make sure that everyone has enough food to eat.

RESEARCH

PROJECT

My summer project mainly focused on constricting a special vector, which will be used for creating PGMS and TGMS line, with the use of transgenic technology. This also resulted with learning about basic molecular techniques in the lab such as plasmid isolation, DNA Recovery, DNA Extraction, Adding on Adenine (A) to the DNA, and many other molecular biology skills.

This process is fairly complicated and does have expensive equipment and chemicals that are needed. However, as previously mentioned, there are different ways to produce hybrid rice seeds (reference page 6). Using the old traditional way of creating hybrid seeds, a sterile plant (A) is crossed with a plant that had more desirable traits (B) to get better producing rice seeds. Continue this process then about 5 more times, making the traditional way a lot of work. I was working in the fields for a couple of days learning and preforming the traditional way of producing hybrid rice (Dong, 2013). One has to remove the 6 anthers from the rice pod and be careful not to touch the stamen, and then leave the rice plant for a couple days for pollination to take place. However, there is a way to perform this in the lab and that is where I focused most of my research on. This involves taking plant B and inserting a TALEN vector. Eliminating the need to pollinate by hand and to cross with other plants.

The reason that this technology was being used because of the time it takes to complete the task. Creating the PGMS or TGMS using TALEN technology can take upwards of 1-2 years, which is a very small amount of time when compared to the traditional way of 5-10 years. They way that this genome editing is produced is through the use of plasmids and other chemicals that help express the process (Yuan, 2013).

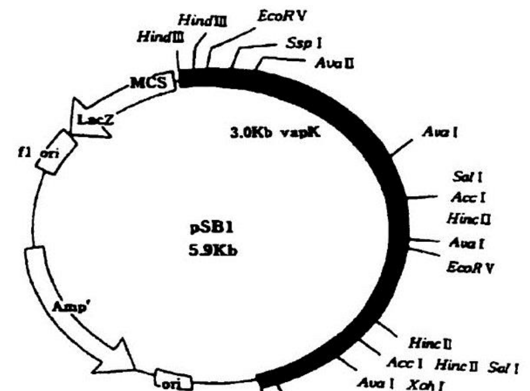
Genetics and molecular biology consist of many procedures, different chemicals, and vast knowledge of working that are needed to perform various experiments. That being said, if you wish to pursue molecular biology or genetics, know that this paper briefly explains the procedures and experiments performed and the molecular reactions, which are the reason why things happen, are not explained in depth solely due to the maximum length of the paper.

I started my research on the third day at the research center. Because of my advanced placement class in biology, I found some of the concepts in the procedures to be very. However, I learned a lot of new things that sometimes were challenging, yet, I was very fortunate to have this experience. The hardest part about my project was when we transitioned to the new lab. We went from a 3 story lab to a 9 story lab and there was a lot of equipment that went in different spots when compared to the old lab.

DNA runs 5' to 3' (five prime to three prime) and can include thousands of bp (base pairs) which are A (adenine), T (thymine), C (cytosine) and G (guanine) (Cermak et al, 2011). Bacteria also play an important role as they provide restriction enzymes and also plasmids. Plasmids are rigs of DNA and act as independent, self-replicating engineers in bacteria. Plasmids allow traits not contained in the chromosome to be passed on from bacteria to bacteria. This is what you want in hybrid rice as you can then easily pass genetic information from one host to another. However, rice is not a bacteria. This is where vectors come in.

A vector is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed (Hai, 2012). In this case, the plasmid is the vector moving the gene of choice that will be expressed in the rice plants. There are different types of vectors that biologists use, and the one that we were using the lab was the TALEN vector.

TALEN (Transcription Activator Like Effector Nucleases) technology is an amazing new way to introduce a foreign gene into an already existing genome. Basically it's the delivery body for the genetic code we want to stick in. It is a free tool that was created and maintained by Iowa State University for designing pairs of "TAL effectors" for "TAL effector nucleases" (TALENs) to target a specific gene sequence (Cermak et al, 2011). The year process is, constructing a vector, transferring it into e.coli, if correct, and then transferring it into agrobacterium, which would infect the plant with T-DNA. T-DNA, often called T-Vector is a vector that had a T bp as the tail. As previously mentioned, T will only pair with A. So in one of my experiments in the lab, I would have to add an "A" tail in order to insert the T vector (reference Methods and Materials for process of experiment). The T-DNA will then be inserted into the rice genome with the help of agrobacterium, infecting the plants new seeds (Dong, 2013).



(Figure 4, single unit vector)

Single unit vectors (*(Figure 4)* *Note: Not an equal representation of the single unit vector I was working with because I used one with 2800bp) are initially used as the starting materials for "Unit Assembly Kit" ("The Synthesis of Customized TALEN via "Unit Assembly", 2013). These plasmids were constructed based on the "pMD18T-simple vector (TAKARA)" (Dong, 2013). There are four different single unit vectors which were named as pA, pT, pC and pG, each corresponding to the single nucleotide target it recognizes that we will find later (Nature Biotechnology, 2011).

The first part is to digest the DNA/plasmids using two different enzymes. For the restriction enzymes that I used to construct, was Hind III and SpeI. These restriction enzymes were provided to us with our kits that the lab purchased (Omega Bio-Tek, 2013). These were selected when I found the TALEN Targeter through a web based program that is sponsored though NCBI. One must sequence the nucleotide and then through the program from NCBI, "blast" then DNA sequence (sometimes they will give you multiple sequences and further steps are needed to see which one will work). Then, information is given by the blast to compare with a vector to find what enzymes that will work with the vector and which ones will work in the DNA sequence (Huang, 2012). This also gives us information for the TAL effectors which bind to a DNA sequence, and identify binding sites in a DNA sequence for a given TAL effector (Doyle E.L., et al, 2012).

Since TALEN works as a dimer, a complete TALEN target site consists of a left TALE binding site, a spacer, and a right TALE binding site. We use another online software to predict the target site. Software sponsored and hosted by Cornell University and published by T. Cermak, et al, 2011. Once we select the target site, we confirm the target site sequence in experimental material by sequencing.

