Elliot Suiter

The World Food Prize

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THE WORLD FOOD PRIZE

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Abstract

The average farmer in China has about 1/2 of an acre to farm on. This is due to the huge population, limited amount of farm ground, and also government regulation. In China, farmers do not own their own farm land; instead, the government allows a family to have a certain amount. If they want to expand, families are unable. For many, 1/2 of an acre is not enough, so farmers in China may end up having another job in the city, or abandoning farming altogether. By improving the genetics of grain producing plants, farm ground may be more productive. By making farm ground more productive, poverty and hunger are reduced while quality of life is improved.

The Chinese Academy of Agricultural Sciences (CAAS) is a far-reaching research organization that strives to significantly reduce poverty and hunger throughout China. To this end, the goal of research conducted in the lab of Dr. Xueyong Li is to isolate genes that control certain traits exhibited by rice plants in order to better understand the rice plant.

My research project was to utilize gene mapping to locate a specific mutation responsible for reduced tiller number in a particular strain of rice plants. The methods employed included DNA isolation, gel electrophoresis, and polymerase chain reaction. Additionally, experience and knowledge were gained through fieldwork and by assisting other lab projects involving fine mapping and transgenic matching.

The results of my project were largely inconclusive due to technical difficulties and time constraints. However, my research work laid some necessary groundwork and can be used by future interns or graduate students to complete the project.

Acknowledgements

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I would like to thank my Global Youth Institute group leaders; Allie Wilson and Paige Myers for making GYI such a great experience. Those three days were when I realized how much interest I actually have in the issues of conservation and helping to end world hunger and that I really

wanted to pursue the Borlaug-Ruan Internship opportunity. Without their leadership, I may not have gotten excited and applied for the internship.

I am so grateful to Dr. Xueyong Li for taking me into his lab this summer and for sharing his scientific knowledge with me. Dr. Li is a great teacher and I was so fortunate that he took the time to answer my questions and explain things to me. I couldn't have asked for a better mentor for my internship.

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Chinese Farmer Profile

The arable land in China accounts for 10% of the worldwide supply, yet this 10% of the world produces 20% of the food. Before 2004, China exported more food than it imported, but in the last 11 years, this trend has switched. Although food production in China is at an all-time high, each year, the amount of imported food grows, and more food than ever is being shipped there (Carter, CIA).

In China, farmers do not own their own land. The average farmer is allowed to lease around one half acre of land from the government, and must try to support their family and make a living from that amount of land. Government policies regulate crop production and pricing in an attempt to maintain the country's food self-sufficiency.

Poverty is common among rural farm families, particularly in the western and inland provinces. The urban versus rural wealth disparity in China is huge, and it is only growing. The Chinese government is employing various strategies to reduce poverty for farmers, including support for agribusiness investment to improve technology and management, the development of farmer cooperatives, and financial assistance for transportation infrastructure. Improvements in seed varieties and increased use of fertilizers and irrigation have helped farmers increase crop yields.

Individual farmers in remote areas often lack information about marketing and prices, so they are often unaware of what crops are in demand. Because of this, they end up planting the crop that produced the most revenue the year before. This wouldn't be a problem, but if thousands of farmers do this, then the market is flooded with one or two crops and there are shortages of others.

Farmers in China also face the harsh reality of a growing urban versus rural wealth disparity. As of 2014, 34% of China's labor force was employed in agriculture (CIA). However, there are higher-paying jobs in the cities, as well as many amenities not found in the countryside. As a result, over 250 million rural migrants have left the agricultural sector in search of more productive jobs in industry and services (World Bank). The government provides additional incentive for farmers to move into cities by offering housing in the city and encouraging farmers to hand over management of their plots to corporations in return for a payment.

In the past two decades, China has become more and more a market economy. Standards of living have increased as more people move to the cities looking for a better life. The government of China views urbanization as a stimulus for economic growth because it increases domestic demand for products. With the growing Chinese middle class, the demand for better, more nutritious food has increased. In addition to the traditional crops such as rice, wheat, and corn, farmers are also growing more fruits, vegetables, and specialty crops to meet the demand. This is why genetic research is so important. With higher yielding rice, the farmer can grow more food to support the population of China, reducing hunger while also improving their own economic state.

Chinese Academy of Agricultural Sciences

The Chinese Academy of Agricultural Sciences (CAAS), established in 1957, is a research organization funded by the Chinese government. Its headquarters are in Beijing, but the Academy oversees 42 different institutions spread across China. The lab I worked at was part of the Institute of Crop Sciences, which is one of the 42 institutes, and also part of the Graduate School at CAAS.

CAAS focuses on research first and foremost, and the graduate school is a portion of all that they do. All in all, CAAS consists of over 5000 researchers and 4300 students ranging from masters to post-doctoral programs.

The overall mission of the organization is to significantly reduce poverty and hunger throughout China. In order to do that, CAAS employs the best and the brightest minds available, and uses advanced research methods to garner the best results.

