The Golden Question in the Crimson Country

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Introduction

We have 6,860,200,577 mouths to feed. An additional 267 more mouths are born every minute. That's how many people are on this planet we call Earth. Each one breathes, works, loves, and eats. And each day, 1 in 6 people go without food and 1 person dies every second due to hunger. That totals 4,000 people dying every hour, 100,000 each day and 36,000,000 each year.

By 2030, the world will consume 50% more food, energy, and water. By 2050, we will need to produce 70% more food just to feed the growing population. In 2030, I will be 37 years old. In 2050, I will be 57. This is the problem my generation and I will be facing. It's the golden question: How do we (by 2050) grow 70% more food and feed 2,300,000,000 more people with less land, more demand for biofuels, and an ever increasing standard of living?

When I stepped off the plane at Beijing Capital International Airport, this was not the question that hit me first. The question that hit me was "Why is Beijing so hot and humid?" Over time, I adapted to the heat and humidity, but my summer would prove to be the most eye opening experience I have ever had. How will we feed the world? Based on my first Chinese cuisine experience,; one spicy dumpling at a time.

Unfortunately, the answer is not that simple. However, at the National Laboratory of Protein Engineering and Plant Genetic Engineering at Peking University in the North-West corner of Beijing, China, they are working on answering this very question. And that is where I had the incredible honor of working and living for two months. Two months that I shall never forget.

The Road to Shao Yuan

Reluctant Beginnings

It was a hot July day in 2008. My agriculture teacher and FFA advisor Mr. Tim Larson was coming to my house to check in with me and my work for my summer agriculture class. At the end of the visit he handed me a packet with information about The World Food Prize Youth Institute. I had never heard of it, and assumed it was a cooking contest and put it in my to-do pile. As the Minnesota summer days went by, I had started to learn about the Youth Institute, but I wasn't very enthused about writing the paper. When I learned 2008 was the first year the University Of Minnesota College Of Food, Agricultural and Natural Resource Sciences was sending high school students to the institute, I decided to talk it over with Mr. Larson and my parents. After realizing I had some time at the beginning of my freshman year with not much to do in class, I decided to try and write the paper. I attended the University of Minnesota's selection day, presented my paper and was fortunately chosen to represent Minnesota at the World Food Prize that October.

In mid-October, there I was, sitting in the Iowa state Senate Chambers. Dressed in a World Food Prize button up shirt like everyone else around me, I remember looking out across the crowd. Sitting before me were some of the world's most distinguished agriculturalists. I will always remember the image of that crowd, all in their respective cultural attire. Here was a gathering of people from around the world, all dedicated to answering the golden question. And here again, that was not the first question in my mind. The first question in my mind was "Why are those trumpets so long?"

I realized the importance of the golden question a couple days later. It was at the World Food Prize Youth Institute. I was ready to present my paper about natural resource degradation in Togo, and as the people kept filing in I sat there and enjoyed my muffin and milk. I vividly remember what happened next. One after another, the Borlaug-Ruan International Interns stood up and told their personal summer experiences. Needless to say I was amazed, and soon preceded to the presentation rooms to present my paper. Looking back on it, that day and the World Food Prize Symposium forever changed my outlook on the world, and its future. That day was the first day I ever asked myself the golden question, without having other questions come before it.

I knew I wanted to be an intern and completed my application after spending the next summer at The University of Iowa doing genetics research on epilepsy and fruit flies. I applied and in February of 2010, I found myself pondering that golden question. My mom and I had just driven through a blizzard to get to Des Moines, Iowa for the Borlaug-Ruan International Intern interviews. Lucky for me, I was first. After an interview that I barely remember due to anxiety, I found myself driving back on ice covered roads to get home. Now, I think about the blizzard conditions and below zero temperatures then compared to the monsoon conditions and 107 degree temperatures in July in China. I could never have imagined what the summer of 2010 had in store for me.

I will never forget preparing for the summer of 2010. In April, there I was, in a doctor's office getting prescriptions for malaria pills and emergency antibiotics. I also believed I was receiving every shot known to man. Again the golden question was not in my mind, but rather "Will food in China be like Chinese food here?" and "Is the Great Wall worth all these needles?" Before I knew it, I was soon on a plane going over Russia bound to land in Beijing.

Head under Water

While I sat next to my newfound Chinese-Canadian friend on the plane, I found myself anticipating China and all it had in store. What would the smells be? What were the people like? Where would I go? What would I do? The questions were endless. Before I knew it, I found myself walking through the doors from customs into the giant meeting area of Beijing Capital International Airport. This was the first time I realized Beijing has many people. To be a bit more accurate, the population is about 22,000,000. I soon found an energetic man at the end of the line holding a sign with my name on it. It was my first time meeting Dr. Kang, the man who helped me throughout my entire journey and who I am very grateful to.