Once that step is done, one mixes the DNA sample with the restriction enzymes and incubate, and preforms electrophoresis to separate after separation (see Methods and Materials for electrophoresis procedure). We can then see what lines of DNA that will be sent in for sequencing. Because the TALEN vector only asks for 2800 base pairs, the rest of the lines will not be used if they do not have to appropriate number of base pairs.

The next steps to complete the PGMS or the TGMS would be to use agrobacterium to introduce that DNA that we made into the rice plant (Dong, 13). Because of the length of my internship, I was unfortunately not able to see through the full stages of the hybrid rice plant. However, this does not mean that it is a failure, as I was able to make something that will eventually become hybrid rice

Because we had extra DNA sequence that we prepared before we did plasmid isolation, we could send in those test tube to be sequenced. This was a step that was not in the literature that was

given to me, but it did save a lot of time. However, the literature that was provided for me did list the steps if we had to pursue it. If we had to we could assist with digestion of the gel into the DNA. Steps labeled below.

1- Digest the pieces of gel that fit 2800bp into smaller chunks. Pulling out the line corresponding to 102bp.
2- Reassemble the small pieces using a ligase. This enzyme will stick it back together again to be used.
3- Once it is reassembled, you have to create a LB Culture and culture the sample with E. coli. This plasmid will be taken up by the bacteria and the bacteria are considered "transformed" into a different type of bacteria that can be sequenced for the vector.
4- Then extract the plasmid (see methods and materials for procedure) and send in the plasmid for sequencing.

Like previously mentioned, please note that this is a simplified version of my internship project and the workings. There is a lot of information to that pertains to TALEN technology, vectors, genetics, and biological implementations that are difficult to explain. The amazing things that can come from trans-genetic technology is endless. Near the end of my internship, CNHRRDC announced that they have reached their third goal for yields on hybrid rice. With the work of their researchers, they created hybrid rice that yields 13 tons/hector (2.5 acre) (Yuan, 13). This was very exciting news for CNHRRDC and there are even more possibilities to come.

METHODS AND MATERIALS

These next methods of constructing the experiments were all taught to me by other workers in the lab and also from instructions that I read. This is not necessarily the way that all of the experiments are performed. I am including the methods in the order that I received instruction on the proper procedure. Also, due note that there is a lot of molecular reactions with all of the solutions, buffers and enzymes that are being added and I unfortunately can't explain all of the molecular biology experiments that I preformed. All methods and materials are accredited to purchases made by CNHRRDC and through the instruction of my mentor Yu Dong, 2013.

Electrophoresis

Why: We use electrophoresis to disperse the DNA fragments, under a spatially uniform electrical field. It allows us to "push" the DNA fragments across the gel and the ones that go farther on the gel, will be smaller. We than can get a rough estimate with the number of base pairs we have because of DNA marker that we also use. Electrophoresis is a rather inexpensive, efficient tool that is commonly used in many biological settings. It was very beneficial to get a lot of experience with it during my internship as it will help in my future career.

Steps:

1- Put "TAE Buffer" into the electrophoresis bin and add Place gel into apparatus
2- Add 3 drops of "DNA Loading Buffer" into the DNA samples and mix on vortex softly
3- Add 10ul of "KBP ladder marker" into the first gel slot (allows for easy identification of the DNA.
4- Add 20ul of the DNA or other specimen into the other slots.
5- Place top on electrophoresis bin turn on machine. 110u for 40min.
    a. Apply the electrical current for separation
    b. Allow to separate
6- Collect data either through on-line detection from the use of the "DNA Loading Buffer"

Plasmid Isolation

Why: Plasmid isolation is a very common molecular biology experiment that I preformed many times. I would perform this experiment to aid future experiments such as sequencing and transferring the plasmid into agrobacterium. Plasmids are very important in biology because they contain at least one DNA sequence and they can serve as the origin of replication. I learned this in the start of my internship and used it when I would check for the left and right tails of the TALEN vector which were all using the ptms-12 gene. Many plasmids had 100% positive identities, meaning identical, which was very exciting to see because we can then use them for the t-vector for the male sterile line.

Steps:

7- Centrifuge the 10ml of e.coli solution at 7,000rpm for 3min and disguard the liquid, leaving a pellet
8- Add 250ul of "Solution I" and suspend in the vibrating vortex
9- Add 250ul of "Solution II," mix slowly and sit for 2min
10- Add 350ul of "Solution III" and mix until white precipitate forms
11- Centrifuge mixture at 12,000rpm for 10min
12- While the tubes are centrifuging, add 200ul of GPS in a separate labeled mini column concealed in a clear tube *(figure 5),* sit for 2min and centrifuge tubes at 12,000rpm for 1min and discard the solution, but keep the tubes
13- Add the clear solution created from step 6 into the top mini column from the GPS. Centrifuge at 12,000rpm for 1min
14- Discard the liquid and add 700ul of "DNA Wash Buffer," sit for 2min and centrifuge at 12,000rpm for 1min
15- Repeat step 8
16- Discard the liquid and centrifuge the empty tubes at 13,000rpm for 2min
17- Place the mini column into a new clear tube, label tube, sit for 2 min and add 40ul of deionized water (ddH_2O) slowly into the mini column
18- Place on metal bath at 65C for 2min and centrifuge at 12,000rpm for 1min
19- Place liquid in the bottom tube on the top mini column and repeat step 12
20- You have now isolated the plasmid and to finish, place in the fridge to use in other experiments

(Figure 5)

DNA Recovery

Why: We do DNA recovery in case the pH of the electrophoresis buffer was too high. Ensure that fresh running buffer is used for electrophoresis. This can also help with the wash solution that was used if it was designed to contain the appropriate amount of components (95% ethanol).

