

*MI FAN MEANS RICE**



Anne Michael Langguth
Iowa City, Iowa
2004 Borlaug-Ruan International Intern
National Laboratory of Protein Engineering and Plant Genetic Engineering
Peking University
Beijing, China

*Quote from personal journal, Thursday, 6/10/04

ABSTRACT

On Sunday, June 4th, 2004, I departed from the Des Moines International Airport on what would prove to be the experience of a lifetime, an eight and a half week internship at the National Laboratory of Protein Engineering and Plant Genetic Engineering on the campus of Peking University in Beijing, China.

As an intern in the National Laboratory, I contributed to the China Functional Genomics Program's Rice Mutant Library, a catalog pinpointing phenotypic traits to locations on the already determined genetic sequence of rice. Using T-DNA insertion, I collaborated with fellow researchers in searching for specific gene function—knowledge that is essential to the creation of higher-yielding, stronger rice plants. In a little over two months time, I learned more than I dreamed possible about molecular biology, the Chinese culture, and my career goals and life ambitions.

To the many people in the beautiful and historically rich country of China with whom I had the opportunity to live, work, and play during the summer of 2004. I always will remember your smiles when discovering a perfectly banded PCR result, your laughs of delight when challenging me to a game of badminton, your diligence when testing different primers, your patience when sorting *arabidopsis thaliana* seeds, and your uncanny ability to rapidly text-message. You have given me an enriched perspective on life, and for this I am most grateful. Thank you.

ACKNOWLEDGMENTS

I would sincerely like to thank Dr. Dingming Kang for his unending support throughout my internship. Dr. Kang was the best mentor, translator, tour-guide, bike repairman, badminton opponent, and friend an intern could have.

Deepest thanks to Dr. Hongya Gu for hosting and supervising my internship in China. Dr. Gu's museum explanations were fascinating, and I will never forget the welcome setting in which I first saw the movie *Troy*.

A big xiexie goes out to Dr. Zhangliang Chen. Dr. Chen proved to me that I quite literally can climb mountains.

With deepest gratitude I would like to thank the World Food Prize Foundation for providing this opportunity, and for allowing me to embark on this journey. Dr. Norman Borlaug's life and work have been an inspiration to me. I always will remember the feeling of empowerment and the desire to help others that his speech at the CAAS and WFP joint conference instilled in me. Ambassador Kenneth Quinn's life experiences have enlightened me and remind me to view events and process information with a global perspective. His treasure trove of knowledge has proven that we can and must learn from the past to create a stronger future. I am grateful to Lisa Fleming for the countless hours of work she invests in making Borlaug-Ruan Internships possible—she truly is a watchful eye and guardian.

Thank you to Ms. Roxanne Hughes for introducing me to the World Food Prize Foundation. The class period in English 9 spent watching the *Student Voices* feature on the Borlaug-Ruan International Internship was clearly life-changing for me.

And last, but certainly not least, thank you to my wonderful parents and sister for their unconditional love, support, and encouragement, and who along with my grandparents and friends helped me feel close to home even though I was so very far away.

TABLE OF CONTENTS

	Page
LIST OF PHOTOGRAPHS	viii
PERSONAL INTRODUCTION TO THE WORLD FOOD PRIZE	1
SECTION	
I. RESEARCH SETTING	3
Peking University	3
The National Laboratory of Protein Engineering and Plant Genetic Engineering	5
II. RESEARCH BACKGROUND.....	8
The China Rice Functional Genomics Program	8
The Rice Mutant Library.....	9
III. RESEARCH INVOLVEMENT	11
Part I: T-DNA Insertion and Rice Seedling Growth.....	11
Part II: DNA Analysis	14
Part III: Phenotype Observation.....	20
Protocol Translation.....	22
Field Observation.....	23
IV. REFLECTIONS ON THE INTERNSHIP EXPERIENCE.....	30
PHOTO COLLAGE	36
APPENDIX A: AGAROSE GEL PREPARATION.....	38

APPENDIX B: GENOMIC DNA EXTRACTION.....	39
APPENDIX C: INDUCTION OF THE CALLUS	42
APPENDIX D: RNA EXTRACTION (HEAT PHENOL METHOD).....	50
WORKS CITED	53

LIST OF PHOTOGRAPHS

Photograph

1. The Shao Yuan International Dormitory	3
2. The National Laboratory of Protein Engineering and Plant Genetic Engineering	5
3. Badminton on Tuesdays.....	7
4. Working in the Tissue Culture Transgenesis Room.....	14
5. Performing a Genomic DNA Extraction.....	15
6. Preparing the Gel Electrophoresis.....	16
7. Smearred PCR Results	17
8. Arbitrary Primer Determination.....	18
9. Successful Specific Primers	19
10. Recording Phenotypic Observations	21
11. Rice Plant Height and Tillering Comparison.....	22
12. Walking Alongside Pinggun Summer Corn.....	27
13. Speaking with Xi'an Farmers	28
14. Enjoying Time with the Daughters of a Xi'an Farmer	28
15. My Roommate, Tomoko, and Me in our Dorm Room.....	31

