Zhong Guo Ren

***Native to China**

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To my parents who raised me, my mentor Dr. Dingming Kang who guided me, my teachers who educated me, Lisa Fleming who believed in me, and to the World Food Prize Foundation which made this experience possible,

I am truly grateful.

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Introduction and Personal Interest

A famous Japanese proverb states “A vision without action is merely a daydream.” Throughout my years growing up I have been exposed first hand to the growing problems of hunger and malnutrition. It is hard to think that I will go home to a house with plenty of food while others are helplessly stranded in the street with meager quantities to eat. For many years the desire to help those in need of food security has been present within me, but, as the proverb states, action on my part had only been within the realm of a daydream.

At the end of the summer of my sophomore year, I received an excited phone call from my mother as I was away at debate camp. She eagerly began to describe to me this program she had learned of that fit my interests by presenting students with not only the opportunity to learn about the devastating effects of malnutrition but to discover the problems first hand. Intrigued, I began to do research on the World Food Prize foundation as well as the problems regarding hunger societies faced at all corners of the globe. It was truly inspirational to learn about all the extraordinary, passionate people who have formed a coalition to battle against malnutrition, helping hundreds of people around the world. I quickly immersed myself in numerous articles as I began my research on the effects of poverty on the paradox of hunger and obesity in China.

As I prepared for the World Food Prize Youth Institute taking place on that October of 2005, my awareness of the tremendous magnitude dealing with the people who were hungry heightened dramatically. The experience of solely doing research for my paper provided a stepping stone for the journey I was about to take with the Youth Institute and my internship.

The Institute proved to be an eye opening experience for me. I sat beside world renowned scientists and World Food Prize Laureates as they discussed the matters of hunger and obesity at hand. It was such and honor and a great source of excitement for me to not only listen to the ideas of these people but to actually get the opportunity to *talk* to them. I soon realized the immense nature of hunger and obesity and realized that even though the solution of stopping global hunger appears simple (i.e. feed them) the complications caused by politics and geography are endless. I also came to the realization as I sat amongst my peers that the responsibilities of malnutrition would be passed down to our generation and require the contribution of each and every person. The Institute soon came to an end but left me with a new beginning as I soon realized the new passion and drive it had instilled within me.

At the end of the presentation where all the interns gave a brief synopsis of their experiences, I excitedly picked up an application form from the table outside of the presentation room. At the time, I had no idea of the remarkable experience that would be awaiting me. My dream of being a part of the effort to stop world hunger was soon going to be a reality.

Destination: Peking University in Beijing, China

It is said that there are four main things China, in particular Beijing, is famous for: Peking Duck, Peking Man, Peking Opera, and Peking University. I was lucky enough to be able to experience the fourth attraction of the list. In the spring of my junior year, I received a letter delivering a message that I had been so anxiously waiting. It stated that in the summer of 2006 I would have the opportunity to work at the National Laboratory of Protein Engineering and Plant Genetic Engineering at Peking University in Beijing, China. I was ecstatic and could not wait for this life changing experience. The truth is I had never heard of the four things China is famous for let alone Peking University in particular. However, in the days prior to my internship, after a little research and long discussions with my Chinese friends, I was amazed to see the respect the name Peking University was associated with. Many of my Chinese friends parents were not only shocked that I would be going to Peking University for the summer but *jealous* that I would have the opportunity to live and work there.

Shao Yuan International Dormitory

Located in the Haidian District, Peking University was founded in 1898 and is considered one of the most prestigious universities in China. Its buildings mimic the

historic nature of China's deeply rooted culture with its imperial structure, numerous ponds, and rising pagodas. Every morning as walked to lab, I would find myself lost in admiration of the simplistic yet breathtakingly beautiful nature of the campus. The sight of towering trees and twisting trails mingling with little children in neon orange shirts as they toured the campus was such a pleasant sight to be greeted by each morning. I was surprised at the diverse nature of the people and activities on campus. I was shocked at the range of languages being spoken as I would squeeze my way through crowds of people heading towards the nearby Wu Mei (a small grocery store nestled at the end of the street of my dormitory).

While on campus, I spent a majority of my time exploring my new surroundings, working at the lab, or reading in my new home: the Shao Yuan International Dormitory. During the first two weeks of my stay, I had a roommate who was from South Korea. As she did not know English and Korean was not one of the languages taught in school, communicating became a daily game of charades. I soon learned the universality of a smile and that despite the barriers language posed, in the end we all share the common traits of humanity. I not only became friends with my roommate before she went back to Korea, but formed friendships with people I passed everyday as well as the people I worked with in the lab. They introduced me to their friends and included me in their lives as though I was we had been friends for years. I soon became well accustomed to the layout of Peking University as well as my lab itself.