Steps:

1- Perform agarose gel electrophoresis
   a. Put "TAE Buffer" into the electrophoresis bin and add gel into bin
   b. Add 3 drops of "DNA Loading Buffer" into the DNA samples and mix on vortex softly
   c. Add 10ul of "KBP ladder marker" into the first gel slot (allows for easy identification of the DNA
   d. Add 20ul of the DNA into the other slots
   e. Place top on electrophoresis bin turn on machine. 110u for 40min
2- Take the gel out of the electrophoresis bin and place it in the "EP" solution for 10min

3- Wash off gel and place it in Gene Shot machine to read for the DNA. Cut out the lighted up portion of the gel (DNA) and place in tube
4- Add 500ul of "Binding XP2 Buffer" into the tube and place in the metal bath at 65C for 10min
5- Add 200ul of "GPS" into separate mini column and tube, wait for 2min, centrifuge at 12,000rpm for 2min and discard liquid
6- After metal bath, transfer the 800ul from step 4 into the "GPS" mini column, label it and wait for 2min
7- Centrifuge the mixture at 12,000rpm for 1min and remove the solution
8- Add 600ul of "SPW Wash Buffer" to the tube, wait for 2min and centrifuge at 12,000rpm for 1min
9- Discard liquid and repeat step 8
10- Remove solution and centrifuge for 2min at 12,000rpm and add ddH_2O into the metal bath
11- Place mini column into new tube and add 30ul of the ddH_2O into the tube
12- Place in metal bath at 65C for 2min
13- Centrifuge at 12,000rpm for 1min, put the liquid that spun to the bottom of the tube into mini column and place in metal bath
14- Centrifuge at 12,000 for 2min and place in the fridge

DNA Extraction

Why: DNA Extraction is a laboratory procedure to isolate and remove DNA from the rice plant. We will do this for many rice plants that we think are a good suits for a possible male sterile line crosses or for other experiments that we want the rice DNA for. This is a very common procedure and can be applied to not only rice, but other things that I might wish to extract the DNA from in the future.

Steps:

1- Grind about 200mg of rice leaves (about 6 leaves) with N_2, grind very fast with the grinder
2- Transfer the tissue into an tube Add 700ul of "CTAB Buffer" and mix well
3- Incubate in metal bath for 30min at 65C
4- Add 700ul of "Chloroform" and mix the solution and centrifuge the tubes at 12000rpm for 1omin to protein denaturation
5- Transfer the upper aqueous liquid into a clean microfuge tube
6- Add 500-600ul of ice-cold "isopropanol."
7- Centrifuge the tubes at 12000rpm for 10min and discard the liquid. Leaving a white pellet which is the DNA and add 1ml or 70% ETOH
8- Add 40ul of ddH_2O and when the pellet dissolves, store the sample at -20C

Detecting Plasmids for Digestion

Why: The reason we would detect the plasmids for digestion is to see which plasmids we have to use. To perform this skill, we add enzymes and buffers to the plasmid that we want to see if we can use it and then run electrophoresis to see if it is the correct size, around 2000bp. Once we get the plasmid, we then essentially "cut" out DNA fragments using restriction enzymes and then insert DNA fragments of interest into the plasmids. This type of genetic engineering is rather complex, however, is very useful and is an import part of this food technology as it rapidly speeds up the process for the male sterile line.

Steps:

1- After isolation of plasmid, detect which restriction enzymes you should use
2- Place the appropriate number of tubes in ice bath and add 8ul of ddH_2O, 2ul of X10 fast digestion buffer, and 1ul of each restriction enzyme
3- Then add 8ul of each separate plasmid to their own separate tube with the above mixture
4- Mix softly on vortex and then centrifuge shortly
5- Incubate on metal bath at 37C for 30min
6- Preform electrophoresis
    a. We are look for each plasmid column to have two bright marks. If some have a bright mark at the 2000bp indicator, then we will use those ones

DNA PCR (Polymerase Chain Reaction)

Why: PCR is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. It's an inexpensive technique that can make segments of DNA. PCR can target specifics parts of DNA. Polymerase (pol) is an enzyme that is often used in DNA replication.

Steps:

1- Place the "PCR Buffer" and "dNTPs" on a bed of ice.
2- Pair up the primers based on previous experiments.
3- PCR Reaction System.
    a. ddH_2O- 10ul
    b. PCR Buffer- 25ul
    c. dNTPs- 10ul
    d. Primers- 1.5ul of each primer pair
    e. KOD- 1ul
    f. DNA template- 2ul
4- Place the PCR reaction system in the designated number of tubes (based on number or pairs)
5- Mix on the mini centrifuge.

6- Add into the PCR machine and set to appropriate settings.

Adding Adenine to DNA

Why: The T-Vector (thymine) needs to attach to the appropriate base pair of DNA in order to stick. In DNA, thymine will only stick to adenine. So for this I had to add Adenine to the DNA in order to add the T-Vector.

Steps:

1- One the PCR product is finished, take out and prepare the below solution
   a. 10XTaq Buffer- 5ul
   b. dNTP- 2ul
   c. Taq- 1ul
2- Add the solution into the DNA and centrifuge shortly.
3- Mix on the vortex.
4- Place in metal bath at 72C for 30min.
5- Preform purification.
   a. Centrifuge the tubes shortly
   b. Place in the fridge overnight

Gel for Electrophoresis

Why: We would not buy the gel that we use for electrophoresis because we preform electrophoresis on a daily basis. Below are the steps to create the gel that is used for electrophoresis.

Steps:

1- Retrieve white box tray for gel.
2- Insert the gel holder into the box tray.
3- Add spacer indicator.
4- Make the liquid that will solidify into gel.
   a. For full box tray,
      i. 80ul of “TAE” into a beaker.
      ii. Add 1%= 0.8g of “Agrobiowest”
      iii. Shake and microwave for about 30sec (until the liquid is all clear).
5- Pour into gel box tray, remove air bubbles and sit for 30min.

LB Culture

Why: In order to culture the e.coli cells and other things that we would need for the experiments, we would need to prepare the petri dishes with a “LB” culture for them to grow.

Steps:

1- Create the liquid for culture.
   a. Tryptone- 10g
   b. Sodium Chloride- 10g
   c. Yeast Extract- 5g
   d. Agar Powder- 15g

2- Mix all together in a beaker and then add 1,000ml of ddH$_2$O.

3- Place in the incubator for 1hr.

4- Petri dishes are ready to be cultured.

Analyzing the DNA Sequence

Why: This is preformed to, analyze the DNA sequence. We would use this online software to look at the DNA sequence to find things such as restriction enzymes and other important information that we would use.

Steps:

1- Go to the website of NCBI

2- Select “blast” from the right column

3- Select “Align” at the bottom of the page

4- Paste the nucleotide sequence into the window provided. (this sequence was given by the NCBI and was found on their website.

5- Copy the new sequences that you have into the window below the window used in step 4.