Dr. Li's lab, which I was a member of this summer, focuses on rice morphology, or plant architecture. The main goal of this research is to improve the rice plant, making it stronger and capable of producing more grain. In order to accomplish this, the lab uses many advanced research methods. The traits focused on in the lab include tiller number, leaf size, plant height, grain weight, and panicle size.

Most of the rice plants grown by the members of the lab are not ready for the market, and in many cases produce much less grain than standard rice plants. These hybrid, mutant, and otherwise genetically modified plants are created strictly for genetic research, trying to learn more about what makes rice exhibit certain traits. Once traits are isolated, and the genes that control them are found, it is much easier to amplify the favorable traits and eliminate the not so favorable ones.

Dr. Li selected a research project for me that fully supported the goals of the lab. In short, my project was to utilize gene mapping to locate a specific mutation responsible for reduced tiller number in a particular strain of rice plants.

Introduction

I loved working in the lab I was assigned to this summer. The other students and researchers took me in as one of their own, and treated me as an equal. The project assigned to me was the same gene mapping assignment that any incoming master's student starts off with. The work I did while in Beijing was both a great learning experience for me, and helpful to the research goals of the lab. This made the time I spent there feel all the more valuable.

The following report is just a snapshot of the work done in Dr. Li's lab. The projects I worked on are all part of the common goal, and I leave behind my research and my footprint. Others to follow will pick up where I left off and eventually come to more conclusive results.

Fieldwork

I arrived in Beijing at a very critical and busy time of the year. In the springtime, rice is planted from seed into clumps in the seedling bed. By clumping, space is saved, and poor survival rates are made up for. If planted in a row, there would be gaps where some seeds did not survive, but the clumping means this can be avoided. When the rice seedlings are about four weeks old, they begin to run out of space, and they must be transplanted and spread out into other fields. I arrived just in time that my first day at work was the first day of this project.

In much of China, the transplanting process can be done with a machine, but since the rice planted by the lab is for research, this job must be done by hand in order to ensure every plant gets put in the proper place.

The rice we were working on was planted in clumps or "families" each descended from a different plant in the M_1 generation. The task was relatively simple: picking each seedling, washing the roots of mud, and tying it into a bundle with the other seedlings in its respective

family. These bundles were then transferred to paddy fields where they were untied and planted in rows.

We set up the field using a system of stakes with parallel lines running across the field. There would be a meter and a half wide strip of crops alternated with 1/3 meter wide walkways. The rows of rice seedlings were planted perpendicular to these lines, making many short rows that could be easily observed and marked for phenotypic differences.

We started with one field of rice (approximately $1/6^{th}$ of an acre), and ended up with almost ten fields completely full of rice, ready to grow to maturity. My first two weeks in China consisted of fieldwork just like this. My first week was at the Chinese Academy of Agricultural Sciences Institute of Crop Sciences fields in Changping (North Beijing). The second week, Dr. Li, Chun Yan and I traveled to the Shandong Rice Research Institute (a collaborator with Dr. Li) with some rice seedlings of a different variety to plant there.

The warmer climate and longer growing season in Shandong Province were better suited for these plants. While there, we also helped set up a few fields (in the manner described above) that were to be planted with seedlings belonging to the Shandong Rice Research Institute.

While the fields at Changping are small and used solely for research, the fields in Shandong are much larger and used for breeding. The end goal of the Shandong Rice Research Institute is to get a variety of rice out into the market. Much seed is produced and also tested to make sure it will perform well. While I was in Shandong, I learned much more about how genetic testing can turn into actual results and yield increases. I also learned that in China, every province uses different varieties of rice best suited for their climate. Shandong does not seem very far away from Beijing (about 500 kilometers south), but the varieties of rice planted at the two locations are very different.

Project Introduction

Dr. Li's lab locates specific genes and checks their functions. These genes are then used in in the process of improving rice yields by institutes such as the Shandong Rice Research Institute. They will work with the genes found by labs like Dr. Li's, trying to use them to improve rice varieties, and eventually bring them to the market. Genes that negatively affect the production of food can be covered up, or cut out of the rice plants. Genes can also be overexpressed, or moved to different portions of the DNA to serve different functions.

After we returned from Shandong, I spent two days getting to know the lab. I helped Chun Yan isolate DNA and run polymerase chain reaction (PCR) and agarose gel. After those two days, I began working on my own project. I was able to work independently and determine how to schedule the various stages of my research. I was by no means alone, though. There was always someone else in the lab working on their own projects, and if I would have a question, all I had to do was ask. They were all very eager to help me with whatever problems I might come across, or find someone who could help me.

I did realize quite soon, that working independently also had potential drawbacks. If I made a mistake without realizing it, my results would be completely off and I wouldn't know why. I could do something wrong for weeks and not even know what my problem was. Thankfully, I learned early on to ask questions, which proved a very useful habit as time when on.

My project was to map a gene to try to find out why a certain trait exists in a rice population. I was given 36 leaf samples from rice plants grown during the 2014 season. Each of these plants was part of the F2 generation of a hybridization between SD11 and Dular. Dular is a common Indicia rice variety, while SD11 is a mutant version of the Japonica Variety SD808.