The smog. Beijing has a lot of smog. Thick, heavy, smelly smog. As I rode in a taxi at 140 km/h hoping I would survive to see Peking University, the smog is what struck me first. But then it was the trees, and then the buildings, and then the cars, and then the people. The list goes on and on. I was like a kid in a candy store trying to take in all of the sights around me. Here I was sitting in the back of a Beijing taxi swerving erratically through traffic and pedestrians while I was surrounded by unfamiliar plants, strange cars, weird smells, Chinese language--everything was so shocking. I look back on that first day while I sit here nearing the end of my adventure and I see a complete 360 degree change. I now know the names of the plants, I'm familiar with the cars, I like the weird smells, I can roughly understand meanings when people speak Chinese, and I no longer fear my life while in a taxi.

After an hour or so of driving through all these unfamiliar surroundings we arrived at Peking University. I was immediately struck with the beauty of the campus. The lotus ponds, the pagodas, the lake, everything was new and exciting. Everything was happening so fast, I didn't have time to process it; it was like I was dreaming. We soon arrived at Shao Yuan where I would be sleeping for 2 months, and I quickly unpacked my bags, looking forward to a filling meal. My first meal in China would prove to be my spiciest meal in China. It consisted of chicken feet, pigs head, eel and the spicy pork dumplings with leek, garlic, vinegar, and chilies.

That night it hit me. I was in China. As kids, I dreamed of tunneling to China or being on the other side of the world. But once I was there, the feelings overwhelmed me. Even though I was sitting awake on a bamboo bed at 2 in the morning surrounded by these strange things, it was surreal. It didn't feel like I was in China, or at least the China I expected it to be. I knew I was in a completely new world. It was the first, but not last, time during my internship where my preconceptions were challenged and proven completely wrong. Over the course of eight weeks, so many notions I had about China and Asia in general were completely erased and replaced with memories that I will forever cherish. But that first night there, I just needed some sleep. The next day would prove to be a very adventurous one.

Surrounded by 1,300,000,000

It was my first full day in China. Suffering from jet lag, I woke up at about 5 in the morning and waited until breakfast. When the time came around, I found myself in a very unique situation. Here I was standing in a cafeteria full of strange foods, trying to pick out what I should eat. On top of it, I didn't speak Chinese. After that morning, I never ordered fried rice balls again. I also quickly learned how to talk with my hands.

Not having anything to do, and still wide awake, I decided to be adventurous. I grabbed my camera and began walking in what I assumed to be a northern direction within the walled grounds of Peking University. I found myself walking though trees I had never seen before staring upon a pond before me. I could hear cars horns and braking sounds in the distance and all I could think of was what was around me.

Not only was I surrounded by strange plants, but Beijing itself, China itself, Asia itself. Standing in a country holding 20% of the world's population, the golden question that I had pondered before yet

again floated around in my mind. How do we, by 2050, grow 70% more food and feed 2,300,000,000 more people with less land, more demand for biofuels, and an ever increasing standard of living? Yet again, I was lost without an answer, but over time I would work towards answering that question. I knew it was time for me to find the lab I would be working in for the next two months.

Home Away From Home

Peking University

One of my first stops during my stay at Peking University was at the museum on campus detailing every aspect of its long, winding history. Originally established in 1898, Peking University was originally known as the Imperial Capital University. In 1912, following the Xinhai Revolution, the Imperial University was renamed National Peking University. In 1919, students from Peking University formed the bulk of the protesters of the May Fourth Movement, which is seen as the movement that started Chinese communism due to its spreading of Marxist and radical views.

After the outbreak of the Second Sino-Japanese War in 1937, Peking University moved to Changsha and formed the Changsha Temporary University along with Tsinghua University and Nankai University. The three schools then moved again in 1938 to Kunming. In 1946, Peking University moved back to Beijing after World War II. In 1952, three years after the founding of the People's Republic of China, Peking University dropped the "National" at the beginning of its name to reflect its public status under the new government. The first disturbances of the Cultural Revolution began at Peking University in 1966, and education ceased between 1966 at 1970.

Today, most national and international rankings place Peking University as one of the best universities in China due to their strong academics. As well as its academics, Peking University is renowned for the beauty of its traditional Chinese grounds and architecture. It currently has 15,128 undergraduates and 15,038 graduate students.

National Laboratory of Protein Engineering and Plant Genetic Engineering

I spent the majority of my time in China at the National Laboratory of Protein Engineering and Plant Genetic Engineering in the north-western part of Peking University. The laboratory was established in 1987 with funding from the State Commission of Planning, Ministry of Science and Technology, and the Ministry of Education of China to support cutting edge research in the life sciences. It is considered part of the College of Life Sciences at Peking University, and passed its first qualification review in 1990, and its major review by the Chinese National Natural Science Foundation in 2001.The lab employs 50 full-time faculty and staff including 21 full professors and 4 associate professors.