6- Select “Blast”. This will give you a sheet with the two nucleotide matched up.
   a. If the nucleotides match up with one another, then you have the correct sequence.
   b. If they do not match up with each other, then you have to repeat step 4 with the other nucleotide sequences you have.

These were the main experiments that I would conduct. They were always different too. Because the vector had to be around 2000bp, I would have to find a plasmid that could be transferred into agrobacterium that would affect the plant with T-DNA which would then be inserted into the rice genome.

Table 2 explains the method that I used during my research project. Step 1 is explained on pages 9-11 about finding the binding site. Step 2 involves the TALEN kit and is used for the

Steps (Simplified Method)
1-TALE binding site
2- Ligation
3- Transform into E.coli
4- Extraction of Plasmids
5- Digestion
6- Sequencing

ligation of the vector arms. In the kit we would mix together the Left arm vector (ex. CC1, GC2,GC3,GG9…) and the right arm vector. Then you put in in a PCR, add some solutions that were provided to you in the kit, incubate and then continue to step 3. Step 3 is to transfer the arms into e.coli using an e.coli cell. Then you culture the e-coli overnight. Once the e.coli has been cultured, it will give you petri dishes that are full of cells that can be used in step 4. You then have to take out samples the e.coli cells and place them into a "NC" solution with an antibiotic. Step 4 is extraction of the plasmids, previously mentioned in "Methods and Materials." This involves a lot of centrifuging and other solutions that are provided in the kit. Step 5 is digestion that is also mentioned in "Methods and Materials." This process involves restriction enzymes along with other chemicals that help for plasmid digestion. Then, too see if we completed the above steps correctly, we then send in the vector in for sequencing.

RESULTS

I was very new to this new technology and to some of the molecular biology concepts. With that, some of my results did not come out as I hoped in the beginning. An example of this would be when I first sequenced the vector arms to see if it was correct, only the right arm was correct. However, my mentor also conducted the same experiment and only his left arm vector was correct. So together we could use the two to create a 2080bp vector for the male sterile line.

When I constructed the arms a second time, I had to transform e.coli, isolate plasmids and preform plasmid digestion many times. I was able to get the Left arm in 16 samples, but the Right arm took 32. This was because some of the samples that I took were not of around 2,000bp and we couldn't use them.

(Figure 6, 2000bp)

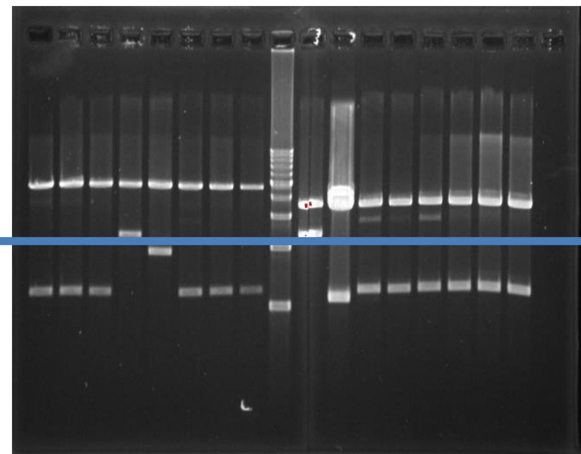


Figure 6 is the results of the second electrophoresis gel sample. The columns represents the plasmids that I isolated with the "ptms-12" gene that we later sent in for sequencing. The blue line represents 2000bp mark. The mark can be identified using a "KBP ladder marker" that I put in the center of the gel (figure 6).

When the results came back for the second time of preforming the experiments, I was very pleased. Figure 7 shows the sequencing of the R1-9 in reverse order and it had 100% positive identities. This is just one of the pictures that were given after the sequencing. In this second experiment, we were able to use all three samples of the left arm and the one of the right arm.

Range 2: 49 to 999 Graphics ▼ Next Match ▲

Score	Expect	Identities	Positives	Gaps
732 bits(1597)	0.0	317/317(100%)	317/317(100%)	0/317(

```
Query  580   RLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNGGG  759
             RLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNGGG
Sbjct  999   RLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNGGG  820

Query  760   KQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVV  939
             KQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVV
Sbjct  819   KQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVV  640

Query  940   AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH  1119
             AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH
Sbjct  639   AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH  460

Query  1120  GLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRL  1299
             GLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRL
Sbjct  459   GLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRL  280

Query  1300  LPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQ  1479
             LPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQ
Sbjct  279   LPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQ  100

Query  1480  ALETVQRLLPVLCQAHG  1530
```

(Figure 7, the results of R1-9 that we sent in for sequencing)

These next corresponding pictures are of the other samples that we sent in for sampling from the ptms-12 gene. They show the tail of the vector and they number sample taken (ex. L1- Left Tail, 3- 3rd sample), the order that it was sequenced, (F-Forward, R-Reverse), and it's identities to the host plasmid.

L1-3

F

Range 2: 90 to 998 Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Positives	Gaps	Frame
696 bits(1517)	0.0	303/303(100%)	303/303(100%)	0/303(0%)	+1/+3

```
Query  1    LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  180
            LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL
Sbjct  90   LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  269

Query  181  PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA  360
            PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA
Sbjct  270  PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA  449

Query  361  LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA  540
            LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
Sbjct  450  LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA  629

Query  541  SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT  720
            SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT
Sbjct  630  SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT  809

Query  721  PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV  900
            PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV
Sbjct  810  PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV  989

Query  901  LCQ  909
            LCQ
Sbjct  990  LCQ  998
```

R

Range 2: 52 to 999 Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Positives	Gaps	Frame
727 bits(1586)	0.0	316/316(100%)	316/316(100%)	0/316(0%)	+1/-2

```
Query  583   LLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGK  762
             LLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGK
Sbjct  999   LLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGK  820

Query  763   QALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVA  942
             QALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVA
Sbjct  819   QALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVA  640

Query  943   IASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG  1122
             IASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG
Sbjct  639   IASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG  460

Query  1123  LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLL  1302
             LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLL
Sbjct  459   LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLL  280

Query  1303  PVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA  1482
             PVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA
Sbjct  279   PVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA  100

Query  1483  LETVQRLLPVLCQAHG  1530
             LETVQRLLPVLCQAHG
Sbjct  99    LETVQRLLPVLCQAHG  52
```