The SD808 seeds had been treated with the chemical mutagen ethyl methanesulfonate (EMS), and SD11 is a mutant selected from the offspring of SD808 that grew up with a reduced number of tillers. The average SD808 plant may have up to 15 tillers, but SD11 has a maximum of about three. Tiller number in the F1 generation that resulted from SD11/Dular hybridization was uniform and normal, however, in the F2 generation, plants segregated into two groups. Three-quarters of them had normal tiller number while one-quarter had reduced tiller number. Tillering is an important aspect of rice growth, because each tiller is limited to one grain-growing panicle. The more tillers a rice plant has, the higher yield potential it has.

It was hypothesized that the reduction in tiller number in some of the F2 plants was caused by a gene that was mutated and had lost its function. It was my job to find and identify this specific gene.

Explanation of Methods - DNA Isolation, Polymerase Chain Reaction, Gel Electrophoresis

-DNA Isolation (see attached protocol, Appendix A)

In order to determine which gene was responsible for the mutation, it was first necessary to isolate the DNA from my leaf samples. First, the samples were frozen in liquid nitrogen, and ground up in a shaker. This process breaks down the cell walls in order to initiate the process. Cetyltrimethyl ammonium bromide (CTAB), an extraction buffer, was then added to this leaf powder, and the mixture was incubated in a 65 degree Celsius water bath for an hour. CTAB is a detergent that breaks down the cell and nuclear membrane, exposing the DNA. CTAB also contains a buffer, which keeps the pH of the solution very close to constant. A buffer acts as both an acid and a base, and is able to resist changes in pH. DNA is very fragile, and changes in pH can render it useless, so keeping it in a buffer is very important.

To get rid of protein and pigment from the extract, a mixture of 24:1 Chloroform:Isoamyl alcohol was added to the extraction buffer solution and mixed well. After centrifugation, the upper aqueous phase contained DNA, whereas protein, pigment, and debris were in the lower organic phase. The aqueous phase was collected from the top of the microtube and transferred into a new tube. The addition of two volumes of ethanol caused the DNA to precipitate, or separate out. The solution was frozen to -20 degrees Celsius for one hour, and then centrifuged.

The DNA congregated at the bottom of the tube. Once the liquid was vacuumed off, there was just DNA left at the bottom of the microtube. Water was added in order to dissolve the DNA pellet.

After DNA isolation was complete, it was necessary to run the genomic DNA using agarose gel electrophoresis to check the quality and concentration of the isolated DNA. Then the DNA solution could be used as a template for the PCR reaction.

-Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a very common and often indispensable method of DNA amplification that relies on thermal cycling to separate and copy specific chunks of the chromosomes.

This is done using various primers which correspond to different markers on each chromosome. The markers used in my process were named Indel (short for insertion/deletion). Each of these primers has a forward and reverse phase that must be mixed together and is driven by the Taq DNA polymerase. For my PCR experiments, five solutions had to be combined: DNA, water, 2X Mix, and both forward and reverse primers.

In prior years, there were even more things to add into the PCR tube. The PCR reaction requires a buffer to keep the DNA stable, dNTPs (the four nucleotides), and the Taq DNA polymerase. Nowadays, these three components come pre-mixed from a bio-supply company in the form of 2X Mix, making my job quite a bit easier. For my experiments, I combined 1 microliter (µl) of each of my individual DNA samples with 3 µl of double-distilled-water, 5 µl of 2X Mix, and 0.5 µl each of a forward and reverse primer.

The heating and cooling cycles of PCR each have a specific function. In the heating cycle, the double helix of DNA is broken apart to become a single strand, exposing the genetic sequence. The genome of rice has been sequenced, and each specific chunk of each chromosome has its own marker. The primers that were used correspond to these markers, and will bind to the single-strand genomic DNA in these specific places during the cooling cycle of PCR. Once the primers join with the DNA, the Taq DNA polymerase will make copies of the DNA fragment between the forward and reverse primers. This is done many thousands of times, amplifying this section of the DNA. By amplifying the DNA, it is much easier to work with, and the marker that is intended to be studied will show up much more clearly on the gel.

-Gel Electrophoresis

Two types of gel were used for electrophoresis – agarose gel and polyacrylamide gel. Agarose gel was used first because it is quick and easy to make, and can be used to determine if DNA has been properly isolated. One percent agarose gel consists of 1 gram solid agarose mixed with 110 milliliters (mL) 1x TAE (Tris-acetate-EDTA) buffer. This mixture was heated in a microwave oven and poured into the gel form. A minute amount of ethidium bromide (EB) was added to this mixture as well. When EB comes into contact with DNA, it becomes a fluorescent marker that

can be viewed under ultraviolet light. A piece of plastic called a “gel comb” was placed so that the teeth displaced the liquid gel and formed wells as it cooled.

The gel was allowed to cool for 20-30 minutes, before the gel comb was removed and the gel was taken out of the form and moved to the electrophoresis box. Five µl of the PCR solution were added to each of the wells in the gel, along with 5 µl of a marker in the first well. This marker consisted of DNA fragments of various sizes that travel at different speeds through the gel. These fragments formed multiple bands that looked like a ladder next to the bands of the testing DNA sample. Based on where the DNA showed up in relation to the marker, the length of segments could be determined.