The laboratory focuses on research areas where scientific research meets China's economic needs. Current focuses of the laboratory include the elucidation of the structural and functional relationship of bio-macro molecules, and molecular biology specializing in protein structure and gene

regulation. The main work in the laboratory is application-oriented basic research. The majority of the research aims to apply modern biotechnology to agriculture and biomedicine for long term benefits.

In the past 5 years the laboratory has hosted 11 national, international, or bilateral scientific conferences, as well as receiving over 100 visiting scholars from various foreign universities and research institutes worldwide. The laboratory is also the Peking-Yale Joint Research Center for Plant Molecular Genetics and Agro-Biotechnology, and the Peking-Hong Kong Chinese University Plant Genetic Engineering joint laboratory.

My Research

Introduction

Rice is one of the main food staples in China and throughout the world. However, as agricultural cultivation expands, rice crops can become more sensitive to poor cultivation practices, weather, and other factors which can lead to drought and high salinity content in the soil, which both drastically reduce crop yields. For my research project at the National Laboratory of Protein Engineering and Plant Genetic Engineering, I worked under the guidance of Dr. Dingming Kang, Dr. Li-Jia Qu, Dr. Hongya Gu, and Dr. Wei Tang on *Arabidopsis* and rice functional genomes. Specifically, my project was to take 7 genes from rice varieties that are naturally resistant to drought, high salinity, and other stresses and insert the genes in other rice varieties.

After talking with Dr. Kang about the various projects I could work on, I chose this one for a few reasons. First of all, I have a huge fascination with genetics and how genetics determines everything living. Second, I personally believe that, in a growing world, genetically modified organisms, such as rice, will be critical to raise yields and feed more people with less land. Finally, I wanted to do a project I could take control of myself. I wanted to be responsible for these 7 genes, and do hands on research trough trial and error. In retrospect, I think I made a great choice.

There are two processes used one after another to insert genes into rice. The first process is isolation, purification, and insertion of the gene into a bacterium plasmid. The second process involved the use of agro-bacteria to transfer the plasmid into the rice's genome. I will describe the two processes in detail below. During my time at Peking University, the majority of my work was with the first process, and I worked with 7 genes from *Oryza sativa*, or common rice.

1. OS01G09080 (OsWRKY100)
2. OS05G49620 (OsWRKY19)
3. OS01G53040 (OsWRKY14)
4. OS09G25060 (OsWRKY76)
5. OS09G16510 (OsWRKY74)
6. OS01G40260 (OsWRKY77)
7. OS07G48260 (OsWRKY47)

All 7 genes are expressed and interact with zinc within the rice plant. All 7 genes are also part of a super family of rice transcription factors having WRKY domains, meaning there is also a comparable gene and transcription factor in *Arabidopsis*.

There are two main ways used to insert the gene into the genome. The first way is through the use of a gene gun, otherwise known as a particle gun; however, this process can be very expensive due to the heavy metals used in the process. During my stay in Beijing, I used the second method, which is a form of genetic transformation using agro-bacteria such as *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. These bacteria are specialized bacterium that under certain circumstances will convert their DNA into transfer DNA (T-DNA) of the tumor-inducing (Ti) plasmid, and then proceed to actually transfer this DNA into the host plant's nuclear genome.

Gene Extraction and Preparation

PCR Clone

The first and most time consuming of the steps is the PCR Clone reaction. The goal of the first step is to isolate and amplify the desired genetic code within a gene. Each genetic code must be isolated and amplified using one of three different polymerases, as well as different annealing temperatures and extension times. This step for the most part uses a guess-check-revise strategy and it can take weeks to find the right combination. For my research project, I was able to get 6 of the 7 genes past this step. The three polymerases that can be used are Taq polymerase, Pfu DNA polymerase, and a Taq/GC polymerase mix with dNTP.

Taq polymerase is a thermostable DNA polymerase named after the thermophilic bacterium *Thermus aquaticus*, from which it was isolated in 1965. Taq polymerase is an enzyme able to withstand the high temperatures used during protein-denaturing conditions in PCR reactions. However, Taq polymerase lacks a 3' to 5' exonuclease proofreading activity, thus giving it a 1 in 9,000 nucleotide error rate. Taq also leaves an A (adenine) overhang at the 3' end of DNA products.

Pfu polymerase is an enzyme found in the hyperthermophilic archaeon *Pyrococcus furiosus*. The main difference between Pfu and Taq is Pfu's superior thermostability and 'proofreading' properties. Pfu possesses 3' to 5' exonuclease proofreading activity, meaning that it works its way from the 5' end to 3' end and corrects nucleotide-misincorporation errors, thus giving it a 1 in 1.3 million base pair error rate. Pfu also results in blunt ended DNA strands. However, Pfu is slower and requires 1-2 more minutes per cycle to amplify the DNA.