PERSONAL INTRODUCTION TO THE WORLD FOOD PRIZE

In the fall of my ninth grade year, I walked into English class to discover the television and VCR poised in position for the class to watch a video. Expecting to see a reenactment of a short story we had been reading, I was surprised when we spent the class period viewing interviews with youth interns who had spent the summer at various agricultural research centers around the world. Not only was I surprised, I was hooked; enthralled by the idea that someone my age could be involved in research abroad, and in so doing, have an impact on an issue as important as world food security.

Through independent research on water quality in Mexico and political instability and its impact on food security in Liberia in preparation for the World Food Prize Youth Institutes, my awareness of food security issues was heightened. Participating in the World Food Prize Youth Institute in October of 2003 solidified my desire to work alongside those in the forefront of research in an effort to work toward achieving global food security and connect with those in need. A highlight of the institute was having an opportunity to become acquainted with a number of World Food Prize Laureates. Interacting with these individuals so renowned in their field made me realize that as a member of the next generation, my peers and I have the responsibility to step up to the plate and do what we can to make a positive difference in the world.

As a result of my participation in the World Food Prize Youth Institute, I had not only enhanced my views on world food security, I now was eligible to apply to become a Borlaug-Ruan International Intern. As I excitedly picked up the application materials stacked outside of our Youth Institute meeting room, and later participated in a selection interview, I could in no way imagine the incredible learning experience I would have that summer. My destination: The National Laboratory of Protein Engineering and Plant Genetic Engineering at Peking University in Beijing, China.

SECTION I

RESEARCH SETTING

Peking University

Founded in 1898 during the Qing Dynasty, Peking University is located in the Haidian District, a northwestern suburb of Beijing. Situated between the Yuan Ming Gardens and the Summer Palace (an imperial summer residence built with funds embezzled from the China Navy), Peking University is a 700 acre academic setting with historic significance amid natural beauty. Known as “Yan Yuan,” the gardens of Yan, the campus is a maze of lotus blossom ponds, red-tiled roofs adorned with carved mythical creatures, hidden pagodas, and winding trails.

My first day on campus, I was shown the route from the Shao Yuan International

Photograph 1.

The Shao Yuan International Dormitory



Dormitory (my home away from home) to the “Sheng Wu Ji Shu Xi”—Biotechnology Department—and attempted to memorize the course by taking note of the buildings along the way. What I failed to take into account when feeling quite confident that my

white buildings with ornate red trim landmarks would help me record the route, was that practically *every* building on campus is a study in Chinese architecture. Needless

to say, following lunch I got lost when trying to navigate my way back to the lab from the Shao Yuan. Though beautiful, the gold painted signs and building markers in Chinese characters did little to help my situation. A helpful soul came to the rescue, directing me back to the Shao Yuan where I phoned the lab for a personal escort (a.k.a. Dr. Kang). Interestingly enough, three weeks later I was quite familiar with the many trails around the Shao Yuan and found myself returning the favor bestowed upon me a few weeks earlier by helping bewildered Americans who had just arrived for their summer studies find their way around campus.

Peking University (PKU) is proud of its revolutionary tradition (the anti-imperialist and anti-feudal May 4th Movement was initiated from Peking University), counting Mao Zedong, Li Dazhao, and Chen Duxiu (founders of the Chinese Communist Party) among former teachers or administrators. Although considered the premier university of China, I had never heard of Peking University prior to receiving notification that I had been accepted to complete a Borlaug-Ruan International Internship there. Initially, I was a bit confused as to whether it was Peking University or Beijing University where I would be interning. I later learned that they're actually one in the same; Westerners began calling it Beijing University after the name of the Chinese capital was changed from Peking to Beijing, even though the Chinese government has officially sanctioned the name to remain Peking—along with other traditional Chinese icons such as Peking Duck, Peking Opera, and the Peking Man Site. I was surprised by the respect that the name “Peking University” commanded

among my Chinese friends. My friend Elisabeth Wang was excited that I would be spending two months in her hometown and at her dream school. Elisabeth was right; I was in for an amazing experience.

The National Laboratory of Protein Engineering and Plant Genetic Engineering

The National Laboratory of Protein Engineering and Plant Genetic Engineering is nestled among the grand, red and white buildings in the architecturally historic segment of campus. To the foreigner (that would be me), the only clue to the

Photograph 2. The National Laboratory of Protein Engineering and Plant Genetic Engineering



building's function is the twenty-foot high, green and yellow DNA

model just to the left of the laboratory's entrance. Founded in 1987, the lab currently has 25 senior scientists, 39 researchers, and 45 full-time staff members.