Gel electrophoresis works based on the porous nature of the medium the DNA will travel through. Gel is a porous matrix that the DNA can slowly travel through when energy is added in the form of electric current. Negative and positive electrodes were hooked up at either end of the gel, which was suspended in Tris-borate-EDTA (TBE) buffer. DNA is negatively charged, so it moved toward the positive terminal through the gel matrix.

The gel electrophoresis basically separated out the DNA by size. The smaller fragments traveled more quickly through the gel, while the larger ones moved more slowly. This difference in travel speed created bands on the gel at different intervals, depending on how long the DNA segment was. These bands could be studied to learn more about the genetic makeup.

While agarose gel is very quick and easy to make, it is unsuited for gene mapping due to the random nature of the gel matrix. Agarose gel is formed by phase change (freezing), which creates a fairly random gel matrix. This makes the gel results less precise, and unable to track minute differences in segment lengths, since the size of the porosity varies.

This is where polyacrylamide gel (PAGE gel) becomes useful. Instead of being formed by phase change like agarose gel, PAGE gel is formed by a chemical reaction, which makes a much more uniform final product. For this enhancement in quality of results, PAGE gel is more difficult and time consuming to produce and test.

To make PAGE gel, 10 mL 10x TBE, 20 mL 40% acrylamide, 1 mL ammonium persulfate (AP), and 100 mL Tetramethylethylenediamine (TEMED) were added to 70 mL water.

The resulting mixture was poured between two panes of glass. Care must be taken, as bubbles can form when this is done. A flat plastic tool was used to work the bubbles out. A gel comb was added, and it was allowed to sit for 30 minutes. During this time, the chemicals reacted with one another and the gel solidified.

The gel (still between the glass panes) was then placed in the electrophoresis box. After 0.5x TBE was added, the combs were removed and the wells were filled with PCR solution. Electrophoresis was run at 180 volts for 1.5 hours.

PAGE gel is clear, and nothing is visible on it, even after electrophoresis. The gel had to be stained in order for the bands to show up. The process used is called silver staining. First the TBE was rinsed off using distilled water. The gel was then submerged in a washing buffer consisting of aqueous silver nitrate (AgNO3) and shaken gently for 10 minutes. After this, the gel was again rinsed with distilled water, and then submerged in a solution called the color substrate. This consisted of sodium hydroxide (NaOH), formaldehyde, and water. This was then shaken gently for seven minutes. During this time, the residual silver reacted with the color substrate, the gel turned a brownish yellow color and the bands appeared.

After the gel was rinsed with water again, it was enclosed in a sheet of plastic wrap to keep it intact. The gel could then be studied, written on, and photographed.

Care must be taken throughout this process, and I had some issues with my gels falling apart. If the mix of chemicals was off, then the gel became very delicate. Piecing these paper-thin gels back together was difficult, so I learned early on to take utmost care throughout this process.

If too much TBE was added to the gel mixture, it would solidify almost immediately, making it impossible to pour into the forms. Sometimes I would rack up enough PCR's all at once to require up to 12 gels. This was a challenge, because of the limited time before the gel solidified. I learned that by adding just a couple mL less of TBE, the gel would take much longer to solidify and I could fill all of the forms before the gel solidified in the flask.

Interpreting Data

After a PAGE gel was run, it was important to analyze the data. I ran gel/PCR in three different manners: using a single pool, three pools, and each sample individually.

Once my DNA was isolated, I started the process of primary mapping. Each DNA sample was tested for concentration using an ultraviolet spectrometer. Ten samples of similar quality were chosen from the 36 samples and combined together to make a pool.

Since each pool consisted of ten different samples, many plants could be tested at once, giving more accurate results with less false positives. With ten different DNA samples combined to make one band, the comparison was much easier, as any outliers would greatly skew the data. Each time a primer was tested, the mother, father, F1, and pool/individuals were done at the same time. Comparing the resulting bands to one another determined the results.

Each primer was tested with the mother (SD808), father (Dular), F1 (SD808/Dular), and the pool (SD11/Dular). When a gel was run, it was important to note if the mother and pool had similar bands. The mother is the variety that was mutated to create my population, so it contains the gene I was looking for. If my F2 population matched the mother, then there was a possibility that the marker is linked.

Many other results can also show up on the gel. If all four bands matched when a gel was run, this meant there was no polymorphism. All of the DNA samples have the same sequence in this location, and no further testing was needed.

The F1 population was a mix of both of the parents' DNA, and showed both bands when the gel was run. If the pool had the same bands as the F1, this meant that the mutation was not in this location, as my pool was a perfect mix of both the unmutated mother and father.

If no bands showed up at all, there was a problem with the primers. To solve this, the mother solution was diluted again to make a new working primer. If there was still a lack of bands, then new primers were ordered from the biotechnology company.