Taq/GC polymerase mix is a combination of the Taq polymerase and GC buffer along with dNTP. The GC buffer allows for high processing rates and high accuracy rates in DNA strands with a high GC-content (guanine-cytosine content). The GC buffer also gives the Taq the exonuclease proofreading activity seen in Pfu. The dNTP further works by removing the A overhang from the 3' end of DNA products.

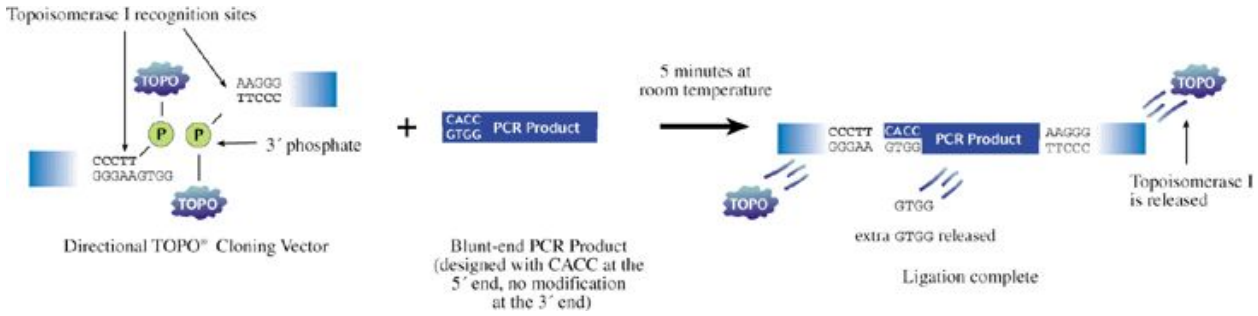
After the PCR Clone reaction, the results are checked using gel electrophoresis and a 1% Agarose gel. Ethidium bromide (EtBr) is added to the gel as a florescent tag which can be seen once exposed to ultraviolet light. EtBr is an intercalating agent which allows it to bind to DNA, and give off light under ultraviolet light.

TOPO Reaction

After the genetic code is isolated and amplified using the PCR Clone, it is put through a second step where it is modified and transferred into a bacteria plasmid. This is done using a TOPO Reaction and Gateway Technology. Although this step only takes 5 minutes to complete, it is the most complicated in terms of inter-workings, and the most critical step.

TOPO cloning technology is the most widely used cloning method in the world. It takes 5 minutes from start to finish, and can be done at room temperature with a 95% success rate. TOPO uses the enzyme topoisomerase I, which functions as both a restriction enzyme and ligase. The biological role of Topoisomerase I is to unwind and wind DNA in order to facilitate DNA replication. While the double helix structure provides a stable way to store genetic information, it is intertwined, thus requiring cutting and untwisting for replication. Topoisomerase I bind to single or double stranded DNA and cuts the phosphate backbone of the DNA. This break allows the DNA to be unwound or untangled, and at the end of replication the DNA is reconnected again. Since the overall chemical composition and connectivity of the DNA does not change, the topoisomerase I is a chemical isomer, only changing global topology, thus its name.

Vaccinia virus topoisomerase I specifically recognizes the sequence 5'-(C/T)CCTT-3' and forms a covalent bond with the phosphate group attached to the 3' thymine. It cleaves one DNA strand, enabling the DNA to unwind. The enzyme then relegates the ends of the cleaved strand and releases itself from the DNA. To use the relegating activity of the topoisomerase I, TOPO vectors (plasmids) are linearized with topoisomerase I covalently bonded to each 3' phosphate. This allows the vectors to readily ligate the DNA sequence with compatible end.



Once the genetic code has been inserted into a TOPO vector, Gateway Technology is used to transfer that vector into the genetic code of *E. coli* strains. The specific TOPO vector used is an entry vector (pENTR). This vector contains the *ccd*B gene used for negative selection. The CcdB protein interferes with *E. coli* DNA gyrase, thereby inhibiting growth of *E. coli* strains.

When recombination occurs in the TOPO reaction, the *ccdB* gene is replaced by the gene in interest. Cells that take up the unreacted vectors carrying the *ccdB* gene will fail to grow, while cells that took up vectors containing the desired genetic code will continue growth. This allows high-efficiency recovery of desired clones.

The vector also includes *att*L1 and *att*L2 sites to allow recombinational cloning of the genetic code of interest with a destination vector to produce an expression clone, a Kozak consensus sequence for efficient translation in eukaryotic cells and a Shine-Dalgarno sequence for initiation in *E. coli*, kanamycin resistance gene for selection of plasmid in *E. coli*, and a pUC origin for high-copy replication and maintenance of the plasmid in *E. coli*.

Transformation

The bacteria vector gained from the TOPO reaction and Gateway Technology must then be immediately introduced to artificially competent *E. Coli* bacteria for genetic transformation to occur. Competence refers to the ability of the cell to take up exogenous DNA from the environment, and artificial competence is induced by laboratory procedures. It involves making the bacteria cell passively permeable to DNA by exposing it to conditions that do not normally occur in nature.