The National Laboratory of Protein Engineering and Plant Genetic Engineering

Throughout my internship, I spent a majority of my time working at the National Laboratory of Protein Engineering and Plant Genetic Engineering. The three story lab consisted of numerous senior scientists, student researchers, and various staff members. Unlike the typical red brick buildings that enclose laboratories in the United States, this laboratory also exemplified the history and character of the Chinese people through its eloquent structure and ornate designs perched on top its flaming red roof. The research being investigated within these walls is as unique as the appearance of the building itself.

The National Laboratory of Protein Engineering and Plant Genetic Engineering

During the first few weeks of my stay, before I started actually assisting with research, I spent my time reading published literature of other members of the lab. This helped me get acquainted with the protocols of our lab as well as the material being studied. Through the course of time and patient guidance, as well as diagrams, of my mentor Dr. Kang, I soon learned the methods and concepts I would need for my research. Additionally, I was especially thankful for the numerous researchers working at the National Laboratory that helped me through experiments when I stumbled across procedures that were written in Chinese. Their warmth and kindness to me were incalculable and as Dr. Kang would often remind me, they really did become my “big brothers and sisters” for the summer. They included

me in all the traditions of the lab such as the weekly devouring of “she gua” (watermelon) every Thursday afternoon in the common room and playing ping pong in the main hall on Sundays. The atmosphere was better than I could have imagined and the experiments I performed there taught me more than any text book. The project I worked on for the eight weeks taught me more than I could hope for.

Scientific Objective

During my internship, with the help of my mentors, I obtained a research project that I would be investigating over the course of my stay. The overall objective of the lab's research is to analyze the sequence and functions of the genes associated with the development of the rice plant. In order to improve rice quality, it is evident that a global effort is needed. Thus, they set out to sequence the genome of the rice plant in collaboration with many labs across the world. However, the rice plant has a complex genomic nature which makes it difficult to standardize techniques when attempting to experiment with it.

Experimental Plants Grown in Laboratory

This necessitated the need for another suitable model plant. With this is in mind, Arabidopsis Thaliana, a flowering plant, was chosen as a model to study genes and their functions. Not only is Arabidopsis Thaliana a good model for study, but has many beneficial properties. These advantages include a short generation time, small size, large number of offspring, and, most importantly, a relatively small nuclear genome (AGI 2000). These advantages have promoted the growth of an international scientific community that is working to characterize the genes of Arabidopsis: the Arabidopsis Genome Initiative (AGI). Many groups have already characterized different chromosomes. In addition, scientists are now trying to understand

the functions of various genes in Arabidopsis. One such process that scientists, particularly in this lab, have started to study is embryogenesis or the formation of the seed. In this study our objective is to clone four genes out of an estimated one-hundred for further investigation purposes.

Aim

The aim of this research project is to clone and characterize the selected four genes: At4g31420, At2g42630, At2g46990, At5g24330, in order to determine their role in embryogenesis.

Rationale

Previous studies have identified around 100 genes that are involved in embryogenesis. The functions of many of these genes remain unknown. However, because 100 genes is too large of a number to study, we needed a method to narrow down our number to a fewer number of genes. We accomplished this by using gene expression profiles from previous microarray analysis. Microarray analyses have shown that these four genes among the genes that are significantly differentially expressed compared to reference RNA. A microarray is a tool for analyzing gene expression that consists of a small membrane or glass slide containing samples of many genes arranged in a regular pattern. Information obtained from the microarray tells us the differential expression of these genes (i.e. which genes are overexpressed and which are underexpressed). If a gene is overexpressed, it signifies that that particular gene is involved in the process of embryogenesis. The four genes we selected to study were among those that were overexpressed, justifying their selection. The information we obtain regarding their sequence and function will allow us to cross apply our findings to the rice plant as well as other crops.