L1-4

F

Range 2: 90 to 998 Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Positives	Gaps	Frame
696 bits(1517)	0.0	303/303(100%)	303/303(100%)	0/303(0%)	+1/+3

```
Query  1     LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  180
             LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL
Sbjct  90    LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  269

Query  181   PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA  360
             PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA
Sbjct  270   PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA  449

Query  361   LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA  540
             LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
Sbjct  450   LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA  629

Query  541   SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT  720
             SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT
Sbjct  630   SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT  809

Query  721   PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV  900
             PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV
Sbjct  810   PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV  989

Query  901   LCQ  909
             LCQ
Sbjct  990   LCQ  998
```

R

Range 2: 50 to 1000 Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Positives	Gaps	Frame
730 bits(1591)	0.0	317/317(100%)	317/317(100%)	0/317(0%)	+1/-1

```
Query  580   RLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGG  759
             RLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGG
Sbjct  1000  RLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGG  821

Query  760   KQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVV  939
             KQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVV
Sbjct  820   KQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVV  641

Query  940   AIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH  1119
             AIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH
Sbjct  640   AIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH  461

Query  1120  GLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRL  1299
             GLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRL
Sbjct  460   GLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRL  281

Query  1300  LPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQ  1479
             LPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQ
Sbjct  280   LPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQ  101

Query  1480  ALETVQRLLPVLCQAHG  1530
             ALETVQRLLPVLCQAHG
Sbjct  100   ALETVQRLLPVLCQAHG  50
```

L1-12

F

Range 2: 86 to 1000 Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Positives	Gaps	Frame
701 bits(1529)	0.0	305/305(100%)	305/305(100%)	0/305(0%)	+1/+2

```
Query  1    LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  180
            LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL
Sbjct  86   LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  265

Query  181  PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA  360
            PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA
Sbjct  266  PVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA  445

Query  361  LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA  540
            LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
Sbjct  446  LETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIA  625

Query  541  SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT  720
            SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT
Sbjct  626  SNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT  805

Query  721  PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV  900
            PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV
Sbjct  806  PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPV  985

Query  901  LCQAH  915
            LCQAH
Sbjct  986  LCQAH  1000
```

R

Range 2: 49 to 999 Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Positives	Gaps	Frame
729 bits(1589)	0.0	316/317(99%)	317/317(100%)	0/317(0%)	+1/-2

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R1-9

F

Range 6: 88 to 930 Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Positives	Gaps	Frame
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R

Range 2: 49 to 999 Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Positives	Gaps	Frame
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Sbjct  999   RLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNGGG  820

Query  760   KQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVV  939
             KQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVV
Sbjct  819   KQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVV  640

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Sbjct  639   AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH  460

Query  1120  GLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRL  1299
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Sbjct  459   GLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRL  280

Query  1300  LPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQ  1479
             LPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQ
Sbjct  279   LPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQ  100