For my research project, I used calcium chloride ($CaCl_2$) transformation. Chilling the cells in the presence of divalent cations such as Ca^{2+} (In $CaCl_2$) prepares the cell membrane to become permeable to plasmid DNA. The addition of calcium chloride to a cell suspension promotes the binding of plasmid DNA to the cell surface, which can then pass into the cell. The cells are incubated on ice and then heat shocked at 42^{o}C thus allowing the DNA to enter the cells. This method works very well for circular plasmid DNA; however, this method does not work for linear DNA such as fragments of chromosomal DNA.

In order for the vector to be maintained and replicated in the bacteria cell, it must contain an origin of replication, such as pUC origin in pENTR, which allows it to be replicated in the cell independent of the cell's own chromosome. Because transformation usually produces a mixture of relatively few transformed cells and relatively abundant non-transformed cells, a method is needed to identify the cells that have taken up the transformed vector. The method usually consists of using a plasmid that contains a gene that codes for resistance to a natural antibiotic. The mixtures of cells are then grown on a medium containing this antibiotic and thus only the transformed cells would be able to grow. Cells that did not take up the plasmid would be killed on the medium.

The pENTR vector I used first codes for a pUC origin to ensure replication and maintenance in the cell. Secondly, it contains the *ccdB* which ensures that vectors without the desired genetic code would be lethal to the bacteria cell. Finally, it contains a gene that codes for resistance to kanamycin, a natural antibiotic. Therefore, these three factors ensure that after overnight culture only colonies with the desired vector and desired genetic code would be growing on a LB+kanamycin plate.

PCR Screen

After overnight culturing at 37^{o}C on a LB+kanamycin plate, the resulting colonies must be numbered for organization and then screened as verification of the transformation and TOPO reaction. In order to number and organize the bacteria colonies, I would draw a grid pattern on a new LB+kanamycin plate and number it from 1-16. I would then randomly choose 16 colonies from the

original plate and transfer cells from those colonies to the new plate with one colony per number. I would then allow this plate to incubate overnight at 37^{o}C.

In order to screen the numbered colonies I would then perform a PCR Screen reaction. I would do 16 micro-reactions in micro-tubes using a PCR Screen program similar to the one used in the PCR Clone reaction. I would also use one of the three buffers yet again, typically the one used in the clone reaction as well; Taq, Pfu, or Taq/GC with dNTP. However, in the clone reaction the two primers I used would be the forward (F) and reverse (R) primer for the gene specifically (e.g. If it was OsWRKY76 I would use 76F and 76R). In the screen reaction, I would only use one of those primers as well as the opposite M13 primer (e.g. If it was OsWRKY76 I would use either 76F and M13R, or 76R and M13F).

That small change is a very important one. Other than that, the reactions and programs are virtually the same. In the PCR Clone reaction, I am taking a linear piece of extracted DNA, and only amplifying one gene within that strand. Therefore, I want to use primers that just attach to the beginning and end of that gene. Therefore, I can use the F and R primer together. However, with the PCR Screen reaction I am trying to amplify a gene with a circular vector. During the TOPO reaction, the beginning of the desired gene is slightly altered by the topoisomerase I and replaced by a M13 DNA marker. This marker makes the gene unique from the rest of the genes within the vector. Therefore, I can use a M13 F or R primer in conjunction with a regular F or R primer to amplify the desired gene and none other.

After the reaction solution is mixed, I prepare 16 micro-PCR tubes with the numbers corresponding to the 16 numbered colonies. A small amount of cells are transferred from each colony to its respective tube and then amplified hundreds of times over. After this I run a gel electrophoresis with a 1% Agarose gel and EtBr to determine what colonies are positive for the desired genetic code. If they are positive, the primers will have isolated and amplified the desired gene and a strong white band will be seen in the gel with a base pair (bp) number corresponding to the gene length (e.g. OsWRKY76 has a bp number of 984, meaning it is 984 base pairs long).

Plasmid Extraction and Sequencing

After the screen, I perform an overnight culture of one of the numbered colonies in a liquid LB broth with kanamycin. The kanamycin prevents any bacteria from growing other than the ones containing the transformed vector. After the overnight culture, the desired plasmid is extracted and purified for further use.

The extraction and purification of plasmid DNA from bacteria, *E. coli* in particular, is typically called plasmid preparation. After this procedure, the plasmid can then be transferred to other bacteria, such as agro-bacteria, for further use. First, bacteria cells are separated from the LB broth using centrifugation. Three solutions are then added in order one after another with mixing in between to extract the plasmid.