Background and Significance

As I became acclimated to the procedures of the laboratory, I soon began to explore an area for my research project. As stated, the goal of this laboratory, in collaboration with other international laboratories, is to improve the quality of the rice by using genetic engineering techniques. In particular, the understanding of the process of embryogenesis will allow scientist to genetically manipulate important genes that control functions of the seeds where farmers encounter problems. For example, often times, when farmers plant seeds in the expectation that *all* of the seeds they plant will develop into healthy plants. However, they find themselves disappointed because all of their seeds failed to grow. When the seeds do not germinate, the farmer loses money not only in the dormant seed but also in the plant he will not have. In this scenario, the understanding and manipulation of the genes involved in embryogenesis will be highly desirable. By producing a transgenic plant, it can be ensured that each and every seed planted will in fact produce a plant. Also, with further of manipulation of other genes involved in plant formation, not only will we have the optimum seed but the optimum plant. This genetic engineering process will benefit many people i.e. the farmer and the consumers all over the world by providing a greater source of food.

Methodology

We began our research of analyzing these four genes by obtaining the genome sequence on an online database where Genbank has been published. GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences. GenBank is part of the International Nucleotide Sequence Database Collaboration, where various international organizations exchange new sequence information, including a description of the sequence and the known function. Using the sequence, we then designed appropriate primers to amplify these genes using a polymerase chain reaction (PCR). If DNA is simply obtained without amplification, the quantity is too minute for the purpose of analysis. Thus, a PCR is a technique for amplifying specific regions of DNA by multiple cycles of DNA polymerization- which is the formation of complementary strands of nucleotides. Each cycle is then followed by a brief period of heating in order to separate the complimentary strands. Thus, this results in multiple copies of our original, required segment of DNA. In order to ensure that the selected segment of DNA is amplified, we use primers, a short segment of nucleotides that are complementary to the segment of interest, to tell the DNA polymerase where to start copying. The following primers were designed for our four genes:

1. Gene Name: At4g31420

Primers for: Forward 5’ to 3’: atcgatattgaagagagaag

Reverse 3’ to 5’: ttttaattatgtgcttgcaac

2. Gene Name: At2g42630

Primers for: Forward 5’ to 3’: gagaatcgaaggaatctgaac

Reverse 3’ to 5’: aaagaactaagaagatatacg

3. Gene Name: At2g46990

Primers for: Forward 5’ to 3’: gagatatgggaagagggagaa

Reverse 3’ to 5’: gactatatattcagatgcatg

4. Gene Name: At5g24330

Primers for: Forward 5’ to 3’: atggtggctgtgaggcggagg

Reverse 3’ to 5’: ggttgacttgtaaaacatatt

After performing our PCR, we then completed an agarose gel electrophoresis to confirm that the right size DNA had in fact been obtained. The concept behind a gel electrophoresis is to separate the DNA molecules based on their surface electrical charge. The gel also contained a chemical called ethidium bromide which gets incorporated into the polynucleotide segments which in turn helps us to see the DNA strand on the UV light exposed electrophoretic. After the gel was put under the light and the correct settings were obtained, the amplified DNA segments were compared to the molecular marker and finally photographed. Then we put our purified DNA fragments into an over expression vector, also known as a plasmid. A plasmid is a tiny cycle of DNA that can also be amplified independent of the genome. In addition, the DNA of interest can inserted into the over expression vector, which is then amplified. In order to amplify the required segments, the plasmid that has been ligated with gene fragments must transfected into the competent cell, in this case E Coli. This recombinant DNA mixture is allowed to transform a bacterial culture, which is then exposed to antibiotics. All the

cells except those which have been encoded by the plasmid DNA recombinant are killed, leaving a cell culture containing the desired recombinant DNA. We did this by spreading our cells onto antibiotic ampicilin plates that contain agar. Then, the bacterial colony is taken from the plate and put into a larger conical flask containing sterile growth medium to expand the desired cell population. To effectively expand our population, the flasks were put into a shaker and then the cells are lysed to get the plasmid DNA. The plasmid DNA contains additional segments of DNA besides the one we have inserted. Thus, restricted enzymes were used to digest the uneeded portions of DNA segments. Following this a PCR was performed to confirm that the plasmid DNA was in fact ligated with the gene. The PCR product was further sequenced to ensure the amplified DNA is indeed our segment of interest. These plasmids are then placed into agrobacteria tumefacien to generate the translated protein product. The agrobacteria containing the gene segment was than transfected in the Arabidopsis thaliana to study the gene function. We then looked for altered phenotypic expression on our transgenic plants. However, due to the short time of my stay, I was not able to complete the last portion of the project but Dr. Kang is continuing the experimentation process.