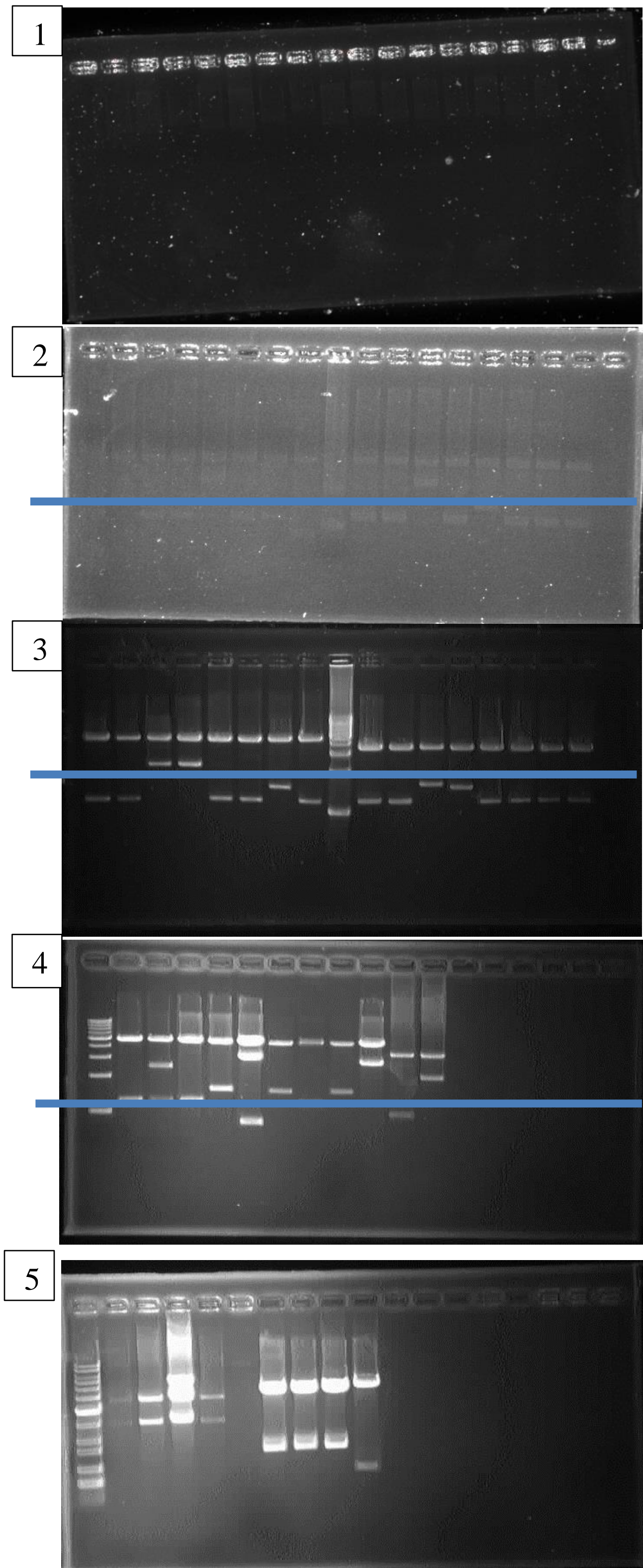
Query  1480  ALETVQRLLPVLCQAHG  1530
             ALETVQRLLPVLCQAHG
Sbjct  99    ALETVQRLLPVLCQAHG  49
```

These pictures on the right are all examples of other electrophoresis results that I preformed. Electrophoresis is a cheap and fast way to look at what you're trying to look at through the process.

We can use electrophoresis to see a number of things. One in particular that I mentioned is for the digestion of plasmids. This can be seen in pictures 2 and 3. The blue line represents the 2,000bp mark and with the 2,080bp vector, we want to t-vector/plasmid to have around 2,000bp so we can send them in to be sequenced.

We can also use this processes to detect if the e.coli is good. For this processes, we run the e.coli in a PCR reaction and then run thorough electrophoresis. We can then see that the columns that have the DNA that are lit up are good and we can use them. This can be seen in picture 5. In this we can see that perhaps the first column of the e.coli and the third column are not good and that we can't use that e.coli. Note that the first column that you can see in picture 5 is a "KBP ladder marker" and does not count as a column of e.coli.

We can also use electrophoresis to show other results that are shown in the other pictures on the right. However, there are many experiments that I do not have the room to mention that use electrophoresis. Molecular biology is a very large field and consists of many experiments. I was fortunate enough with the help of lab personal to have had good results with most of the experiments that I conducted.



FINAL TEST

The questions outlined in bold represent the questions that were given to me at the CNHRRDC from my mentor at the beginning of the internship. At first, some of them I did not know how to respond to, but by the end of my internship I knew the answers for all of them.

Why did China develop hybrid rice?

The growing population meant that there would be a growing number of stomachs. Rice is a crop that has come to mean so much to the Chinese people for thousands of years. Rice is used for food security and also holds great economic importance. Along with food distribution, products of the rice plant are used for a number of different purposes, such as fuel, thatching, industrial starch, and artwork.

I believe that hybrid rice was developed to meet the demand of the rice needed to keep people from going hungry. Although that there are still thousands that are hungry in this world, there are less people going hungry if it wasn't for the development of hybrid rice.

What is three-line system and two-line system? How to breed hybrid rice combination?

In the two-line method, the two lines are involved in a cross for hybrid rice seed production. One line is genetically controlled by recessive genes, and the other is any inbred variety with a dominant gene. The male sterile can be controlled by temperature and are known as thermosensitive genic male sterile (TGMS) lines. The male sterile line can be developed by hand, or in the lab. By hand is very time consuming and my project dealt with more of the lab research and development.

The use of the three-line hybrid rice breeding technique is very different than the two-line. To use three-line and two-line rice hybrids, farmers have to buy fresh seed every season. This seed is produced by a seed production agency in the public or private sector. Three line rice production method was used at the research center. However, two line method is more favored.

What is Professor Yuan's contribution to the development of hybrid rice?

Yuan Longping started the research of indica hybrid in the 1960s. At the beginning, he discovered male-sterile rice. It was Yuan Longpings breakthrough in 1973 that revolutionized rice. He was the first person to develop indicia hybrid rice. With Dr. Yuan, China became the first country that is capable of producing hybrid rice. This earned him the title "Father of Hybrid Rice". His accomplishments made possible the feeding of 22% of the world's population on only 7% of the world's total arable land.

Dr. Yuan conducted two observation tests of the spring and autumn. Six years, more than 1,000 varieties and more than 3,000 crosses and they still do not breed sterile plants. Dr. Yuans took

those six years of experience and formed lessons about hybrid rice to create a sterile breeding plant.

Some scientists thought that there is no heterosis in rice, why did he succeed in hybrid rice?

Heterosis, is an outbreeding enhancement, and for rice, each of the phases marks a new breakthrough in rice breeding and will result in a marked increase in rice yield if it is attained. In the field of rice heterosis, many rice scientists have attempted to explore new technological approaches for increasing the yield potential of rice. The most successful attempt was using the two-line method.

Dr. Yuan was able to succeed because he tried for years and years and then succeeded in the two line breeding method. When I went out into the fields and have manually crossed the rice plants, I have seen how time consuming it can be. This is probably why many scientists have failed, because it takes a lot of time.

What's the target of Chinese Super Rice? What's the development status of Chinese Hybrid Rice?

The target of Chinese Super Rice is to obtain 15t/ha. There is a system of phases that Yuan developed on to creating the super rice. Phase 1 (1996-2000) was complete with the rice yielding around 10t/ha. Phase 2 (2001-2005) was complete with the rice yielding around 11.5t/ha. Phase 3 (2006-2013) was complete with the rice yielding around 13t/ha. This year will be the start of phase 4 (2014-?) with the hope of yielding around 13t/ha. This can hopefully be accomplished in the next coming years. How they get this projection is based on an equation. Grain yield= Biomass X HI.

What's the effect of hybrid rice on World Food Security? How to promote hybrid rice in the world?

Hybrid rice has one of the largest impacts on world food security. About 60% of the world's population depends on rice as their staple food. This is a crop that was developed in China that will save thousands of lives. We can calculate that if the conventional rice were completely replaced with the cultivation of the hybrid rice, the total rice production in the world would be double and could meet the food requirements of one billion more people.

How to promote the use of hybrid rice in the world is through organization and through the spirit of hope. Hope that we can make a difference if more hybrid rice was cultivated. The work of the employees at CNHRRDC is also very important as they go to developing countries to educate others about hybrid rice.

FUTURE WORK AND LESSONS LEARNED

There are many lessons learned and the future work that can be done. Because my research project was very new technology, I await what the future scientists and future interns can experience in hybrid rice technology as the technology its self can only improve and the future of feeding nations that depend on rice as their main source of food.

Future Work. On the 7th week of my internship, I was invited to attend a lecture by Dr. Yuan himself. He talked about how the past brought so much to him and the future of China in the present. We were given some literature about how his history and how he made the impossible possible. In that lecture we would also learn about the future of hybrid rice.

The CNHRRDC just successfully completed its third phase of hybrid rice improvement, this phase was set at a goal that they would successfully construct hybrid rice that would yield 13t/ha from 2006-2015. It was complete not to long before my arrival for my internship. They now are starting phase 4 which has a goal or creating hybrid rice that would have a yield potential of 15-18t.ha. They wish to accomplish this phase in hybrid rice history by the year 2020. This can be thought of as super hybrid rice. A crop that will feed more than 60% of the world's population.