The first solution contains four chemicals at pH 8.0: glucose, ethylenediaminetetraacetic acid (EDTA), ribonuclease (RNase), and lysozyme. Glucose causes osmotic shock that leads to the rupture of cell walls and membranes, EDTA inhibits nucleases, RNase degrades RNA, and lysozyme causes the rupture of the bacteria cell wall.

The second solution contains two chemicals at alkaline pH 12.0: sodium hydroxide and sodium dodecyl sulphate (SDS). The sodium hydroxide lyses the bacteria cell completely, and the SDS causes the alkaline pH which in turn denatures chromosomal DNA but not the covalently closed circular plasmid DNA.

The third solution contains only sodium or potassium acetate at acidic pH 5.4. This has three outcomes; it neutralizes the alkaline pH, precipitates protein and forms SDS-protein complex, and renatures chromosomal DNA which aggregates with the bacterial protein.

The solution is then centrifuged at 12,000 RPM for 15 minutes to pellet the protein and DNA aggregates. The plasmid DNA will be left in the liquid, which is then collected and moved onto further purification. Once the liquid is transferred into a specialized spin column tube, it is centrifuged yet again to separate the liquid and plasmid DNA. Silica membrane is then added to the tube suspended in a buffer with high salinity content.

In high salt content buffers, plasmid DNA will bind to the silica membrane. Any remaining material can then be washed away, purifying the plasmid. Under low salt conditions through the use of ddH_2O, plasmids can then be eluted from the silica membrane and collected.

Once this procedure is done, a solution is left that contains pure transformed vectors with the desired genetic code. A sample of this solution is then sent for sequencing, and if the results come back as they should, the vectors can be moved on to the second process, which uses agro-bacteria to insert the desired gene into rice plants.

Agrobacterium Transformation

Importance of Genetically Modified Organisms

We as a world face a growing problem. It is yet again the golden question. How do we by 2050 grow 70% more food and feed 2,300,000,000 more people with less land, more demand for biofuels, and an ever increasing standard of living? There is no one answer, but a solution to part of the problem may be genetically modified organisms (GMOs). GMOs can give higher yields, increased pest resistance, increased tolerance to biotic and abiotic stresses, increased nutrition, as well as countless other benefits.

In an interview published on July 13th, 2008, Norman Borlaug said he believed genetic modification of organisms was one of the only ways to increase world food production as the world runs out of unused arable land. According to Norman Borlaug, GMOs are not inherently dangerous “because we’ve been genetically modifying plants and animals for a long time. Long before we called it science, people were selecting the best breeds.”

Agrobacterium transformation in particular has proven to be very successful. *Agrobacterium* is listed as being the original source of genetic material in these USA GMO foods: soybeans, cotton, corn, sugar beet, alfalfa, wheat, canola, creeping bentgrass (for animal feed), and rice. *Agrobacterium* is also listed as the original source of genetic material in Golden Rice.

Agrobacterium

Agrobacterium is a genus of Gram-negative bacteria that use horizontal gene transfer to cause tumors in plants. The two most commonly used in genetic transformation are *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. When a plant is wounded, it excretes chemical compounds such as acetosyringone into the soil surrounding it. Acetosyringone is recognized by the VirA protein on the *Agrobacterium*, which is a transmembrane protein encoded in the VirA gene on the Ti plasmid. This causes chemotaxis with the bacteria, which is the reaction to and resulting orientation and locomotion to the chemical attractants. Without chemotaxis there would never be cell to cell contact. The bacteria then chemotactically move toward the chemical exudates.

Attachment is then a two step process. Following an initial weak and reversible attachment, the bacteria synthesize cellulose fibrils that anchor them to the wounded plant cell. Four main genes are involved in this process: *chvA*, *chvB*, *pscA*, and *att*. These fibrils also anchor the bacteria to each other, helping to form a microcolony. After development of fibrils, a Ca^{2+} dependent outer membrane protein called rhicadhesin is produced. This also aids in the sticking of the bacteria to the cell walls.

The *Agrobacterium* infect the plant through its Ti plasmid. The Ti plasmid integrates a part of its DNA, known as the T-DNA, into the chromosomal DNA of its host plant cells. In order to transfer its T-DNA into the target plant cell, *Agrobacterium* uses a T-pilus. Once the bacterium is exposed to acetosyringone, it leads to a signal transduction event which triggers the production of a T-pilus. This T-pilus is then used to transfer the T-DNA into the target cells cytoplasm. Nuclear localization signals (NLS) are then recognized by the plant's importin alpha protein, which in turn associates with importin beta and the nuclear pore complex to transfer the T-DNA into the nucleus. Once inside the nucleus, the T-DNA targets areas of the chromatin that are being actively transcribed and incorporates itself into the host genome.

Plant Tissue Culture

Plant tissue culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant. The use of plant tissue culture allows the regeneration of an entire plant from undifferentiated cells that have been genetically modified using *Agrobacterium*. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate an entire plant from single cells, otherwise known as totipotency.

