The Third Generation of Hybrid Rice: Unlocking the Possibilities of Chloroplast Transformation

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Table of Contents

I. Acknowledgements

I would like to thank Yuan Longping for granting me the opportunity to come to China and to learn from the community that he has created at the CNHRRDC. His legacy has taught me so much about the importance of hybrid rice in the global fight against hunger. I also thank him for his dedication not only to the people of China, but also to the people of the world, and his dream that even something as small as a grain of rice can hold such great potential.

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I would like to thank my friends and co-workers Qi Wen and Li Xiangyuan for giving me so many experiences and so many memories during my time in China. They have not only helped me and supported me in my laboratory work, but they have also allowed me into their lives. I truly appreciate the open welcome that they gave me the very first time I met them, and for continuing to show me friendship during my time in China. I am glad to say that the most important thing that I will take with me from China is the time that I have spent in the company of friends.

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I would like to thank the entire CNHRRDC community for showing me such hospitality, kindness, and friendship during my stay in China. Due to their open welcome, I have had so many experiences and I have made so many memories that I will cherish and carry with me for the rest of my life.

II. Personal Introduction

Looking back at my childhood, it seems almost inevitable that agriculture would become part of my life. I come from Wisconsin, one of the most agricultural states in the country. My childhood home was a farmhouse located in the rural countryside. My parents owned a greenhouse where they grew organic herbs and tomatoes to sell to local grocery stores. Even my ancestry is based in agriculture; I come from a long line of Norwegian farmers. Yet somehow, I never felt connected to agriculture growing up. There were even times when I resented agriculture. I didn't

think that being a farmer would bring me the excitement or adventure that I craved from life. I couldn't see how agriculture would help me achieve my goal of travelling the world.

When a former teacher of mine informed me about a program called The World Prize Global Youth Institute, what initially drew me to the program was the mention of the Borlaug-Ruan International Internship. At that point in my life, what I desperately wanted more than anything was to travel, to experience other countries, other cultures. For the first time, it seemed like the possibility to see the world was within my grasp. So over the summer of 2013, I decided to write a paper for The World Food Prize Global Youth Institute. When I made it to the national level that fall and qualified to apply for the Borlaug-Ruan International Internship, I knew that I was on the verge of something life-changing. After I applied, interviewed, and received the news that I would be sent to study hybrid rice in Changsha, China for two months during the summer of 2014, I struggled equally with emotions of excitement and terror as I awaited the day of my departure. I felt that my life would forever change once I got on that plane. I was right.

III. Introduction to Hybrid Rice

In the field of agriculture, the hybridization of crops to obtain an offspring with superior qualities is a concept that has been well known for many years. For a long time, however, it was believed that rice, a self-reproducing crop which has the ability to pollinate itself, would not respond favorably to the practice of hybrid breeding. This assumption was proven wrong in 1961 when Yuan Longping discovered a natural hybrid rice plant that possessed large panicles, strong stems, heavy grains, and a fifty percent higher yield than normal rice varieties. Hybrid rice is the product of crossbreeding two genetically dissimilar parents. The benefits that result from hybridization are part of a phenomenon known as "heterosis" or "hybrid vigor". Hybrid vigor is a desirable trait because it means that the hybrid possesses the best qualities of both parents. Due to heterosis, hybrid rice plants have the ability to produce more grain, be more resistant to diseases, and grow rice more effectively than normal rice varieties. This makes hybrid rice technology not only an innovation in agriculture, but also an innovation in the fight against world hunger and food insecurity.

3.1 The CNHRRDC

The China National Hybrid Rice Research and Development Center (CNHRRDC) located in the Furong District of Changsha, Hunan, China carries out some of the world's most innovative research in hybrid rice technology. The main activities of the center are:

> "breeding new hybrid rice combinations with high yield, good quality and multiple resistances by three-line and two-line systems; exploring the utilization of distant heterosis in rice through molecular biotechnology; researching into the mechanism of heterosis in rice; studying the technology for multiplication and purification of parental lines and production of hybrid seeds; studying the technology for high yielding cultivation of hybrid rice; collecting and evaluating genetic resources of hybrid rice; evaluating the seed purity of parental lines and rice hybrids; testing and analyzing rice grain quality; integrating hybrid rice technology and marketing hybrid rice seeds; training on hybrid rice technology" ("Hunan…").

The official motto of the research center is “Realistic, Innovative, Diligent, and Enterprising” (“Hunan…”). China National Hybrid Rice Research and Development Center strives to be “a worldwide center of scientific research, germplasm resources, technical services, academic exchange and personnel training for hybrid rice” (“Hunan…”). The center is strongly supported by the Chinese government, and has built an international network with other institutions and countries throughout the world. It continues to conduct ground-breaking research and is considered to be a leading authority on the subject of hybrid rice.

The CNHRRDC was founded in 1995 by Professor Yuan Longping, also known as “the Father of Hybrid Rice”, because of his vision to “develop hybrid rice for the welfare of the people all over the world” (*The…*). Living through China’s great famine of 1958-1961 as well as years of political turmoil in China, Yuan saw the toll that it was taking on the Chinese people, especially the farming communities (*The…*). He decided to dedicate his life to developing higher yields of China’s staple food crop, rice. Yuan has spent his life working amongst the people he strives to help. Many of the farmers and villagers across China revere him as a national hero. They were the ones who gave him the title “the Father of Hybrid Rice” (*The…*). A humble man, Yuan says “the peasants in our country have a very rich experience in how to grow rice…I have a lot to learn from them” (*The…).* Even though he has achieved so much already, Yuan Longping still says “one of my dreams is to make hybrid rice help more people in the world” (*The…).* To this day, Yuan Longping actively serves as the Director General of the CNHRRDC, even at the age of eighty-four.