Going to China to preform molecular biology experiment that express the assembly of the male sterile line was the biggest lessons and gift one could give me on this internship. However, just going out into the cities and farms has taught me something even more valuable. It's why we need to focus our attention on improving food security around the globe and that it can only be done together.

Everyone has a story, a story that can't be told on an empty stomach. So many people do extraordinary things just for us to have enough food to eat every night. The research that is constructed at the lab and in many other countries around the world is one of the biggest accomplishments and improvements that we can make to help fight this issue. More and more people are born every day and we need to put forth our best efforts to ensuring that we have the technology and capability to feed each and every one of them.

In my research paper I wrote for the WFP in the summer of 2012, I mentioned "working with the people and not for the people." This still stands true after I've completed my internship in the China National Hybrid Rice Research and Development Center and even more important that we do this together as one. One thing that stood out to me was that CNHRRDC will send representatives to other countries to work with them and not for them. Sharing what can not only benefit them, but many others, in hopes to change the world.

PERSONAL EXPERIENCES

PICTURES- RESEARCH

These are pictures of the Ptms-12 gene that I transferred into e.coli cells that have been cultured. This is a very important step as it will allow us to extract the plasmid. You can kind of see in the top picture that some of the cells are blue and some are white. I will only use the white ones to turn into e.coli to be extracted to form plasmids.

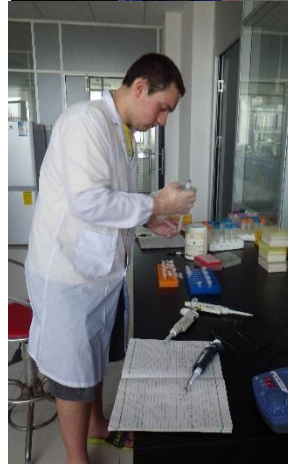
In these three pictures, you will see that I am preform experiments. In the first picture I am transferring the t-vector into e.coli. It is the experiment that was follows the picture above.

In the bottom two pictures, I am extracting plasmids. For the plasmid isolation, gel extraction and some other experiments, we use kits that have the necessary solutions and chemicals that we need to finish the experiment.

A lot of the experiments require accurate measuring and patience, as the slightest mistake can ruin the entire days or weeks work.

The top picture is of me excitingly making the gel that we use for electrophoresis. I would often make the thing that are necessary for us to complete the experiments, such as "LB" culture plates, gel for electrophoresis and sterilization or tubes and machines.

The bottom picture is of me centrifuging some DNA. In order for all the chemicals inserted into the tubes to be conjoined, I would often centrifuge them many times.



This is a picture of me doing other experiments with in the lab. Some might include southern blot, DNA recovery, the addition of "A" tale on to DNA and many others. The experiments would often take a lot of time and specification, but that gave me time to start other projects.

PICTURES- CULTURE/INTERNATIONAL EXPERIENCE

They would have big fireworks every Saturday. The London and Beijing Olympic fireworks were made near Changsha, China!

Beautiful temple I visited in the mountains.

Bike riding through the city with my mentor's family. Very long but very worth all the busses we had to take!

I was able to visit the local zoo where I saw many animals. I attended elephant show and was able to pet and feed the elephants!

Dr.Xin was nice enough to invite me on one of her family vacations to a different province! It was a great trip! Also meet with district governors of China.

Fortunately I had time to visit the rice fields many times. This was not associated with my lab work, but I would take any chance I got to go to the rice fields.

Picture me at the "Floating Mountains." They received their name after the movie Avatar.

Saw the panda at the Zoo! I was able to get a picture with it too. Pandas are a very special animal. Especially in China.

The lab group was very nice to me and would invite me along on all of their trips. One trip was rafting down a river! Great time!!

Planting rice! A very new and beneficial experience. Very different from planning other crops in the U.S

These are all the Bangladesh participants. Some of the nicest people during my stay.

I was able to attend a traditional Chinese wedding while I was in China, very different form weddings in the U.S.

This is a pot that was used for a game that I played in the city. The game involves a pot of water and coins.

Beautiful designer China that was for sale. It was very expensive and the designs were so elaborate. I did not purchase any to take home.

CULTURAL EXPERIENCE

During my internship, I did learn a lot about molecular biology. However, the cultural aspects that I was introduced to along the way was a great plenty. I was fortunate enough to have travel to different provinces with in china and have seen differences from those provinces. Some of the cultural experiences I had would be with food, people, daily life and language.

Food. Coming from a small town in Minnesota, I was not quite accustomed to the Chinese food. However, the Chinese food is full of great flavor, vegetables, and also had a great presentation. In contrast to an American diet, they like to eat different parts of the animals. Sometimes this did come as a surprise, yet it never was a disappointment. The food in Changsha is very famous and I was fortunate to have some very traditional Changsha Food. When you eat, you sit at a round table and in the center is a glass plate that will hold the dishes served for that meal. It was very different from American Chinese food, but so delicious.

People. Going to China as a foreigner, you do stand out and get some attention. The times when people would come up to me and say "you have a beautiful smile" or "you're so beautiful" were a great plenty. The people there were very friendly and hospitable. Not once did I have a dangerous situation or feel not safe. The most important aspect of the people that I have come to increase in my life is their sense of community. The people of China have a sense of community and unity that keeps everyone together. I've noticed how effectively they work together and how kindly they socialize with people that they may not know. They live a different life, a life that suits them very well and most importantly, keeps them happy.

Daily Life. Compared to Minnesota, Changsha is very hot! Because of the extreme temps, they rest during the day after lunch for a couple of hours. This was very new to me, but I do like the idea. I don't think in college they will let me rest after dinner. During the day a lot of people also tend gardens. For some, the produce that they grow is their only income. You can walk do many streets and find someone selling fruit at every corner.

Toilets. I was fortunate enough to have a westernized toilet in my hotel room. However, some places do not use the westernized style. It was a different experience using a "squat toilet," but I did get used to it after 2-3 times.

Night Life. Compared with the day life, the night life is much more active. This is mainly because of the extreme temperatures that they experience during the day. At night it is a lot cooler and that's when more people come out. This can be a little different at first, experiencing

that many people at 10-11pm at night, but it makes it very fun. It’s almost like being at the fair with all the people, flashing lights and all the street food.