Emphasizing a strategy of

cooperation among all aspects of science—molecular biology, chemistry, physics, and computer science—the focus of the National Laboratory's research primarily revolves around agricultural, environmental, and pharmaceutical biotechnology.

My first two weeks in the National Laboratory were spent becoming familiar with the laboratory setting and learning procedural protocols under the knowledgeable

and patient tutelage of Dr. Dingming Kang. Though I often ended up memorizing equipment processes—the Chinese signage wasn't of much help to me—and using hand gestures and nods to make myself understood, I was impressed by the English skills possessed by every member of the laboratory, and came to appreciate that science truly is an international collaboration (particularly the field of molecular biology)—with English being the common language used for all research. That being said, while an American student must demonstrate strong skills in mathematics and science in order to excel in the research field, in China, proficiency in English also is a prerequisite for scientific study.

On several occasions, I was the excuse for the lab to brush up on their English skills. The lab team with which I was involved decided to conduct all of their future (at least while I was on the team) meetings in English. It was during the second week of my internship that protocol books from the United States surfaced. Though the researchers in the National Laboratory communicated with one another primarily in Chinese, I found that aspiring to learn and progress transcends language barriers. While my colleagues in the laboratory were trained in scientific settings throughout China and my scientific training had occurred in classrooms in the U.S., we shared a common curiosity and drive to discover more—despite the fact that our first languages were not the same and we had been attending schools on opposite sides of the world.

I can't envision a more welcoming work setting. Eager to assist in any way I could, every effort was made to enhance my understanding of their work and involve me in their research efforts. In addition to including me in their research, Tuesdays from ten to twelve in the morning found me participating in lab-sponsored badminton matches at a gym across campus. I am grateful to have been included in the play, as it was through active participation in events such as "Badminton on Tuesdays" that I had the opportunity to become better acquainted with members of the research team. I came to realize that a wild leap across the court followed by a missed birdie results in a good laugh no matter what language is spoken, or where the match occurs.

**Photograph 3. Badminton on
 Tuesdays**



SECTION II

RESEARCH BACKGROUND

The China Rice Functional Genomics Program

In conjunction with the mission of the World Food Prize Foundation and its quest for global food security, my work in the lab involved research contributing to the China Rice Functional Genomics Program (CRFGP). Though a relatively small portion of the National Laboratory's work (I joined with seven other researchers), the CRFGP is a nation-wide project that employs six renowned labs in China and a total investment of more than 150 million RMB (approximately \$20 million) from the Chinese government. Officially started on January 1st, 2000, the goal of the CRFGP is “to isolate genes relating to the important agronomic traits of rice, based on functional genomic approaches, and to establish public databases to provide genomic and literature data about *Oryza Sativa* [rice]” (1). Targets for the next 5-year period for the CRFGP include: (a) establishing a biological database for rice genes—the Rice Mutant Library, (b) isolating at least forty full-length genes controlling important agronomic traits, and (c) training young scientists to be qualified in the fields of molecular biology and modern crop breeding.

Apart from the fact that rice is one of the world's most important food crops—about one-third of the world population depends on rice for more than 50% of caloric intake (2)—rice often is chosen as a model system in cereal genome research because

of its relatively small size. The rice genome is estimated to contain about 430 mega base pairs (Mbp), whereas the genome of sorghum is estimated at 1000 Mbp, maize at 3000 Mbp, barley at 5000 Mbp, and wheat at 16,000 Mbp (2, 3).

The Rice Mutant Library

CRFGP-related work at the National Laboratory at Peking University revolves around the establishment of a Rice Mutant Library through the process of T-DNA insertion. When completed, the Rice Mutant Library will be build upon the existing Rice Genome Database that can be accessed through the National Center for Biotechnology Information (NCBI). The Rice Genome Database contains the nucleotide sequences for all genes in the rice plant—a fantastic feat in itself, and one that is essential for further genetic research to continue—however, the ever-growing Rice Mutant Library takes the information one step further; identifying the phenotype derived from specific nucleotide sequences. That is, to identify the exact function of each gene. It is only when specific genes related to flowering, fertility, reproduction, metabolic control, and stress responses in rice are identified that research can continue into the development of transgenic (genetically modified) crops with the potential for greater yield (3).

My work at the National Laboratory involved T-DNA insertion with rice seedling embryos. T-DNA insertion, a process first developed in 1995 (4), is a high-tech, detail-oriented, and time-consuming task employed to discover the function of a

gene. Determining the function of a gene through T-DNA insertion is a multi-step procedure, and over the course of my eight and a half week internship at the National Laboratory, I had the opportunity to be involved in each step of the process.

Part One of the T-DNA insertion process involves the insertion of T-DNA into a rice seedling and then growing the seedling to sufficient size. Part Two is the analysis of rice seedling DNA to discover what gene was affected by the T-DNA insertion. Part Three entails observing the growing seedling to determine if there are noticeable phenotypic differences as a result of the T-DNA insertion.