3.2 The Benefits of Heterosis

The benefits of heterosis are expressed mainly in superior morphological characteristics, superior physiological behavior, and superior grain yield. Superior morphological characteristics are typically observed in a more vigorous root system, a strong tillering ability, and large panicles with heavier grains (Yuan, 2003). The root system of hybrid rice is typically superior to normal rice in both quality and quantity (Yuan, 2003). The growth rate of roots in hybrid rice plants tends to be greater, and some studies have also indicated that rice hybrids are superior to their parents in total root dry weight, root number, length, diameter, and pulling force (Yuan, 2003). This helps hybrid rice plants get better access to the water and nutrients they need. As for a strong tillering ability, tillers are the stems developed from the main stem (Zhu). Tillering is an indication that the rice plant is becoming more complex (Zhu). Hybrids have been known to have a fast growth rate of tillers, and thus it has been believed that this may contribute to their high grain yield (Zhu). Hybrid plants are also superior in their large panicles and heavy grains. All of these morphological characteristics help to contribute to superior physiological behavior and the ultimate goal of higher grain yield.

Heterosis also gives hybrid plants superiority in physiological behavior. Hybrid plants have been known to show higher root activity, larger photosynthetic area, more photosynthetic efficiency, lower respiration intensity, and better distribution of assimilates (Yuan, 1995). During the period of tillering to heading, hybrid rice plants have consistently demonstrated higher root activity than the parental lines (Yuan, 1995). Hybrid rice plants have also shown to have a greater green leaf area than that of the parents, resulting in greater photosynthetic efficiency (Yuan, 1995). Lower respiration intensity and better distribution of assimilates also aid in the superior performance of

hybrid rice plants compared with their parents (Yuan, 1995). The advantages in the physiological behavior of hybrid rice plants compared with their parents help to demonstrate the true benefits of heterosis.

One of the most important aspects of heterosis in hybrid rice plants is the property of superior grain yield. Throughout the years, hybrid rice has consistently shown more than a twenty percent yield advantage over the conventional varieties. In the provinces of southern China, the average grain yield of hybrid rice is near to 7.5 tonnes per hectare (Li, Jiming). This is approximately twenty percent higher than the grain yield of the leading local varieties (Li, Jiming). Heterosis is important because the ultimate goal of hybrid rice breeding is to produce high enough grain yields to feed the increasing world population. Perhaps future developments in hybrid rice breeding may improve the vigor of hybrid rice and possibly lead to a higher grain yield that can sustain the world.

3.3 What Other Benefits Does Hybrid Rice Offer?

Other than the properties of hybrid vigor, there are many diverse advantages of hybrid rice that make it an important resource to the world. With the production of hybrid rice there come many economic opportunities for farmers in both the commercial production and the seed production of hybrid rice. In particular, hybrid rice seed production compared with normal seed production requires about thirty percent more labor (Virmani). Due to the labor intensiveness of F_1 seed multiplication and production, the hybrid rice program has introduced more rural employment opportunities and increased the income of farmers. Another important advantage of hybrid rice is its potential for use in adverse ecologies. In some countries, the use of hybrid rice has actually uncovered better hybrid vigor in unfavorable conditions than in favorable conditions. Hybrid rice performed well in Egypt under saline conditions, yielding thirty-five percent more than the conventional varieties (Food…). This means that the resistance of hybrid rice could make it possible for rice to be grown not only in favorable, irrigated conditions but also in unfavorable soils and climates. More land could become available for agricultural use, more farmers could be able to make a living off of the land, and more rice production would feed more people. These and the many other benefits of hybrid rice make it a topic valuable not only to scientists, but to all citizens of the world.

3.4 The Three-Line System and the Two-Line System

There are two main methods of producing hybrid seed: the three-line system and the two-line system. Developed in 1974, the three-line system was the first system that was used to breed hybrid rice. To produce hybrid rice seed using the three-line system there must be a cytoplasmic-genetic male sterile line (the A-line or CMS line), a maintainer line (B-line), and a restorer line (R-line). The CMS line does not possess pollen and therefore cannot self-fertilize, however it can produce seeds when pollinated by other varieties of rice. The maintainer line has the ability to pollinate the CMS line and produce offspring that retain the male sterility of the CMS line. This ensures the continued production of the CMS line for future use in hybrid breeding. The purpose of the restorer line is to pollinate the CMS line in order to produce the F_1 generation of hybrids. To produce rice in the three-line system, the CMS line and the maintainer line are planted in one isolated plot while the CMS line and the restorer line are planted in another. Then the maintainer

line pollinates the CMS line to generate more of the CMS line, while the CMS line in the other plot is pollinated by the restorer line to produce F_1 hybrids. This process is repeated over and over again. The three-line system is the oldest method of breeding hybrid rice, and has continued to be utilized for nearly forty years.

There are some benefits and some disadvantages to the three-line system. One of the benefits is that stable male sterility is able to be maintained throughout the breeding process. One of the disadvantages is that there are limited germplasm sources, since only a select few restorer lines are able to pollinate the CMS line. Other disadvantages are that the system requires an extra step for seed production of the parental CMS line, and that the breeding of the CMS line can be time consuming. These defects in the three-line process made it necessary for researchers to search for a more efficient system of rice breeding: the two-line system.

In 1994, twenty years after the creation of the three-line system, another method of breeding hybrid rice was developed called the two-line system. This system was developed after the discovery of EGMS (environment-sensitive genic male sterility). This type of sterility is controlled by nuclear gene expression, which is influenced by environmental factors. There are two varieties that exhibit EGMS, the PGMS line (photoperiod-sensitive genic male sterile line) and the TGMS line (thermo-sensitive genic male sterile line). In the breeding process, the EGMS line serves as the seed parent while any fertile line can serve as the parent that will pollinate the EGMS line. After the fertile line pollinates the EGMS line, an F_1 hybrid is formed. Therefore, the hybrid breeding process can be completed only using two lines instead of three.