First a callus of the plant must be induced. A callus of cells is a mass of undifferentiated cells, which typically cover a plant's wound. Using a callus of cells for genetic transformation has two main benefits. First, the callus can be manipulated under certain conditions to grow an entire plant which can then be used to continue the genetic line. Second, the callus naturally secretes the acetosyringone which attracts *Agrobacterium*.

In order to produce a callus, first plant cells without walls (protoplasts), such as cells from plant leaves, must be cultured under aseptic conditions in a laboratory setting on a solid medium containing all necessary plant nutrients. An excess of auxin in the medium will cause a proliferation of roots, while an excess of cytokinin will yield shoots. However, a right balance between auxin and cytokinin will cause an unorganized growth of cells, thus resulting in a callus. A callus can then be sustained on a gel medium for a substantial amount of time, much in the same way bacteria are grown. The medium typically consists of necessary macro- and micronutrients for the given cell type, such as nitrogen, phosphorus, and potassium.

T-DNA Transfer

After a callus has been induced, it is possible to take the pENTR vector obtained from the first process and prepare it for transfer to *Agrobacterium*. A modified Ti plasmid is used for transfer to the *Agrobacterium*. The modified Ti plasmid is 'disarmed' by deleting the tumor inducing genes found naturally in the plasmid, leaving only the two boarder repeats of the T-DNA which is needed for the transformation. The part of the pENTR vector containing the desired genetic sequence is then transferred into a plant transformation vector that contains the T-DNA region of the disarmed plasmid, together with selectable marker, such as herbicide resistance.

In the next and most important step, the *Agrobacterium* with the incorporated modified Ti plasmid are introduced to the callus for 15 minutes. During this short time, the *Agrobacterium* transfer the T-DNA to the rice callus, where the desired genetic sequence and herbicide resistance is incorporated into the plant's nuclear genome. The callus is then transferred to a medium containing the herbicide the T-DNA coded resistance for. If a callus failed to incorporate the T-DNA into its genome, it will die, while those that have successfully incorporated the T-DNA will begin growing shoots. These calluses can then be transferred to a medium containing a careful balance of auxin, which promotes the growth of a root system, which in turn begins entire plant growth.

Finished Product

After transformation and further growth, the rice plant can then be transplanted into carefully controlled soil under laboratory conditions where it is tested for its resistance to salt, drought, or any other factor. If a particular line of rice gives promising results in the lab, it can then be transferred to further field testing. Due to the actual incorporation of the desired genes into the nuclear genome, if the field tests come back positive, the plants can be bred, reproduced, and sold on a mass scale.

Impact of my Research

According to the United Nation's Food and Agriculture Organization (FAO) food security exists when all people, at all times, have physical and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life. Also according to the FAO, over 852,000,000 people are chronically hungry due to extreme poverty, while over 2,000,000,000 more lack food security due to varying degrees of poverty. 17,000 children alone die each day from starvation.

Numerous factors affect food security. Approximately 40% of the world's agricultural lands are seriously damaged. Himalayan glaciers that supply water to 2.4 billion people could be gone by 2035 as temperatures rise. Wheat stem rust from strain Ug99 sweeping across Africa and Asia could destroy the majority of the world's wheat crops, leaving millions to starve. The majority of the world is facing a worldwide water shortage, and thus many countries are beginning to import grain due to lack of self-sufficiency.

Genetically modified organisms (GMOs) and genetically modified foods can be very powerful tools. They can raise yields, lower costs for the producer, increase resistance and tolerance, as well as many other things. However GMOs can also be very dangerous. They must be assessed carefully for their impact on food security, poverty, bio-safety, and sustainability of agriculture as a whole. Also, data from the FAO show the majority of current GMOs and pipeline GMOs do not directly address the needs of developing countries, but rather those of industrialized countries. Further, in 2009, eight countries grew 95% of the world's global transgenic crops. The majority of transgenic crops are grown in North America, the United States of America specifically, but progress has been made towards expanding their use elsewhere. However, there still exists a very large gap. For example;

Plant	Percent Modified in the US	Percent Modified Worldwide
Soybeans	93%	77%
Field Corn	86%	26%
Cotton	93%	49%
Papaya	80%	0%
Canola	93%	0%
Sugar Cane	0%	21%
Sugar Beet	95%	9%

The research I did at the National Laboratory of Protein Engineering and Plant Genetic Engineering not only worked toward addressing the problems facing food security, but also meeting the needs of developing countries and developing shareholders. Rice is the most consumed staple grain in the world, and has the second highest worldwide production behind corn. Over 600 million tons of paddy rice was produced in 2004. However, compared to corn or wheat, very little of the world's rice crops are genetically modified.