The general theory behind the process of T-DNA insertion is that when a gene is inserted with T-DNA, it will either lose its ability to function, or experience an enhanced ability to function. Whether the gene gains or loses functional ability depends on where the T-DNA happens to insert itself. Enhanced function is the result of T-DNA locating itself in the promoter (beginning segment) of a gene, whereas a loss of function results from T-DNA interweaving into the original nucleotide sequence after the promoter codon.

SECTION III

RESEARCH INVOLVEMENT

Part I: T-DNA Insertion and Rice Seedling Growth

NOTE: For ease of understanding the entire T-DNA insertion process, the actions described in this paper have been placed in procedural order—Part I, followed by Part II, and lastly, Part III. In the laboratory, however, I was first introduced to the DNA Analysis process (Part II), and only after gaining a thorough understanding of this topic, did I move on to conduct Part I and Part III of the procedure.

To begin the procedure of T-DNA insertion, I placed the part of a rice seed deemed the “immature embryo” into a medium with specific plant hormones and left it to grow into a callus. Akin to the human stem cell with its ability to mature into virtually any organ, the plant callus has the potential to differentiate into more than one thing—a seedling, or more calluses, depending on which hormone is placed in the medium.

While the callus was growing to a sufficient size for the T-DNA insertion, I prepared an agrobacterium (the strain GV3101 is used in the National Laboratory) that contained a plasmid with marked T-DNA. The raw agrobacterium containing the plasmid is not prepared in the National Laboratory, as the construction of plasmids is a completely different line of work. Interestingly enough, the National Laboratory orders their SKI015 agrobacterium plasmids from the Salk Institute in California.

Each agrobacterium plasmid contains the T-DNA to be inserted, an antibody resistance sequence, and a marker Bar Gene sequence. Once the lab receives the agrobacterium, it must be readied for its insertion into the callus.

The process involved in priming the agrobacterium is a procedure in itself. First, the strain was left to grow in the liquid medium LB, and then centrifuged to form a precipitate. The resulting precipitate was then resuspended with the new medium 1/2 MS and poured into a petri dish. Once the medium containing the agrobacterium was settled onto the dish, the calluses were immersed in the solution for approximately five to ten minutes to allow the bacteria to infect and transfer its T-DNA into the calluses. Just as a virus infects and inserts its T-DNA into the host cell of an animal, the agrobacterium interweaves its T-DNA into the genomic sequence of the host plant callus cell.

After agrobacterium emersion, the individual calluses were placed on a new plate to grow for approximately one to three days. Following the initial growth period, the calluses were rinsed with double distilled water and then left to grow for about one month in an antibiotic solution to determine which calluses were inserted by the T-DNA and which were not. Only the successfully inserted calluses would survive, as the agrobacterium plasmid contained an antibody resistance sequence, and thus, any calluses that had successfully picked up the T-DNA would have the antibody resistance as well.

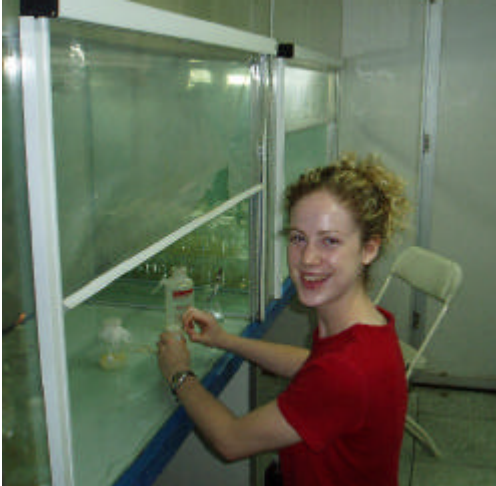
Following antibody testing, regeneration of the calluses continued, this time using a different hormone mixture (a combination of IAA, 2.4D, NAA, ABA, and BAP) to encourage the calluses to grow into seedlings. It is the seedlings that grew into the transgenic rice plants, as they now contained the T-DNA insertion. Once again, antibiotic resistance was tested in the rice genome. This time, however, the seedlings—not the calluses—were placed in an antibiotic medium for a period of one-half to one month. The seedlings were then transferred to the outdoor rice fields for sustained growth.

It was during my fourth week in Beijing that I advanced to the next level of the National Laboratory hierarchy—work with the aforementioned fragile calluses. Announcement of my advancement arrived in the form of Dr. Kang ushering me down to a double-doorwayed-for-sterility room in the basement (otherwise known as the Tissue Culture Transgenesis Room). Hand-written notices in Chinese script were posted on the doors reminding researchers of the detailed protocol for the room. All I could do was follow Dr. Kang's instructions. Because I couldn't read the labels to determine which materials were non-toxic and which were toxic, I opted to treat *all* substances in the room as potentially hazardous and not to be handled without proper precaution.