The two-line system also has some advantages as well as some shortcomings. One of the advantages is that seed production is simpler and more cost effective because there is no need for a maintainer line in seed multiplication. Another benefit is that any fertile line can be used to pollinate the EGMS line in the two-line system. In the three-line system, very few restorer lines have the ability to pollinate the CMS line. Therefore, the two-line system is more efficient than the three-line system in this way. Also, due to the fact that EGMS is governed by major genes, the trait is easily transferred to any genetic background. Therefore, there is genetic diversity in the female (EGMS) parents which helps in reducing potential genetic vulnerabilities within the hybrids. A disadvantage of this system is the fact that male sterility is controlled by environmental factors. Therefore, any sudden change in the environment will influence the sterility of the EGMS lines. Also, hybrid seed production in the two-line system and multiplication of the EGMS lines are restricted by the region and the season they are grown in. These shortcomings in the two-line system have made it necessary for scientists to continue researching ways to improve the hybrid breeding process, even to begin working on a new generation of hybrid rice.

3.5 The Significance of Hybrid Rice

China is the most populous country in the world, supporting twenty-two percent of the world's population (Shihua). Yet only seven percent of the world's arable land is located in China (Shihua). How is it that China has been able to combat the threat of hunger despite this imbalance? The answer is hybrid rice. China was the first country to successfully implement a hybrid rice program. Since the creation of the first generation of hybrid rice by Chinese scientists

in 1974, China has realized more than a thirty percent yield advantage in hybrid rice over conventional pure line varieties (Yuan, 2003). Even though Chinese rice lands steadily decreased from 36.5 million hectares in 1975 to 30.5 million hectares in 2000, the national average yield was raised from 3.5 tonnes per hectare to 6.2 tonnes per hectare (Yuan, 2003). Thanks to hybrid rice, China has thus far been able to sustain its people despite a shrinking amount of arable land and a constantly expanding population. However China, the most populated country in the world, recently released data that its population had increased to 1.39 billion. In the coming years, hybrid rice will be needed more than ever to help China feed an ever growing population.

There is also much potential that hybrid rice holds for the rest of the world. Rice is the most important staple food crop for more than half of the world's population ("Proceedings…"). However, in many parts of Africa, Asia, and Latin America, the demand for rice is soon expected to surpass production ("Proceedings…"). This is a result of world-wide population growth colliding with land scarcity and declining rice yield growth (Food…). In order to help prevent hunger from becoming more prevalent than it already is, hybrid rice must continue to be researched and discussed as one of the keys to ending world-wide food insecurity.

IV. Research

4.1 The Third Generation of Hybrid Rice and Chloroplast Transformation

Over the past few years, scientists at the CNHRRDC have been working on what is referred to as "the third generation of hybrid rice". This new third generation system is expected to produce hybrid rice that is more high-yielding, more resistant, that has more stable and efficient gene expression, and that has fewer effects on the surrounding environment (Li, D.). The third generation hybrid breeding technique will also surpass the breeding methods of both the three-line system and the two-line system. It will solve the problem of the three-line system's limited access to germplasm, and the instability of the male sterile line strands in the two-line system ("3rd…"). Scientists at the CNHRRDC believe that this can be accomplished with the potential of the nuclear male sterile line. All of the present male sterility systems have problems in either restoring male sterility or in obtaining a high degree of male sterility in all parts of the parent under different environmental conditions. A system that can produce absolute male sterility in the parent line is necessary in order to ensure that there is no chance of the parent self-reproducing and contaminating the F_1 generation. Ultimately, the objective is to achieve complete nuclear male sterility while still allowing for the introduction of desirable genes into the rice genome for enhanced qualities. This difficult goal makes it necessary for multiple phases to be used in producing the third generation.

A technique called chloroplast transformation is also being explored as an important part of creating the third generation. Chloroplast transformation is essentially the process of introducing foreign genes into the chloroplast genome. For many years, the common method of genetic modification in rice has been the introduction of a novel gene into the nuclear genome. Chloroplasts make good candidates for the third generation breeding technique because even though both the nucleus and the chloroplasts contain genetic material, their differences in structure and behavior give chloroplast transformation several advantages over the traditional nuclear breeding method. Some of these advantages are high gene expression, low risk of

environmental contamination, lack of gene silencing, a more stable transformation process, and the expression of multiple genes in a single transformation event (Anitha). Most importantly, chloroplast transformation is also a way for desirable genes to be transformed to the rice plant despite complete nuclear male sterility (Anitha).

Each chloroplast contains nearly 100 copies of its own genome (Verma). With approximately 100 chloroplasts contained in each plant cell, this amounts to around 10,000 copies of the chloroplast genome per cell (Verma). This allows chloroplasts to accumulate a very large number of gene copies after the transformation process, giving chloroplasts the advantage of high gene expression. Chloroplasts also have lower risk of environmental contamination. In the past, genetically modified organisms created by nuclear breeding methods have been a concern because of the possibility that foreign genes could be integrated into other plant species through pollen, creating the possibility of invasive species and ecological damage. Chloroplasts (along with the genetic material that they contain) are not passed through the pollen of a plant. Instead chloroplasts are inherited maternally, preventing them from spreading through pollination to nearby plant species and containing the genetically modified material. Another advantage is that chloroplasts are not affected by gene silencing because their transformation process is much more stable. In chloroplast transformation, foreign genes are integrated into the chloroplast genome by homologous recombination. This a process in which similar nucleotide sequences are exchanged between two similar molecules of DNA. Homologous recombination allows for a specific area of the chloroplast genome to be targeted for the insertion of genes without changing or damaging the genome as a whole (Verma). As a result, the transformation process is more stable and thus gene silencing does not occur. Chloroplast transformation also allows for multiple genes to be expressed in a single transformation event through the use of polycistronic mRNA (Verma). Most importantly, since the chloroplast genome exists outside the nucleus and is inherited maternally, there is still a way for new genetic information to be transferred to rice even when the nucleus is completely sterile. Due to these multiple advantages and the vast potential of developing chloroplast transformation technology, it is very likely that chloroplast transformation could help scientists realize the dream of the third generation of hybrid rice.