Setting up a PCR

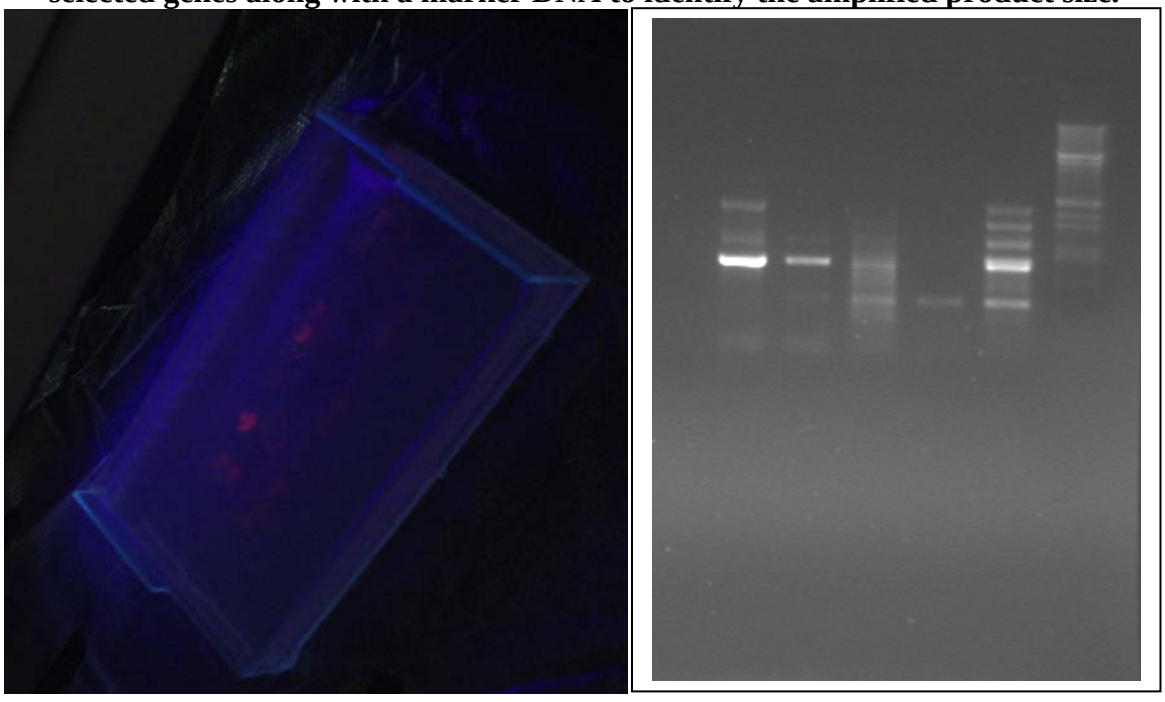
Results/Discussion

During my stay at the Peking University, I worked on the preparation and standardization of PCRs for the selected four genes. However, prior to running the PCR, I had to standardize my methods in order to obtain a consistent PCR product. This involved calculating what the optimum temperatures are for our selected genes. After selecting a few possible temperatures, we would run the genes in a PCR gradient machine. This machine allowed different rows on the machine to have different temperatures. I would write my own programs, named DEEPAJ, in accordance with the PCR I was running. Thus, from this we would find what the best temperature was and use it to do further PCR analysis. It was also important to keep in mind that it was not always the temperature that was the problem when the band would come out fuzzy. Many times the problem was that we had other impurities and unwanted polynucleotide strands. In this case, I would add additional enzymes in order to eliminate the unwanted substances. The following figure demonstrates the PCR product of the four genes.

Working with a coworker to collect the seeds for the experiment

The insertion of these genes into agrobacteria allows us to determine the functions of the genes by seeing the phenotypic expression of Arabidopsis Thaliana, which contain the agrobacteria. The overall significance of this project is to one day use this information to genetically improve the yield and resistance of crops, such as rice, thus allowing a better livelihood for thousands of people all over the world. Specifically with embryogenesis, this research allows us to understand the formation of the seed thus making manipulation possible. Often times, in the rural setting, farmers waste time and energy, in addition to money, as they try to plant seeds that in the end remain dormant. With further understanding of embryogenesis, this excess waste of resources can be prevented. Also, this optimization will not only improve the seed formation but provide a

Two representative photomicrographs of the PCR gel, one under the UV light and the other black and white image showing amplified DNA corresponding to the four selected genes along with a marker DNA to identify the amplified product size.



nutritious food source. Thus, this research is an integral part of the progression towards improving food quality and availability.