The Tissue Culture Transgenesis Room included six hooded cubicles, as the utmost precautions in sterility must be taken when working with the calluses and tissue cultures. Before work with the calluses could begin, all flasks and utensils had

to be AutoClaved—that is, sterilized in a foreboding, metal machine at high

Photograph 4. Working in the Tissue Culture Transgenesis Room



temperatures. In the United States, the heat sterilization procedure is not involved in this line of work, as disposable plastic flasks are used instead of reusable, and thus less expensive, glass flasks. After being AutoClaved, the supplies and the flasks containing the calluses were left to sterilize with UV light and air control under one of the six hoods. When the time came to actually handle the calluses, the workspace as well as

the researcher's hands were doused in 75% ethanol while the steel forceps used to transfer the calluses were dipped in 100% ethanol and then run through a flame.

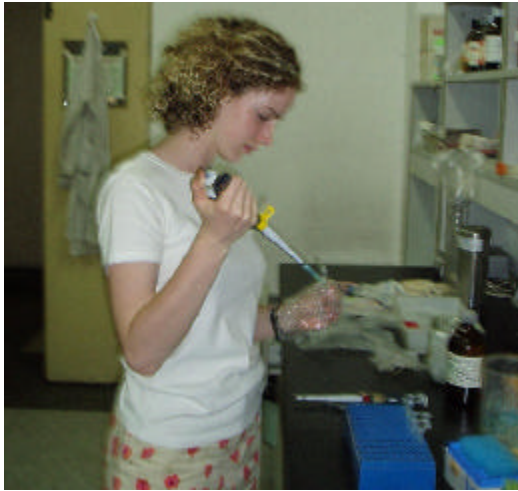
Ethanol serves an important role in the lab, as it is effective in killing most pathogens, thus preventing bacterial contamination from having an effect on research outcomes.

Part II: DNA Analysis

After the rice plant had grown to a sufficient size, its leaves could be cut for DNA extraction. The first leaf cuttings I was given to work with were from rice plants Y₄361, Y₄362, Y₄364, Y₄374, and Y₄379 (the number denoting specific seedling specimens). Later, after I had a better understanding of the extraction

procedure, I also became responsible for cuttings from plants Y₄165 and Y₄166. The extraction process involves precision in both timing and temperature—at first, it is

Photograph 5. Performing a Genomic DNA Extraction



critical that the samples be kept cool, while later, the samples are heated at specific temperatures for precise amounts of time. After the original rice leaf trimmings were rinsed and washed numerous times in CATB buffers, Phenol-Alcohol mixtures, and ethanol, a small pellet of white substance was left at the

bottom of each tube. And by small, I mean *very* small. So small, in fact, that the first time I ran an extraction procedure solo, I thought I'd rinsed away the product! The next morning, however, with a quick shake of the tube, Dr. Kang appeased my fears.

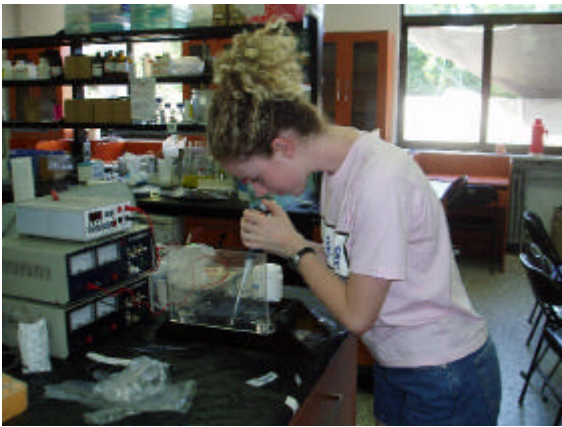
Following DNA extraction, a Polymerase Chain Reaction was run on the DNA using a primer determined by the marker Bar Gene sequence incorporated into the agrobacterium plasmid with which the callus had been injected. Polymerase Chain Reaction (PCR) is a method by which researchers can make an otherwise small sample of DNA large enough for use in procedures (5)—or in the case of the lab, see whether the extraction process was successful. A PCR machine is essentially a complex tool for rapid heating and cooling. Researchers often create their own programs that heat and cool the samples to their personal preference. I've left my

permanent mark on the National Laboratory, as three of my programs are now stored on the PCR machine hard drive—programs ANNE1, ANNE2, and ANNE3.

With the guidance of Dr. Kang, I was able to input programs to maximize temperature control throughout the PCR process. The real work of a PCR, however, is done by a *Thermus aquaticus* (nicknamed *Taq*) Polymerase. *Taq* is bacterium that naturally grows in hot springs, and thus is able to withstand extreme temperatures (upwards of 95°Celsius)—a necessity when running a PCR program. Using the original DNA strand as a template, the *Taq* Polymerase synthesizes two new strands of DNA. This results in a duplication of the original DNA, with each new molecule containing one old, and one new strand. When allowed time to denature and synthesize several times, the PCR program (using the *Taq* Polymerase) can create a substantial amount of duplicate DNA from what otherwise would be a sample too small for use in the desired procedures (6).