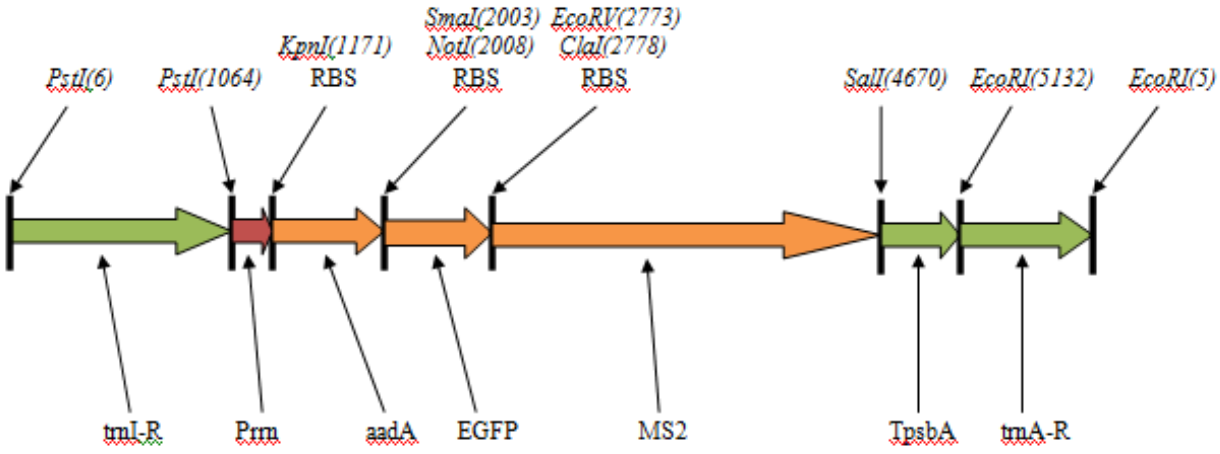
4.2 Research Focus and Objectives

The first successful chloroplast transformation was achieved in 1988 in the single celled green algae named *Chlamydomonas reinhardtii* ("Proteomics…"). Since then, chloroplast transformation has come to be successfully achieved in several crops including tobacco, tomatoes, potatoes, rapeseed, *Arabidopsis thaliana*, and soybeans ("Proteomics…"). However, chloroplast transformation has yet to be successfully implemented in the rice breeding process. There are some difficulties that have thus far prevented scientists from realizing the full potential of chloroplast transformation in hybrid rice.

One of the main difficulties is the challenge of constructing a successful chloroplast transformation vector. Typically, a successful vector would contain several target genes between two recombination fragments. All of the possible recombination fragments are part of the rice genome. The difficulty is identifying which genetic sequence of recombination fragments will successfully insert the three genes into a rice plasmid. This is why my supervisor and I decided to focus our research on chloroplast transformation vector construction. We chose to work on a specific vector called $AEMS_2$ which contained three genes: a gene for fluorescence (so that we

could discern whether the vector had been inserted effectively), a gene for fertilization, and a gene for resistance. These three genes had already been successfully inserted into *Arabidopsis thaliana* (a type of small flowering plant), but there was difficulty inserting these same genes into rice due to a lack of effective recombination fragments. Our objective was to create successful rice chloroplast homologous recombination fragments that would allow this specific vector with these three genes to be inserted into rice chloroplasts.

Figure 1: AEMS$_2$ Target Vector



Target Vector

4.3 Methodology and Procedure

4.3.1 Phase I: Nuclear Transformation

There are two phases which must be undertaken to achieve the third generation. In order to move on to our specific goal of chloroplast transformation, we first had to complete the first phase of the third generation. The first phase is transformation to the nucleus. This phase involves vector construction, transformation, obtaining the seed with fluorescence, and sorting the non-fluorescent seeds from the fluorescent seeds. The reason that this phase must be part of creating the third generation is that nuclear transformation can help verify which seeds possess complete nuclear male sterility and which do not. This was done by transforming a vector with a gene for fertilization attached to a gene for fluorescence into the nuclei of rice cells. If the cells were able to express the fluorescence trait, we knew that the rice did not have complete nuclear male sterility. If they did not express the trait, then we knew that nuclear male sterility existed in those rice cells. During my internship at the CNHRRDC, the only step of the nuclear transformation phase that I took part in was the seed sorting stage. During this stage, we used the green light from a dual fluorescent protein flashlight along with red barrier filter glasses in order to view the DsRed fluorescence in the rice. This made it easier to sort the fluorescent seeds from the non-fluorescent seeds. After we collected the non-fluorescent seeds, we germinated them and then extracted the DNA. After we had the DNA, we were able to perform PCR to verify that the non-fluorescent rice did not have any presence of the fluorescence gene or the fertilization gene. The

sorting process yielded positive results which allowed our research team to continue on to the next phase.

4.3.2 Phase II: Chloroplast Transformation

The second phase of creating the third generation is chloroplast transformation. This phase also involves vector construction, transformation, and obtaining the seed. The majority of my internship was spent on the first part of chloroplast transformation: constructing the vector. The first step in the process of constructing the vector was determining the nucleotide sequence of the target vector. The sequences of all three genes were already known because of their previous insertion into *Arabidopsis thaliana*. The sequences of the recombination fragments were able to be determined because all of the possible fragments were part of the rice genome, which had already been previously sequenced. Therefore, we were able to determine the entire vector sequence (including the three target genes and the recombination fragments) with relative simplicity. After the sequence of the target vector had been ascertained, the next step was to design and synthesize the primer. During PCR (polymerase chain reaction), the primer is used to determine the DNA fragment to be amplified and then aid in the replication process. Selecting and synthesizing the correct primer to recognize the target fragments was important in the success of PCR. Once the correct primer was chosen, PCR could begin.

PCR or polymerase chain reaction is the process of amplifying a fragment or fragments of DNA to generate thousands to millions of copies of a particular DNA sequence. In our experiment, we needed to amplify the recombination fragments in order to verify that the fragments we chose were appropriate for insertion into rice plasmids. Using the primers that we previously chose based on the nucleotide sequences of the fragments, PCR was performed to amplify the recombination fragments. After that, the next step was to choose a restriction enzyme that would recognize and cut the amplified DNA only at a particular sequence of nucleotides. It was necessary to first cut the DNA into fragments in order to accurately measure it and obtain results during gel imaging. Once the DNA was cut, we began the process of gel electrophoresis. Gel electrophoresis is used in PCR in order to obtain a visual image of the length of the DNA fragments. We made a gel and used 100bp and DL2000 markers as a reference to measure the length of the fragments in our sample. After electrophoresis had been performed, we stained the gel using ethidium bromide and took a gel image. If the gel image confirmed the target length of our recombination fragments with the two markers, then we were able to move onto the next step: actually inserting the vector into a plasmid.