After I tested each marker using the single pool compared with the mother, father, and F1 population, I interpreted the data obtained. I then individually tested the markers where the pool and mother bands were similar. This process was the same as before, but much more time consuming. I would still have the mother, father, and F1 population, but also each of the 36 individual DNA samples.

By lining up each of the DNA samples next to one another on a single PAGE gel, it was much easier to see which band the majority of the plants had. This method also eliminated the possibility of false positives, as all of my data was out in the open.

Primary Mapping Conclusion (see attached photos, Appendix B)

After I had gone through all of the markers using the single pool method, and all of the likely markers using each individual DNA sample, I realized something must be wrong. I still had not found a definitive result. I talked with Dr. Li, and he gave me the idea of going back through the remaining markers that I had not yet tested individually, this time using three pools. The idea of using three different pools was that it would be more accurate and would provide less false positives than using just one pool, but would take less time than testing each DNA sample individually again.

About a month into working on my project, I had worked through all of the markers, all of the individual PCR's, and all of the markers again using three pools. Every test I did turned out to be a false positive. With less than two weeks remaining in Beijing, I talked to Dr. Li again about the problems I was having, and we decided that my DNA samples were not very strong. This could have been caused by multiple factors, but most likely was due to degradation over time, or the methodology in isolating the DNA. The samples I had been given at the beginning of the summer were grown during the 2014 season, so they were already almost a year old when I started my experiments. Plus, once I isolated them, they were stored in a four degree refrigerator instead of the -40 degree freezer they had been in, allowing the DNA to degrade even more quickly.

While I was present at CAAS this summer, a new master's student came to the lab. She started her own gene mapping project with another set of DNA, and was having similar problems with

the bands showing up too lightly on the gel, or being a smear (instead of a clearly defined band), indicating poorly amplified DNA and possible contamination of RNA.

Dr. Li did some searching and found a different technique for us both to try that used not one, but three different buffers to initially break down the cell and expose the DNA. With more DNA being extracted from each cell, the amplification process was much more likely to work properly. After working with the new student to isolate some of her samples, we came to the conclusion that the problem did not lie in the isolation, but instead in the age of the leaves. We isolated six samples of Dular (wildtype): three with fresh leaves and three that were a year old. Only two of these samples ended up having usable DNA, and they were both from the newer plants.

There are many possible reasons why I was not able to find my gene this summer. Based on my experiences and discussions with Dr. Li, I think the most likely explanation was the age of my DNA samples and their degradation. Other possible explanations for not finding my gene are environmental factors and the primers I used.

Given the small sample population (only 36), many environmental factors could have influenced phenotype. When searching for a gene with lower recombination rates such as mine, it is a good idea to have a sample population upwards of 500 strong. The sample could have been affected by any number of environmental factors that could reduce plant tillering ranging from the simplest such as the field being flooded for too long, or the summer being too hot, to things like fertilizers, soil quality or plant density in the field. Environmental factors are not as likely to be the actual cause for the phenotypic differences, but taking all possible explanations into account unless proven otherwise is what science is all about.

The primers I used for my experiments could also be a problem. They were designed based on the genetic makeup of common rice varieties. The SD11 mutant and its offspring have never been mapped before, and there could be other, unknown problems causing poor results.

This is where all of the field work becomes so important. It gives the lab new DNA samples every year, and also provides the ability to test the new mutants, hybrids, and transgenics that they create in the lab. I did not find the gene for SD11/Dular, but new samples will be grown, and another master's student can try again, while avoiding some of the setbacks that I encountered.

Fine Mapping

After my attempt to primary map SD11/Dular, I had about two weeks left in the lab. In order to help me learn about the process involved, Dr. Li gave me some new samples to fine map. This grouping was also a mutant population with reduced tillering, but this time between S2-226 (mutated Nipponbare) and Dular. Another student had already primary mapped this population, and determined the mutation to exist between markers 8-8 and 8-9 (chromosome eight). All I had to do was narrow the search, and find the exact location of the mutation.

This new population was much larger, at 144 plants, so the results were much more likely to be accurate. This also meant that everything I did to this population took four times longer than my old population of 36 plants. DNA isolation alone took two full days, which is not a good sign when you're under a time crunch.

The process of fine mapping was identical to primary mapping, with the only difference being the primers used. Special primers needed to be designed to cover shorter portions of the chromosome, so the mutation could be really zeroed in on. While the standard Indel primers I had been using all summer covered about 200 bp (bit pairs) of DNA each, the seven primers designed for my fine mapping project covered an area of just 20 bp each.

I started by doing PCR and PAGE gel for each of my 144 DNA samples plus Nipponbare, Dular and F1 with three of the new primers, 8-8, and 8-9. I put the results of these gels in an Excel document to more easily view the results. Looking at the bands that showed up and comparing each DNA sample with itself in each primer could narrow the location of the mutation once again. It was determined that the mutation probably existed between markers 8-8 and 8-821. When I ran the other four new primers to narrow this once again, I ran into a problem. The primers designed for my use allowed too many bands to show up for each DNA sample: about five to six bands when there should be just one to two.