When completed, the PCR program resulted in a small amount of liquid that

**Photograph 6. Preparing the Gel
Electrophoresis**

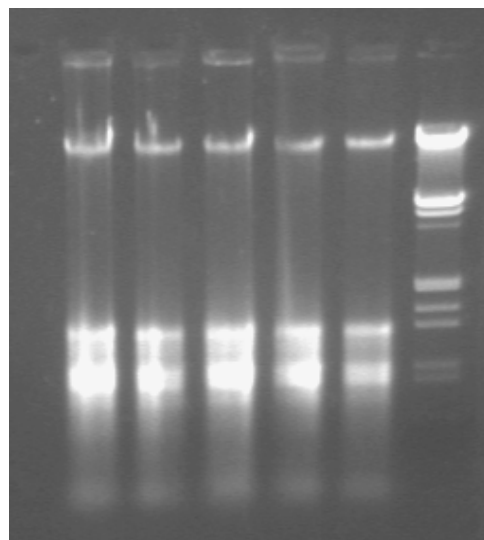


could then be run through gel

electrophoresis—a technique in which molecules (in this case DNA) are forced across a span of gel by an electrical current (7). DNA is a negatively charged molecule, so when an electrical current is applied to a gel inserted with

DNA, the DNA moves to the positively charged end. Small DNA molecules travel quickly, and thus farther along the gel, whereas large molecules of DNA do not shift as much. The movement of the DNA creates a series of bands that can be examined under UV light to determine their clarity; and ultimately, the quality of the DNA extracted. If samples were not kept under the proper temperatures, or if inadequate amounts of chemicals were used in the extraction process, the PCR result would be smeared and ineffective (see Photograph 7).

Photograph 7. Smearred PCR Results



In this PCR (performed on 6/15/04), the bands are too smeared to be of viable quality. The well on the far right contains a molecular marker—showing what the clarity of the bands *should* be. Following Dr. Kang’s suggestion, I added more RNase to the samples to “unclog” the DNA, resulting in clearer bands for my next PCR.

Y₄379 Y₄364 Y₄361
 Y₄374 Y₄362 Molecular Marker

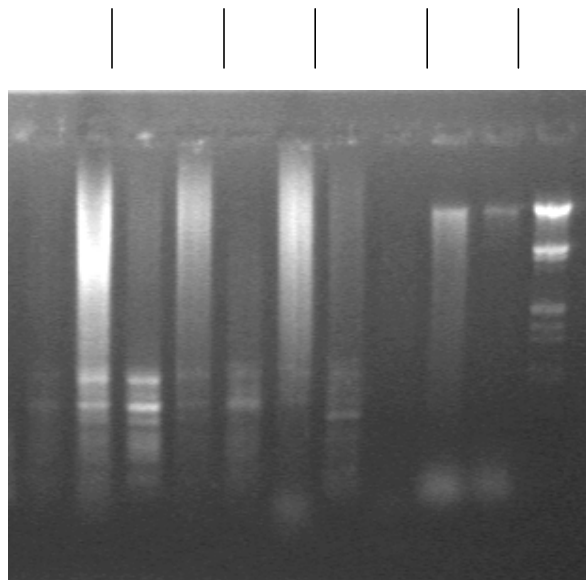
If the PCR extraction result proved to be of viable quality, I would then move on to the next step, running another type of PCR program. This time, the PCR process used was Thermal Asymmetric Interlaced PCR (TAIL-PCR). TAIL-PCR incorporates

the use of a sequence specific primer (Dr. Kang generally advised me to use LS₁, LS₂, and LS₃) along with an arbitrary primer (we utilized various forms of AD primer) to isolate an unsequenced segment of DNA located next to a sequenced one (8). At this juncture, we did know the T-DNA sequence inserted into the original callus. The sequence we were trying to ascertain was the sequence located next to the known T-DNA, which would unlock the secret as to the gene to which the T-DNA had attached. As with the PCR process, once the TAIL-PCR process was completed, a gel electrophoresis would be run to determine if the extracted sequence had been spliced cleanly enough to continue with purification. TAIL-PCR seemingly is a process of trial-and-error—even more so than regular PCR. I ran nine different programs on my seven rice samples (using AD 1, 1-1, 2, 2-1, 2-2, 2-3, 2-4, and AD 2-5 twice) before finding an arbitrary primer that adequately spliced the DNA (See Photograph 8).