In order to use the recombination fragments that we had taken gel images of, we first needed to extract them from the gel and perform gel purification. After this was accomplished, we were able to recombine the target genes and the recombination fragments with a rice plasmid and then transfer the modified plasmid to E. coli cells where it would be able to grow. Then we performed clone PCR on the E. coli cells in order to multiply the number of E. coli and the plasmid inside of them. After the cloning PCR, we extracted the plasmid from the E. coli cells. Then we used enzymes to cut the target vector out of the plasmids. Finally, if all of these steps were successfully completed, we were able to send the target vector off for sequencing in order to verify whether the vector had been successfully transformed into the rice plasmid. If the DNA sequencing step of the process was reached and the vector showed the correct sequence of the

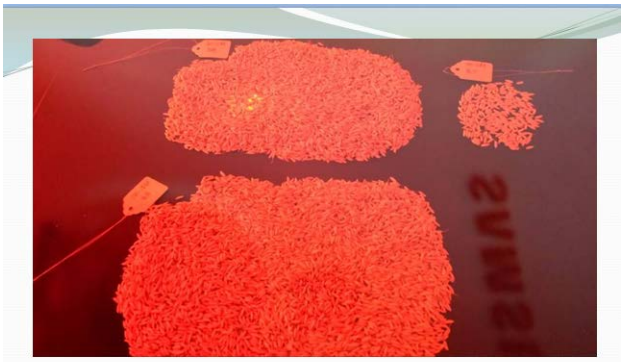
genes as well as the sequence for effective recombination fragments, then the experiment was considered successful and our objective was fulfilled.

4.4 Results

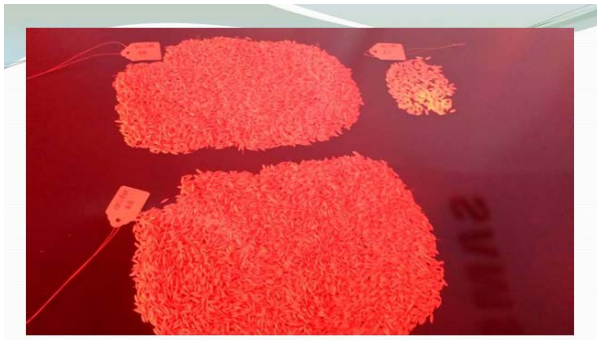
The results from the first phase of the third generation process, nuclear transformation, yielded positive results. In sorting the rice with red fluorescence from the rice without red fluorescence, it was determined that all of the seeds showing red fluorescence did carry the target genes and all those without red fluorescence showed no trace of the target genes. After first sorting the seeds by the appearance of red fluorescence, we were able to verify the presence of the target gene by performing PCR. These gel images indicated with 99.9 percent accuracy that the seeds with red fluorescence had the presence of the target vector while those without red fluorescence did not carry any trace of the target vector in their DNA. This means that our sorting method had an accuracy of 99.9 percent in determining the success of nuclear transformation. These results were an important find in the experimental process of creating the third generation. Without these positive results showing the outcome of the nuclear transformation process, we would not have been unable to move on to the next stage of the research, the actual chloroplast transformation.

Figure 2: Visual Results of Nuclear Transformation Seed Sorting Method

Top left: original group of seeds, without sorting
Top right: sorted seeds with red fluorescence
Bottom left: sorted seeds without red fluorescence



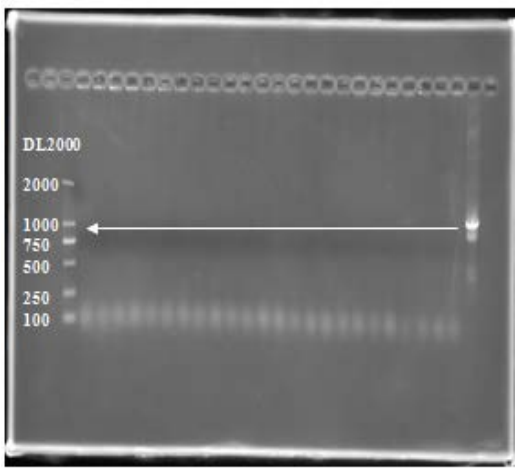
Unsorted original group of seeds – only some show red fluorescence



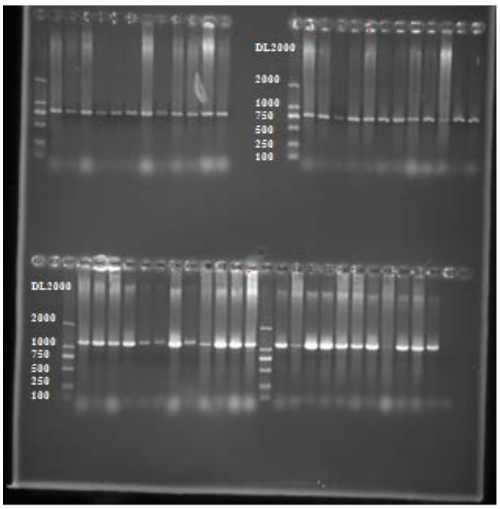
Sorted seeds that show red fluorescence

Sorted seeds that do not show red fluorescence

Figure 3: PCR Results of Nuclear Transformation



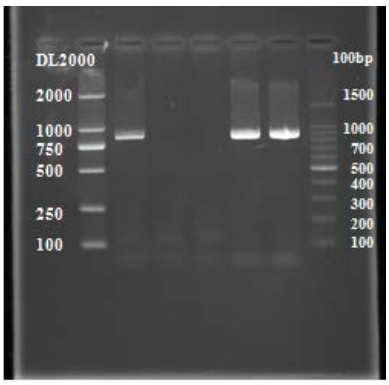
DsRed-Negative for Red Fluorescence
800bp