Unfortunately, by the time I found this problem with the primers, it was too late to order new primers, so my project was once again cut short. However, as with my primary mapping project, some foundational work has been done, and another student will pick up where I left off in the future.

Challenges Faced

Over the summer, there was definitely a learning curve associated with many lab procedures and the use of equipment. I made a few mistakes and had some setbacks along the way that I would like to highlight in this section of my report.

Many times, I would run a PCR overnight, so that the results would be ready in the morning, and I could make a gel. I suppose I was more tired than usual one morning, and forgot to add water to my PAGE gel mixture. I didn't realize this at first. When the gel heated up in the Erlenmeyer flask, immediately solidified, and started smoking, I knew I'd done something wrong. I eventually went back to the lab table and found 140 ml of water measured out, and ready to make my gel.

There was about a week and a half of work when none of my gels would show bands. I came up with possible theories for the problem, tried different combinations, and ended up ruling out the gel, the 2X Mix, and the PCR temperature as possible causes. Eventually I found that my primers were the problem. I had diluted them about three weeks prior and then kept them in the four degree Celsius refrigerator. It was thought that this would be fine for the short amount of time that I would be there, but science isn't always perfect. I had to start over, diluting new primers,

and then storing them at -40 degrees Celsius. This setback took quite a bit of my eight weeks and prevented me from getting as far in my research. However, it is all part of the learning experience, so my efforts were not wasted.

I learned on my trip just how big the world is and the importance of accurate communication when dealing with complex scientific processes. If I did not recognize foods, or equipment and processes in the lab, translation apps became necessary. There are so many things that either don't have a translation, or that I don't recognize even in English. There was a progression to figuring things out. First I would see if anyone in the lab knew the translation and if not, then the translation app came out. If I didn't even recognize the English name, Wikipedia became necessary to make me a pseudo-expert on the topic (just enough to quell my curiosity).

Projects Assisted

At many times throughout the summer, when my project did not need attending, I would have the chance to work with my lab mates on some of their projects. Everyone was always happy to have my help, and I was able to learn a lot more lab procedures and get a more rounded experience overall. The other members of my lab were working on many different projects, but a lot of what they were doing was studying protein functions of certain genes, and transgenic matching, which is what I helped with quite a bit.

After a gene has been identified, transgenic matching is used to check its function. The new gene is isolated from the DNA of the plant being studied, and implanted into stem cells of a normal rice plant. If the resulting seedling grows up with the targeted trait, the gene is known to be correct. I also helped grow calluses, sorted seeds, planted seeds and watered plants in the greenhouse, and helped with bacteria culture growth, as well as the field work my first two weeks there.

The Big Picture

I simply worked on mapping a gene while I was at CAAS. This is the first step taken in the lab by all new students, and is just a small introduction to all of the work that is done there. Knowing the location of a genetic mutation is minimally useful on its own. It is what is done with this information that truly helps improve food security.

The Chinese people do not believe in wasting food. Meals are served family style, and everyone uses their chopsticks to eat from the serving platters directly. Unlike much of the world, where you eat a meal until you are full, in China, you eat a meal until the food is gone. Everyone pitches in to help finish the food that was ordered.

This is a great way of keeping consumption and food waste in check. There is no ordering of extra food that you just throw away. If everyone avoided wasting food as the people in China do, food security would be impacted greatly.

Personal Impact

I initially thought of the World Food Prize youth programs as a quality learning opportunity, but not something that would be a high priority in my life. My plan all along was to write a paper, go to the Iowa and possibly the Global Youth Institute, and that would be the end of it. At that time, I was not interested in pursuing the opportunity that is the Borlaug-Ruan Internship. I did not think I was qualified enough to get such an internship, and even if I did get it, I felt I would have trouble leaving home for so long.

This thought pattern changed once I got to the Iowa Youth Institute in my roundtable and started discussing the papers of both myself and my group. I was really surprised by how enthralled in the conversation I became. It was exciting how a group of high school age students were able to come together, never having met before, and have such a high level discussion. We talked about the similarities of our papers, and besides the obvious lack of water and food, we kept coming back to education. Education was a theme in almost every one of our papers as a necessary step in solving both hunger and poverty. It made me realize how lucky we are in the U.S. to have access to such a good educational system, but also how universal of a need it really is. It also helped me to recognize that by not taking each educational opportunity afforded to me, I was doing myself a disservice. This is what initially got me excited about attending the Global Youth Institute in the fall, and getting everything out of the experience that I could.

By participating in the Global Youth Institute, I really came to understand just how awesome the program truly is. I had never been around so many people with whom I could have insightful discussions about worldly topics, and furthermore, I had not realized that I was so passionate about helping conserve our world's resources and feeding the population. I talked with multiple people who had been previous Borlaug-Ruan Interns, including my group leader Allie Wilson, and the positive response was tremendous. I did not hear about a single intern having a negative experience. During my time at the Global Youth Institute, I decided that I would try my best to be a 2015 Borlaug-Ruan Intern. I went home after the weekend, excited about what I had learned, and began doing some research about the Borlaug-Ruan Internships. Seeing the different locations and types of research that I could potentially experience, only made me more eager for the potential summer to come.