For my research, I worked on developing a rice line that would be able to grow in high salinity conditions, drought conditions, and other negative conditions. As rice cultivation expands, the demand and need for these traits dramatically increase. These are the conditions millions of farmers worldwide are facing on a daily basis. The research I did during my time in Beijing may someday help pull millions of people out of hunger, poverty, and food insecurity by giving them access to crops that will help them overcome these conditions. With the golden question facing us in the coming years, it is critical that we address the needs of the world and its farmers.

As the 2010 Borlaug Dialogue was inspired by the last words of Nobel Laureate Dr. Norman Borlaug, we too as scientists and agriculturists worldwide must heed his words; "Take it to the farmer".

Tapestry of Life

The Ending

On one of my last days in China, I was looking back to that first full day in China. It was in fact a day full of firsts and new experiences. Not only was it the first day I saw the lab and explored Peking University, but it was also the first day I was exposed to the poverty in China. I also remember all of the things that went by so fast during the internship, yet each memory contains in itself a memory of poverty and hunger. Poverty is worldwide, but until I opened my eyes and experienced it, I could have never seen it. I had to experience it firsthand.

In thinking of my experience and going through my memories over the last two months, I tried to pick only one to put in my paper, but I cannot pick just one. Each memory can be seen as a puzzle piece and is unique, interesting, and beautiful in its own way. When seen as a whole, each puzzle piece interlocks with another one. Each one adds onto another. When approached from a linear time perspective, each memory is definite and defined. But when reflected upon, the memories blend into a tapestry with hundreds of different colored threads, each one representing a different story.

My time and experience in China from the first day, to the last day is also a beautiful tapestry. It cannot be summed up with one story or one narrative. Each experience and memory is a different thread. I will always remembermy bike rides with Dr. Kang through Beijing traffic. The political and cultural discussions we had while walking through fields of cotton. My research and work in the lab. The Chinese people and culture are amazing. Finally, I was privileged to see a Korean opera at the National Center for the Performing Arts. The list goes on and on and cannot be encompassed in this paper.

As with any tapestry, the imperfections make each unique and beautiful. My struggles during my time in China had the biggest impact on me. My daily search for clean water opened my eyes towards the importance of water security. The poverty I witnessed just outside my dorm reminded me how lucky I am. The hunger surrounding me, and the hunger I experienced motivated me. I now want to be an active global citizen. I want to make a difference. I want to answer the golden question.

The Golden Question

We have 6,860,200,577 mouths to feed. An additional 267 more mouths are born every minute. 1 in 6 people go without food and 1 person dies every second due to hunger. Those are some very moving statistics. How do we, by 2050, grow 70% more food and feed 2,300,000,000 more people with less land, more demand for biofuels, and an ever increasing standard of living?

I don't believe there is only one answer to this question. It will require work in plant biology, genetics, biochemistry, physiology, ecology, plant breeding, agronomy, forestry, food science, health science, education, politics, social science, economics, law, and agri-business as well as many other areas. This summer, I had the amazing opportunity to work towards getting one of those puzzle pieces, one of those tapestry threads, into place.

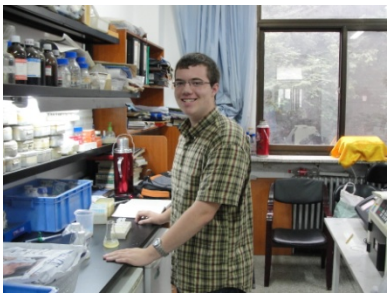
As I flew home to the United States, I remember looking out of the airplane window to see miles upon miles of interconnected, interlaced farm land. Each farm a puzzle piece, each row of plants a colored thread. As members of the human race, we too are part of a puzzle, part of a tapestry. Every person, every ethnicity, every occupation is interconnected and interlaced. Individually, each one is unique, interesting, and beautiful in its own way. But together, each one interlocks with each other. Each one is a thread in a tapestry of millions.

In actuality, the answer to the golden question could also be very simple and I challenge the same to each and every person in the beautiful tapestry called life.

The answer is: Believe

Open your eyes. Open your heart. Open your mind. Look beyond what you can see. Feel what you have not felt before. Think differently from what you have known. Experience the world around you, and all it has to offer. Finally, believe in your ability to make a difference and that the impossible is possible and we will succeed.

Pictures

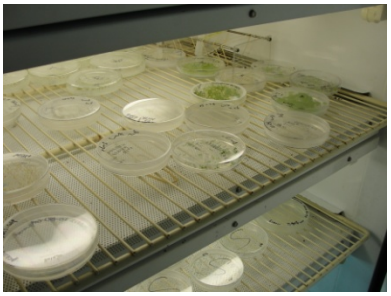


Work with transgenic rice at Peking University

Work in cotton fields south of Beijing, China

National Laboratory of Protein Engineering and Plant Genetic Engineering

Dr. Dingming Kang



Plant tissue cultures of rice

Dr. Wei Tang

Birds Nest National Stadium

Plant Biology Conference

Street Fair in Tianjin, China

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