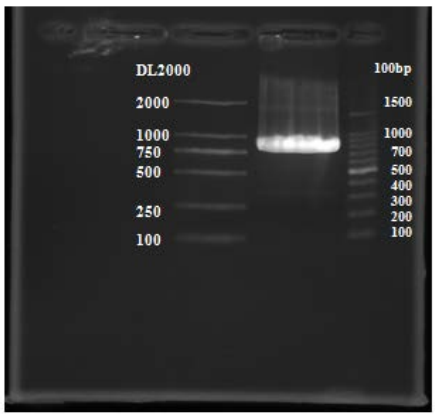
DsRed-Positive for Red Fluorescence
1200bp and 800bp

In our end results using the recombination fragments trnA-R, we were looking for a gel image that would show that our sample was a length of 800 bp. Eventually we were able to successfully achieve gel electrophoresis where the trnA-R measured 800 bp, as can be seen in the gel images below. After we had a successful gel image, we performed cloning PCR to verify the initial results. The positive results from this verification allowed us to send off the trnA-R sample for sequencing. The official results of the complete DNA sequencing will provide the final proof we need to determine whether the recombination fragments were successful.

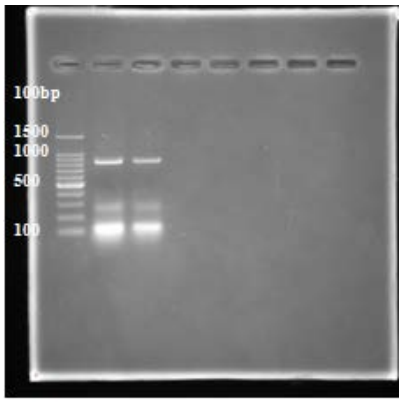
Figure 4: PCR Results trnA-R Recombination Fragments



PCR Product – trnA-R
800bp



PCR Product – tnrA-R
800bp



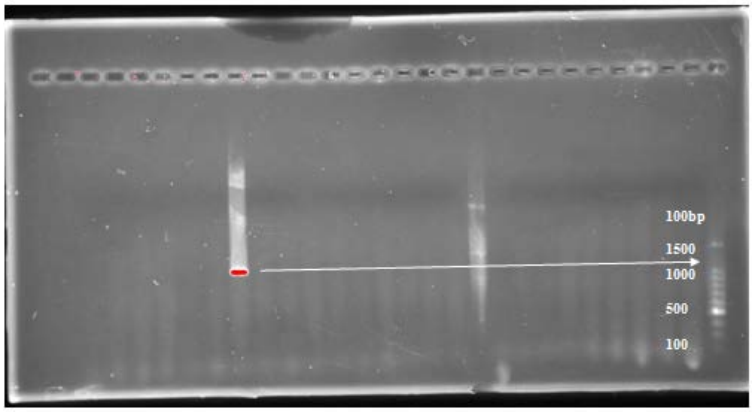
Cloning PCR – trnA-R
800bp

Our results from the recombination fragments trnI-R were obtained in the same process as the results for trnA-R, except we were looking for a measurement of 1300 bp in the gel image. As the image below shows, eventually we were able to successfully achieve gel electrophoresis

where the trnI-R measured 1300 bp. Then we performed cloning PCR to verify the initial results. The positive results from this verification allowed us to send off the trnI-R sample for sequencing.