Language. A lot of people in China know conversational English. Students in school will study English as a second language also. However, I did find at times it was difficult to communicate. In the lab was sometimes difficult because some chemicals are in Chinese and some people of the scientific terms were different. I did know a little conversational Chinese when I went over and the people in the lab were eager to teach me more throughout my internship. At the end, no matter what the topic is, I did learn a lot more than what I came there with.

You can lean so much about the Chinese culture, as culture is the way of life. These examples were just a few differences that I saw how they lived than the way that we live in America. I think that they greatest thing is that their different. It was good to get out and explore new ways and ideas. I’ve seen that both styles suit the people well and have their advantages and disadvantages, but both are correct. I was lucky to see many other traditional Chinese ceremonies such as a tea ceremony and a Chinese wedding. Thing that are so unique to me that I wouldn’t have the chance to experience in America. It was funny because the people in China were so interested in American culture and I was so interested in theirs. At the end, no matter what the topic is, I did learn a lot more than what I came there with.

FINAL THOUGHTS

It was a short 3 months before my departure that I learned that I would be a Borlaug-Ruan International Intern. Something that was just a dream when I was writing my research paper for the Minnesota GYI the summer before. In my interview with the WFP staff, they mentioned many places, but never China. Then came the news on Easter, that I would be attending the China National Hybrid Rice Research Center in Changsha China. I was speechless. I didn't know what to think. I was thinking about the places mentioned in the interview and was mentally preparing for them, but not once did China cross my mind.

After completing my summer Borlaug-Ruan International Internship, I don't think the WFP could have placed me at a better research center. The warmth and hospitality that I received from everyone at the research center and everyone across the nation of China was overwhelming. I was so blessed to also have a great mentor that cared so much about me and that I had a good time. The other people at the lab were also so nice and would invite me to see places so that I would have a good time around China. And I can't forget the 25 government officials from Bangladesh that treated like their son. They provided so much when sometimes we have so little to do. When I left China, I was a part of the "research family," "Bangladesh family" and a friend to many. Without these people, I don't think I could have enjoyed this internship as much as I did.

At the 2012 Global Youth Institute, Ertharin Cousin gave one of the most influential speeches that I have ever heard in my life. It was a speech about hope and that we have the power to make a difference, about people "who believe that their life is going to be better, that things will be different for them. We once believed the same dream where I came from here in the United States, and it did get better… and shame on us if we don't make it better for them." (Cousin, 2012) This is nothing less than the truth. In my eight weeks, every single one of those researchers at the lab and in the office were making a difference no matter what they were doing. Rice is the staple food for many nations and leading national such as China and the United States have that ambition and determination to make it better. To make a better future, a future when everyone can go to sleep on a full stomach and so that that those dreams can come true. With research centers such as CNHRRDC and the work of thousands of people, we can take the hungry to hopeful.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to the World Food Prize Foundation. In particularly Dr. Norman Borlaug for whom the great organization of the WFP was founded by. He was a humanitarian, Nobel Peace Prize Laureate and founder of the WFP. Also Mr. John Ruan, Chairman of the WFP, and Ambassador Kenneth M. Quinn, a former US Ambassador to Cambodia and current President of the WFP. With the help of these extraordinary people, I had an incredible opportunity granted to me and the other Boralug-Ruan Interns.

Many thanks and cheers to Mrs. Lisa Fleming, the Director of Global Education Programs of the WFP, for always being there to advise, coordinate and be there for us when we were away. I don't know how she can get any sleep worrying about the 22 interns that are overseas.

Thank you to Professor Yúan Lóngpíng, the father of hybrid rice, and founder of CNHRRDC. Your hospitality and generosity has for allowed me to learn so much about the need for change and a way to a fuller life. Thank you to Dr. Yèyún Xīn for arranging and coordinating my internship at CNHRRDC and for inviting me along on your family vacations.

Also, a big thank you (非常感谢- Chinese Thank You) to Yu Dong for being my mentor and showing me so much hospitality and generosity while I was fulfilling my internship at the CNHRRDC. It was with your spirit and kindness my stay in Changsha, China was very pleasant. Also thank you to ever staff member and student at CNHRRDC, especially my Bangladesh and African friends, for helping me make my stay so memorable in those 8 weeks!

Thank you to all the World Food Prize Staff, Ambassador Quinn, Lisa, Keegan, Catherine and many others for choosing me as a 2013 Borlaug-Ruan Intern and for trusting me to represent the World Food Prize in China. For without your help, support, and friendship I would not have had the global experience and the determination to make a difference.

I appreciate all my teachers at Medford High School in Minnesota for the support given to me these last months of school. Special thanks to my Ag teachers, Mr. Tim Larson and Mr. Chris Ovrebo for the constant support and drive to pursue the World Food Prize and for all of the help they have gave me through the great organization of FFA.

I would like to express my endless gratitude to my family. My sisters Moranda and Marissa Morris and to my father and mother, Mr. Glen Morris Jr. and Mrs. Melodie Morris, for their endless support of my dreams and for providing me with a wonderful life.

Lastly, thank you to the other people who I did not have room to mention that helped me gain an experience of a life time (cooks, maids, shop keepers, etc) while I was at my internship in China. My experience in Changsha China at the China National Hybrid Rice Research and Development Center allowed me to work with the people and to understand the immense dedication and determination of making the difference for millions.

I would like to thank all of my friends at the research center (Xing Yu, Goa Young and many more). Also a huge amount of thanks to all of my friends at home (Sara, Sara, Sarah, Nicole, Brandon, Deanna, Cede, Taylor, Megan, Tyler and many more) for always texting/skyping with me so that I would have someone to speak fluent English with!

Now that we can hear the music playing, it's time for this paper to come to an end. Lastly I would like to thank you for reading this as I know 38 pages of molecular biology and other information can be a handful. I hope you enjoyed what I had to say and that you make a journey of your own no matter wherever or whatever that may be. We truly are all in this world, together as one, and if not working as one, what are we? I can tell form my experiences that we are strong, confident, independent, determined, helpful, and special. No matter what we do, no matter where that may be, we are all making a difference.

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