I'll never forget the moment I learned that I was selected to be a Borlaug-Ruan Intern in Beijing. It was incredibly exciting news and I couldn't wait to get started. The following weeks were filled with a flurry of activities and preparations for the trip. Up until the night before I left for Beijing, I was not nervous. The trip seemed so far off, just like Beijing. The moment it finally hit me was when my family was trying to decide where to take me out for dinner the night before I was to leave. This was going to be my last meal with my family for two months. My last chance to eat a steak and drink readily available cold water for the rest of the summer (I didn't know that at the time). I was suddenly quite nervous for what was to come. Thankfully, my brother talked me through it, and I was good to go.

I was very fortunate in travelling to Beijing, as another intern from Iowa, Sam Reicks, was also to spend the summer in the city. This meant we were both able to leave from Des Moines, and be on the same flights all the way there. When Sam and I left the airport terminal in Beijing, we

found ourselves in front of a huge line of people all holding signs or waiting for loved ones. Sam found her name right away, and was greeted very excitedly by two girls from her lab. For me, it was not so easy. It took what felt like hours to find the graduate student from my lab, although it couldn't have been more than a minute or so. Thoughts of being alone at the airport or Dr. Li forgetting to send someone to pick me up raced through my head. I did not have long to worry though. As soon as I found my name and introduced myself, Cao Jian was taking my luggage and rushing me through a crowd of people to the street where we hailed a taxi and were on our way to the lab. It was a blisteringly hot Monday afternoon, and the driver of the taxi seemed not to mind, with the windows barely cracked and the air conditioning off. It was about an hour drive from the airport, and the taxi dropped us off at my dorm building.

I had been away from home before traveling to China but never so far, and certainly not for so long. My number one fear was being left alone in my dorm room, especially that first night, before I even knew where the lab was. It was not long before these concerns, too were dispelled. Cao Jian showed me around campus, and the surrounding areas where I could find a supermarket, restaurants, or an ATM. He introduced me to Dr. Li and the rest of the students and researchers. I was so surprised by how many people spoke very good English. I felt very lucky for that.

Everyone in my lab dropped everything that night and took me out for dinner for delicious dumplings (which became my favorite), and it was such a great experience. The members of my lab were so welcoming. I couldn't believe how accommodating everyone was, and how much they truly seemed to care about my wellbeing. They told me if I needed anything, to just ask. Dr. Li asked me if I missed my family. I politely replied yes, and he told me not to. He said that our lab was my family in China, and they would take care of me and make sure I had a good experience. This really helped make my transition easier. After dinner, Dr. Li and Cao Jian took me to a supermarket just outside of the university gate to get me whatever I thought I might want or need in order to feel more at home in China. By the time I returned to my dorm room for the night, I was feeling very reassured about being in China and excited to begin my work in the lab.

Moving to a foreign country for the summer, and leaving America for the first time, I expected vast differences. I expected it to feel different, and for every aspect of life to be unfamiliar and foreign to me. What I learned through my whole experience is that it isn't that different. People still have the same ideas and dreams. There weren't the blaring cultural faux pas that I expected. There was almost nothing that I did in China that was considered culturally wrong. There were the obvious differences, with the food, chopsticks, and peoples' driving manners, but honestly the biggest challenge as a foreigner was the language barrier, and even that was not too big of a problem. Once I figured out how to speak so that my lab mates could better understand me, and I got used to their accents and using translation apps as a tool, I got along great. In this, I learned that anywhere I go, I can meet new people and cope with changes. Adaptation is key in situations like this. If you don't learn to adapt and love the situation you are in, even if it may not seem ideal at first, then eight weeks could seem like a very long time. Once I made the decision that it's okay to try new things and step outside what I was accustomed to in America, I was really able to flourish and have a great experience in China.

This summer, my time management skills have improved greatly. A lot of what I did in the lab was a waiting game, with PCR taking almost two hours, electrophoresis taking an hour and a half, and gel taking 30 minutes to solidify. I learned to take advantage of lunch and dinner breaks, by setting up PCR and gel to run during those breaks. Then after dinner, I could just work on recording my results, planning the next day's experiments, and getting organized in general.

Over the course of the summer, I was so fortunate to be able to travel in and around Beijing on the weekends. The members of my lab enjoyed the chance to get out of the office and show me around the city. I was able to learn so much about the interesting culture and history of China while seeing beautiful architecture and scenery. The amount of history in the Beijing area is astonishing to me, especially with my simplistic Iowa roots. I loved being able to take it all in and learn as much as I could.

My friends in the lab also enjoyed being able to improve their English with me as I learned and improved my pronunciation of words in Mandarin. I learned to love so many different types of food that I had never had before, and haven't been able to find since.

There is nothing quite like living on the other side of the world and I am grateful that I had the opportunity to do so!