Figure 5: PCR Results trnI-R Recombination Fragments

PCR Product – trnI-R
1300bp

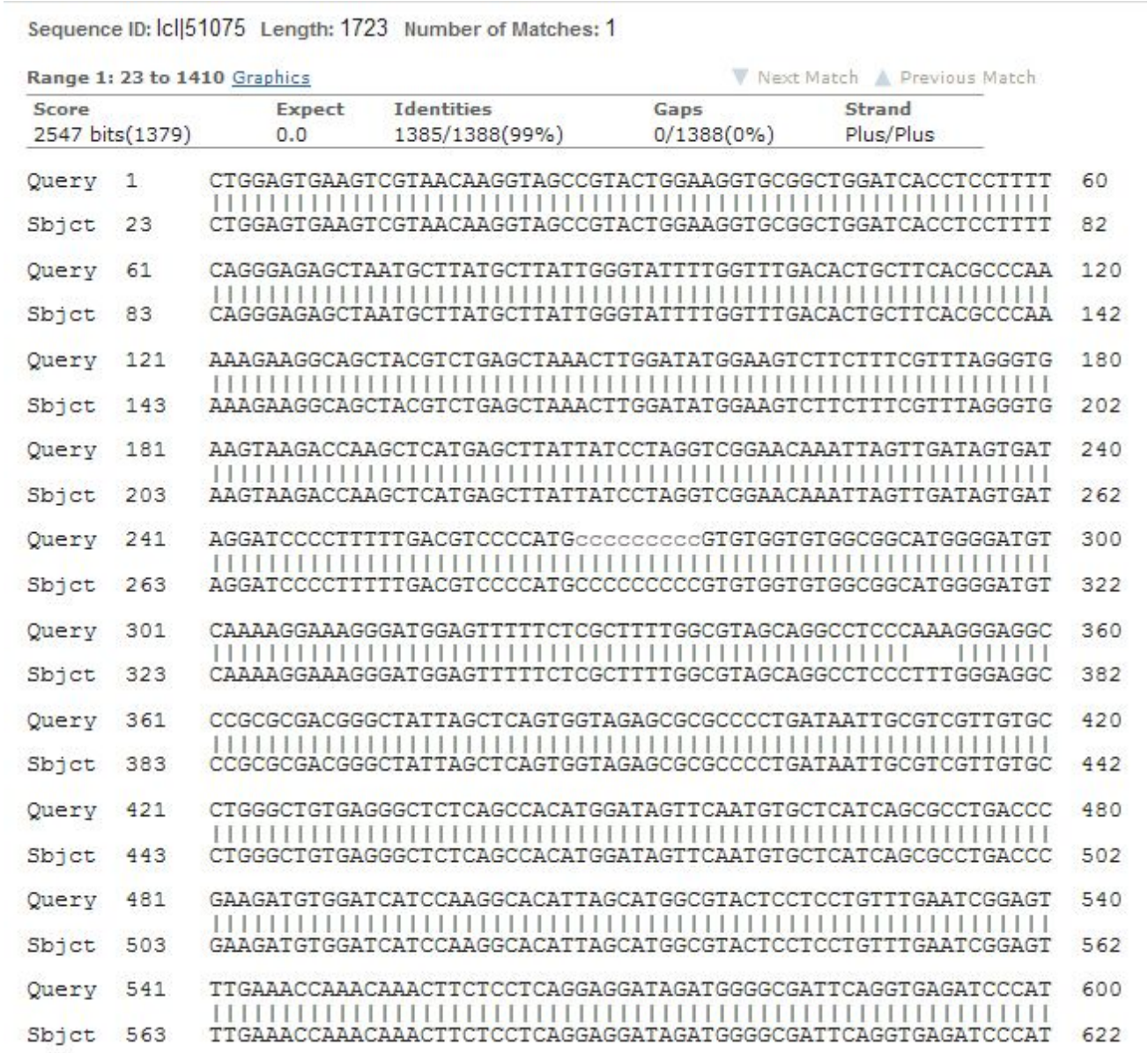


Cloning PCR – trnI-R
1300bp

4.5 Conclusion

About a month after I returned home from China, my supervisor Kuang Feiting contacted me with the official results of the DNA sequencing we had been waiting for. In the sequencing results from trnI-R, there were 3 base errors. These, however, will not influence the function of the vector, which means that we were successful in our experiment. The trnA-R sequencing results showed success as well. Now that the expression of the vector has been confirmed in E. coli cells with the first part of chloroplast transformation, the transformation vector will be delivered into leaves through particle bombardment (Patra). Once the transformed plant has matured and produced seed, the seed will be harvested and then checked for the expression of the transformation vector (Patra). This will complete phase two of chloroplast transformation. I believe that if the recombination fragments from our experiment result in a successful trial transformation of the vector into the actual rice plant, then we will have come one step closer to the third generation, and maybe even one step closer to fighting food insecurity.

Figure 6: Final Results of DNA Sequencing for trnI-R Recombination Fragments



Sequence ID: lcl|51075 Length: 1723 Number of Matches: 1

Range 1: 23 to 1410 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
2547 bits(1379)	0.0	1385/1388(99%)	0/1388(0%)	Plus/Plus

```
Query  1    CTGGAGTGAAGTCGTAACAAGGTAGCCGTACTGGAAGGTGCGGCTGGATCACCTCCTTTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  23   CTGGAGTGAAGTCGTAACAAGGTAGCCGTACTGGAAGGTGCGGCTGGATCACCTCCTTTT  82

Query  61   CAGGGAGAGCTAATGCTTATGCTTATTGGGTATTTTGGTTTGACACTGCTTCACGCCCAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  83   CAGGGAGAGCTAATGCTTATGCTTATTGGGTATTTTGGTTTGACACTGCTTCACGCCCAA  142

Query  121  AAAGAAGGCAGCTACGTCTGAGCTAAACTTGGATATGGAAGTCTTCTTTCGTTTAGGGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  143  AAAGAAGGCAGCTACGTCTGAGCTAAACTTGGATATGGAAGTCTTCTTTCGTTTAGGGTG  202

Query  181  AAGTAAGACCAAGCTCATGAGCTTATTATCCTAGGTCGGAACAAATTAGTTGATAGTGAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  203  AAGTAAGACCAAGCTCATGAGCTTATTATCCTAGGTCGGAACAAATTAGTTGATAGTGAT  262

Query  241  AGGATCCCCTTTTTGACGTCCCCATGccccccccGTGTGGTGTGGCGGCATGGGGATGT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  263  AGGATCCCCTTTTTGACGTCCCCATGCCCCCCCCCGTGTGGTGTGGCGGCATGGGGATGT  322

Query  301  CAAAAGGAAAGGGATGGAGTTTTTCTCGCTTTTGGCGTAGCAGGCCTCCCAAAGGGAGGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  323  CAAAAGGAAAGGGATGGAGTTTTTCTCGCTTTTGGCGTAGCAGGCCTCCCTTTGGGAGGC  382

Query  361  CCGCGCGACGGGCTATTAGCTCAGTGGTAGAGCGCGCCCCTGATAATTGCGTCGTTGTGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  383  CCGCGCGACGGGCTATTAGCTCAGTGGTAGAGCGCGCCCCTGATAATTGCGTCGTTGTGC  442

Query  421  CTGGGCTGTGAGGGCTCTCAGCCACATGGATAGTTCAATGTGCTCATCAGCGCCTGACCC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  443  CTGGGCTGTGAGGGCTCTCAGCCACATGGATAGTTCAATGTGCTCATCAGCGCCTGACCC  502

Query  481  GAAGATGTGGATCATCCAAGGCACATTAGCATGGCGTACTCCTCCTGTTTGAATCGGAGT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  503  GAAGATGTGGATCATCCAAGGCACATTAGCATGGCGTACTCCTCCTGTTTGAATCGGAGT  562

Query  541  TTGAAACCAAACAAACTTCTCCTCAGGAGGATAGATGGGGCGATTCAGGTGAGATCCCAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  563  TTGAAACCAAACAAACTTCTCCTCAGGAGGATAGATGGGGCGATTCAGGTGAGATCCCAT  622
```