Photograph 8. Arbitrary Primer Determination

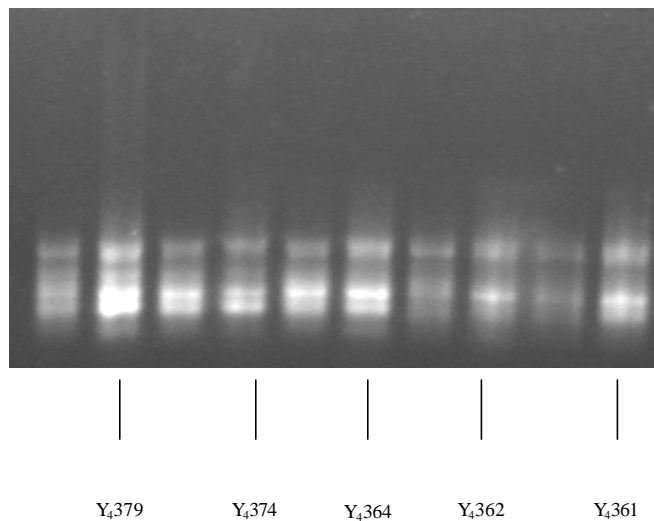
In this TAIL-PCR (performed on 6/22/04), the far right sample is clear and ready for use, whereas the others are too smeared. To remedy the problem, a different arbitrary primer (AD) was used.

Y₄379 Y₄374 Y₄364 Y₄362 Y₄361



Arbitrary primers, however, were just the beginning. Even if the arbitrary primer used was able to splice the DNA perfectly, the TAIL-PCR results weren't of good quality unless the specific primers cut in places that would best "showcase" the arbitrary primer results (See Photograph 9).

Photograph 9. Successful Specific Primers



In this TAIL-PCR (performed on 7/13/04), the AD primer used—in this example AD_{2.5}—was successful; no streaks are apparent. However, each sample seems to be cut in the same place. In the gel shown, the good news was that one of the primers was working, making a clear cut. The bad news was that one of the primers (in this case the LS₁, LS₂, and LS₃ specific primers) wasn't working—they're all spliced at the same location.

Although the trial-and-error method can be used to find a successful specific primer, Dr. Kang opted instead to review research documents from previous Peking University students to see if their work provided any information that would be useful in our TAIL-PCR process. Several research summaries written by Yan Zhang, a recent graduate of the Peking University College of Life Sciences, seemed especially relevant. In the documents, Ms. Zhang highlighted her work with the specific primers TR-57, TR-103, TR-135, RL-1, RL-2, RL-3, and RL-4 in rice T-DNA insertions.

Based on this information, Dr. Kang proceeded to order the new primers from the Shanghai-based Bio-Asia company.

If the extracted DNA can be correctly spliced, the DNA will be purified and put through the 377 DNA Sequence Analysis machine to determine the exact genomic sequence of the DNA. The Sequence Analysis machine, the specialty of lab member Li Zhang, produces a four-color printout of the genetic sequence from an inputted DNA sample. For example, if the Sequence Analysis machine determines that the genetic code of an inputted DNA sample is “atgccgta,” then the researcher will navigate to the NCBI Website and type “atgccgta” into the rice genome database to determine which genes match that sequence. My limited time in the lab did not allow me to witness the purification of my seven DNA samples; however, following my departure, other researchers in the lab have continued my work.

Part III: Phenotype Observation

Equally important as the genomic research, is the observation of morphological, metabolic, and physiological traits in the mutant (that is, T-DNA inserted) rice plants. Each category of traits must be carefully examined for its potential impact on organ function in rice—with the overarching goal being that of finding a mutant rice plant that exhibits a new trait with relevance to crop yield.

Key morphological traits that are studied to create high yield in rice are the tillering capacity of the plant (its ability to create numerous shoots and thrive), how

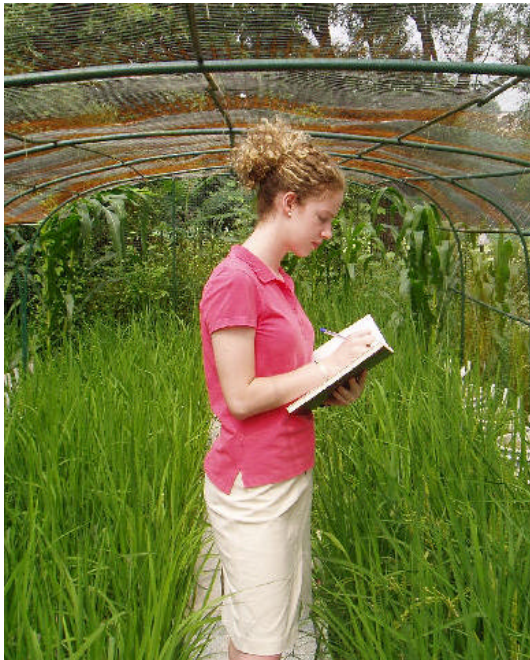
many spikes each rice plant produces (the spike is the part of the plant where the seed is located), how many seeds are on each spike, and the size of the rice seeds.