Reflections on the Internship

My days in the lab taught me more then I could hope for ranging from scientific techniques as well as cultural aspects of my coworkers lives. Every afternoon, after eating lunch, one of my new friends at the lab, Ye Chang, would teach me phrases in Mandarin. Unfortunately, due to the difficult nature of Mandarin, I did not become proficient but did learn a numerous useful phrases throughout the process. I think one of things I appreciated the most during my internship was the patience of the Chinese people. When Ye Chang would give me lessons, we would figure out ways to translate the sounds of the characters into English, coming up with our own system of graphing tones. Even at my dining experiences the people extended to me numerous courtesies.

Most days, I would eat lunch at a dining hall on campus located down the street from lab. The day after I arrived, Dr. Kang took me to a dining hall for lunch and I was definitely in for an experience. People flocked into the building, squeezing through the doors. Truthfully, I was a little mortified at first because I had no idea how I would ask for food. However, my fears turned out to be unwarranted. The people who were working there were not only helpful but welcomed me with a huge smile every time I would enter the dining hall. The man behind the counter would animatedly point out different items to me to make sure we clearly understood each other. His kindness along with the numerous people who I cam in contact with daily made my internship experience wonderful beyond my dreams.

Through the course of my internship, not only did I experience the hospitality of the Chinese people but I also gained insight on the tremendous nature of the issues concerning hunger. I realized that the solution to solving hunger is not just feeding those

that are hungry. As a famous Chinese proverb states: Give a man a fish and you feed him for a day. Teach a man to fish and you feed him for a lifetime. I think this proverb captures the essence of the problem and reiterates how research, such as that is being done in the National Laboratory, is a crucial part to finding success. However, in addition to the scientific portion, I have realized that it is also essential for countries to overcome their geographical boundaries and truly form a global coalition to fight against hunger. As I would sit with Ye Chang in the afternoons learning how to say basic phrases such as "good morning," I realized that in order to have a united global front and overcome these barriers moments like these on a larger scale would be necessary.

Often times, when communicating cross culturally, many misconceptions are embedded on either side. The natural workings of our mind seem to cause us to come up with preconceived notions before we have truly understood the situation. I must admit that I was guilty of harboring misconceptions about China. However, not only did I learn that many of my conceptions on China were false, but that many of the Chinese people I worked with also have false notions about America. It has become evident to me that in order to make world wide progress it is essential for us to clarify our misconceptions so we can have a clear understanding on other nations, thus strengthening everyone's efforts against hunger by working together.

On the more individual perspective to each farmer, it is necessary to realize the conditions of the farming communities in each nation as well as what problems they are facing. The understanding of the difficulties they encounter will not only facilitate our ability to help find solution but will also help more governments and people in general realize what a valuable asset the farming community is and how their success is vital for

our own. Furthermore, the pinpointing of the problems will also help in the research setting. As scientists, such as those working in the National Laboratory, discover what the functions of various genes are they also need to know what current plants are lacking. In the case of embryogenesis, uncovering what the specific functions of those four genes on is great; however, if the scientists are unaware that the problem with the seeds the farmer is encountering is that the seeds remain dormant they will never be able to fix the problem. Thus, I have realized that communication, not only between countries, but between various disciplines is necessary for progress to occur.

At the end of my internship, I arrived at a conclusion with the help of the numerous friends I had made throughout the summer. Despite the differences in our language, our food, our customs and our culture, in the end we are all human and share the passion to make a difference. Throughout my stay in China, banners for the upcoming 2008 Olympics that were to be held in Beijing were plastered on the front of every door. Now even though the Olympics may at often times seem like just a game I now believe it stands for so much more. It is a tradition and common goal among countries nestled in all corners of the globe to try their best and see their dreams to the end. I believe the same concept is pertinent in the global effort against hunger. Throughout the world, people have the desire to make a difference and help those in need. However, unlike the Olympics, we often take the back seat in a trance that we can have no part in the fight. The passion is present but we all need to work harder on the effort on our behalf.

Before my internship, by feeling on my own action were best described by a famous Japanese proverb which states: “A vision without action is merely a daydream.”

The desire to help those in need of food security has been present within me, but, action on my part on felt like a daydream. However, thanks to my experience as an intern, the desire to more actively take part in the global effort was roused and I felt as though I was leaving the realm of a daydream. Not only have I begun to take action on my vision but my internship put my vision in a new light, providing me with more motivation and desire to make a difference than I could have hoped.