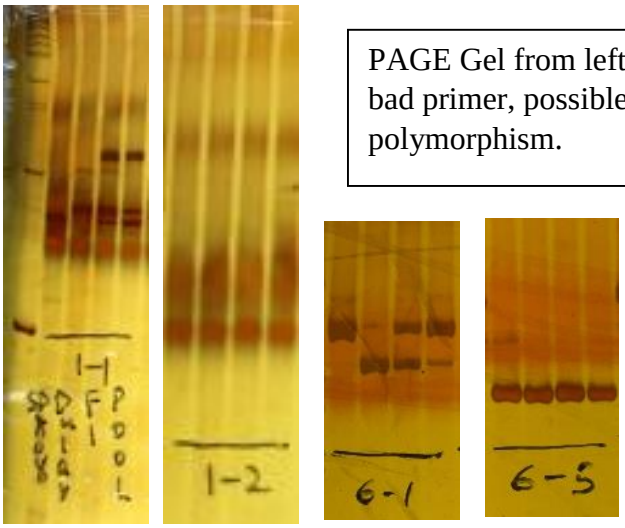
In Conclusion

My summer in Beijing is one I will never forget. The people I met, the work I did, and the culture I experienced were all so amazing. I feel so fortunate to have been chosen for this amazing internship. I can't imagine anywhere else I would have rather gone and spent my summer.

Appendix A: GENOMIC DNA MINIPREP （FOR PCR）

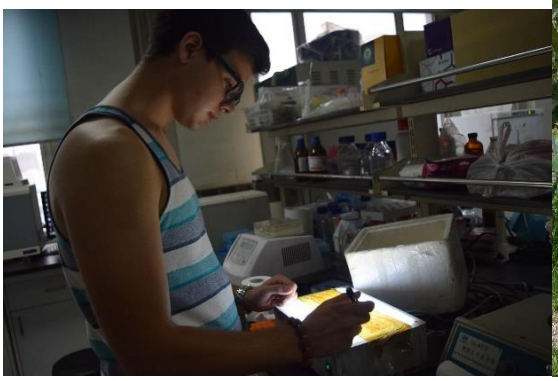
1. Prepare extraction buffer, CTAB: 200 ml of 1M Tris, pH 7.5; 200 ml of 0.25M EDTA; 400 ml 5M NaCl; 20 g CTAB; 200 ml MQ Water.
   (CTAB: Cetyl trimethyl ammonium bromide)
2. Grind 100 mg fresh tissue into fine powder in liquid nitrogen. Transfer powder into 1.5 ml EP tube.
3. Add 700 µl of extraction buffer (CTAB), 1µl RNase A, recap the tube and shake or vortex.
4. Incubate in a 65℃water bath for 30 to 60 min; shake several times during incubation.
5. Add 700 µl of Chloroform:Isoamyl alcohol (24:1), and shake/vortex.
6. Spin in microfuge at 10,000 rpm for 10 min.
7. Pipet off the aqueous phase into a new microtube. Add 2/3 to 1 times the volume of cold isopropanol and invert gently until the DNA precipitates.
8. Spin at 10,000 rpm for 10 min, pour off supernatant, and wash with 70% ethanol. Dry the DNA in air and re-suspend in 100 µl of water.

Appendix B: Photos



PAGE Gel from left to right: No mutation, bad primer, possible mutation, no polymorphism.

Normal rice plant on left, mutated S2-226 plant with reduced tillering on right.



Works Cited

Carter, Colin A. "China's Agriculture: Achievements and Challenges." *Giannini Foundation of Agricultural Economics* (2014): 5-7. University of California. Web. 2 Aug. 2015.

"China Projects & Programs." The World Bank. N.p., 2015. Web. 8 Aug. 2015.

Cho, Jaeil, and Taikan Oki. "Application of Temperature, Water Stress, CO2 in Rice Growth Models." *The Rice Journal*. N.p., 3 May 2012. Web. 1 Aug. 2015.

Gale, Fred. "China: Policy." *USDA Economic Research Service*. N.p., 30 May 2012. Web. 27 Sept. 2015.

Gale, Fred, Growth and Evolution in China's Agricultural Support Policies, ERR-153. U.S. Department of Agriculture, Economic Research Service, August 2013.

Kimura, Shingo, Keijiro Otsuka, and Scott Rozelle. "Efficiency of Land Allocation through Tenancy Markets: Evidence from China." *Economic Development and Cultural Change* 59.3 (2011): 485-510. Stanford University. Web. 1 Aug. 2015.

Mengxiao, Zuo. "Crop Diversification in China - Zuo Mengxiao*." FAO Corporate Document Repository. Food and Agriculture Organization of the United Nations, n.d. Web. 29 Aug. 2015.

Shih, Mi. "Making Rural China Urban." *The China Story*. N.p., 18 June 2013. Web. 29 Aug. 2015.

"The World Factbook." Central Intelligence Agency. Central Intelligence Agency, 2014. Web. 30 Aug. 2015.

Vorotnikova, Ekaterina. "Land Allocation Dynamics in China among Five Top Staple Crops: Corn, Soybeans, Wheat, Rice, and Cotton from 1985-2009." (n.d.): n. pag. University of Florida. Web. 27 Sept. 2015.