Figure 7: Final Results of DNA Sequencing for trnA-R Recombination Fragments

Range 1: 94634 to 95454 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1517 bits(821)	0.0	821/821(100%)	0/821(0%)	Plus/Minus

```
Query  12     ACTTTCATCGTACTGTGCTCTCCAAAGAGCAACTCTTCTCAAAATCTCAAAACAAAAGGT  71
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95454  ACTTTCATCGTACTGTGCTCTCCAAAGAGCAACTCTTCTCAAAATCTCAAAACAAAAGGT  95395

Query  72     GCTGAGTTGGAATCCCATTCTAAGGATTCTTGTGGTTCCGGGGAATCCAGCTACAGGAGA  131
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95394  GCTGAGTTGGAATCCCATTCTAAGGATTCTTGTGGTTCCGGGGAATCCAGCTACAGGAGA  95335

Query  132    ACCAGGAACGGGGAGCTCTCCCCTTTTTCCGCCCGACTCTTTGATCTTAAACTTAAGAAT  191
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95334  ACCAGGAACGGGGAGCTCTCCCCTTTTTCCGCCCGACTCTTTGATCTTAAACTTAAGAAT  95275

Query  192    GCTGGTTTTAAGAACGAGTGATTGCCCTTCTCCGACCCTTACTGCCCAACCGGAGAGCGG  251
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95274  GCTGGTTTTAAGAACGAGTGATTGCCCTTCTCCGACCCTTACTGCCCAACCGGAGAGCGG  95215

Query  252    ACGGCTAATGTGTTCCACTTATTGAACAGGGTCTATGGTCGGTCCGTGACCCCTGGACGC  311
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95214  ACGGCTAATGTGTTCCACTTATTGAACAGGGTCTATGGTCGGTCCGTGACCCCTGGACGC  95155

Query  312    CGAAGGCGTCCTTGGGGTGATCTCGTAGTTCCTACGGGGTGGAGATAATGGGGTCGGTCC  371
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95154  CGAAGGCGTCCTTGGGGTGATCTCGTAGTTCCTACGGGGTGGAGATAATGGGGTCGGTCC  95095

Query  372    ATGGATTTTCCTTCCTTTTGCCACATTTCGCTCAAAGGGTTGAAGGGAGATAGTGCATCA  431
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95094  ATGGATTTTCCTTCCTTTTGCCACATTTCGCTCAAAGGGTTGAAGGGAGATAGTGCATCA  95035

Query  432    AGCTATTCGCAAGGGCCAACTTGATCCTCTTCCCCAGGGATCCCAGATGAGGGAAGCCTA  491
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  95034  AGCTATTCGCAAGGGCCAACTTGATCCTCTTCCCCAGGGATCCCAGATGAGGGAAGCCTA  94975

Query  492    GGAGAGCCGCCGACTCCAACTATCGTCCATGTACGATCCATACTAGATCTGACCAACTGC  551
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  94974  GGAGAGCCGCCGACTCCAACTATCGTCCATGTACGATCCATACTAGATCTGACCAACTGC  94915

Query  552    CCATCCTACCTCCTCTACCTTTTTGACAGCCCATCTTTTTGTCTCAGTAGAGTCTTTCAG  611
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  94914  CCATCCTACCTCCTCTACCTTTTTGACAGCCCATCTTTTTGTCTCAGTAGAGTCTTTCAG  94855

Query  612    TGGCATGTTTCAGTCCTCTTCCCCATTACTTAGAAAAAGTGAGCCACCGGTTCAGGTACA  671
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  94854  TGGCATGTTTCAGTCCTCTTCCCCATTACTTAGAAAAAGTGAGCCACCGGTTCAGGTACA  94795

Query  672    AGATACTACCATTACCGCCTGGACAATTAGACAGCCAACCCGTAATCGCAACGACCCAAT  731
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  94794  AGATACTACCATTACCGCCTGGACAATTAGACAGCCAACCCGTAATCGCAACGACCCAAT  94735

Query  732    TGCAAGAGCGGAGCTCTACCAACTGAGCTATATCCCCCCGAGCCAAGTGGAGTATGCATG  791
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  94734  TGCAAGAGCGGAGCTCTACCAACTGAGCTATATCCCCCCGAGCCAAGTGGAGTATGCATG  94675

Query  792    AAAGAGTCAGATGCTTCTTCTATTCTTTTCCCTGGCGCAGC  832
              |||||||||||||||||||||||||||||||||||||||||
Sbjct  94674  AAAGAGTCAGATGCTTCTTCTATTCTTTTCCCTGGCGCAGC  94634
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V. Personal Reflection

When all is said and done we usually tend to reflect upon the days when work was rewarding, when our experiments were successful and our results were meaningful. Of course there were days such as these during my time in China, but I also feel it important to remember the days when work was sometimes stressful or disappointing. There were days when I would walk into a room and co-workers would be having a heated debate over a lab table or in front of a computer screen with results displayed on it. Their voices would become louder and louder and they would gesture with their hands in a heated or passionate manner. There was one time when I witnessed even Professor Yuan Longping engage in such an argument. I was playing volleyball with some of the other researchers from the center after dinner. Professor Yuan and his wife were watching in the audience, and had even stepped in to play a few serves. Everyone was having a good time. Then a woman walked into the building, went straight to where Professor Yuan was sitting and said something to him. Professor Yuan started to converse with her in Chinese. I could tell that what she had said was not good news, because his voice kept getting louder and louder until he was almost yelling. I was shocked because I couldn't tell what was being said or why the conversation had escalated so quickly. My supervisor and her co-workers explained to me that the woman had lost a very important sample that Yuan Longping had spent months developing and analyzing. At first I was shocked that such arguments would take place in such an open display and in a professional workplace, but then I thought more about where I was and my purpose for being there. People at the CNHRRDC are not just professional scientists and researchers, they are members of a community. They live in a country where food security is not guaranteed, and where the effects of hunger can be seen on a daily basis. Most of my co-workers come from rural farm villages, and they can see how their relatives struggle to make a living so that they can put food on the table. In the United States, an open display of conflict and frustration such as this would be deemed inappropriate. In China, however, it demonstrates the passion and significance that people feel for their work. I think that this is one of the aspects of my experience that changed my perspective the most. In high school, science was just a class and a grade that I needed to get to college. In China, science is a means of feeding your country, your community, your friends and neighbors, your family. That is the context in which science should be viewed all over the world, and I am so grateful that China has given me this realization that I might otherwise have been blind to.

VI. Photos

VII. Works Cited

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