Metabolic traits observed relate to chemical growth patterns, and physiological traits have to do with the plant's ability to endure saline environments, high or low temperatures, limited water resources, or mineral deficits.

The mutant rice plants with which I worked at the National Laboratory were in their F_1 generation—the first group of seedlings to contain the inserted T-DNA.

Because F_1 generation rice plants are heterozygous (with half of their chromosomes

Photograph 10. Recording Phenotypic Observations



from the inserted T-DNA, and half from the parent plant), it is difficult to

determine whether observed morphological changes in the plant are the result of environmental factors or actual genetic differences. In the F_2 and F_3 generations, traits can be more accurately attributed to the genomic sequence, because the plants are homozygous for the inserted T-DNA.

Even so, an exciting observation was made in five of the F_1 generation plants with which I worked: plants Y₄361, Y₄362, Y₄364, Y₄374, and Y₄379 were all significantly shorter and possessed fewer tillers than plants of comparable ages (See

Photograph 11). During my two months at the lab, however, we did not have sufficient time to determine whether these characteristics were due to external variables, or the inserted T-DNA. Only after the F_2 and F_3 generations are grown will the researchers be able to ascertain whether genes relating to height and tillering have been identified.

Photograph 11. Rice Plant Height and Tillering Comparison



Tubs containing the shorter and thinner than average F_1 generation of rice plants Y₄361, Y₄362, Y₄364, Y₄374, and Y₄379. (7/15/04)

Tubs containing rice plants of the same age as those to the left. These plants, however, exhibit typical morphology. (7/15/04)

Protocol Translation

In addition to participating in research procedure, a portion of my time at the National Laboratory was spent translating lab protocol into English. Having access to standard lab procedures in English is valuable to the laboratory because the protocol can then be used to explain their research efforts to foreign visitors, international students (like myself) can more readily participate in their research, and the guidelines can facilitate the preparation of research papers written and published in English. I

was provided the current English translations of lab protocol, and would proceed to decipher the procedure described. Dr. Kang would explain to me any concept or procedural step that I was unsure of, and double-check to ensure that the outline I had gleaned from the current overview was correct. I would then type the protocols, and after Dr. Kang's assistance in editing and revision, prepare a hard copy for the lab's use. I completed four protocols during my internship—that of Agarose Gel Preparation, Genomic DNA Extraction, Induction of the Callus, and RNA extraction (See Appendixes A, B, C, and D for complete protocols).

Field Observation

During the final two weeks of my internship, I had the opportunity to travel outside the city limits of Beijing. It was during these journeys that I was able to observe (albeit a brief glimpse) the living conditions more typical of two-thirds of the Chinese population. On these brief, yet incredibly educational sojourns, the rural and urban divide became evident to me. I had read that the income ratio of the urban workforce to the rural workforce is nearly four to one, however, I hadn't imagined that the disparity would be so evident. Although the observations I made regarding the living conditions in rural China can in no way be considered definitive or comprehensive, the few places I visited validated the need for food security measures to continue.

Observing agricultural practices currently in use (e.g., farmers toting canisters of pesticides on their backs) and speaking with regional farmers reinforced my desire to push forward in the laboratory. Though my research was only a small part of a vast collaborative network, being with the people that had so much to gain from work such as the Rice Mutant Library allowed me to observe firsthand the pressing need for agricultural innovation.

A fascinating trip outside the Beijing Municipality occurred in association with a weekend excursion to the coastal city of Penglai in Shandong Province, a trip sponsored by the director of the National Laboratory. As others were drifting asleep or playing card games during the 12-hour mini-bus drive, I found myself glued to the agricultural panorama passing before my eyes. Images that remain with me from this bus trip include spring green fields with workers wearing wide-brimmed straw hats hunched over tending to the crops, oxen tied to trees alongside the road, shepherds carrying homemade herding equipment (a twelve-foot long stick with a rope attached to the end) while tending their sheep, farmers with skin as tan as the soil they were working pedaling bikes back to their one-room houses for lunch.

Having grown up in a farming state, while traveling in the countryside I couldn't help but notice the lack of heavy farm equipment. In the evenings, it was a common site to see farmers walking up and down the rows of their fields with sprayers in hand and plastic tanks of pesticides on their backs, but never with any machinery that would lessen the need for extensive manual labor. My observations relative to manual

versus mechanized labor would be addressed later, in a small village outside of Pinggun in the Beijing Municipality.

A second interesting visit outside the city was to the peach growing district of Pinggun, about a two-hour drive from the Peking University campus. It was here that I was first able to converse with locals about their farming practices, the changes in farming they had witnessed during their lifetime, as well as what they believed the future had in store for agricultural practices in China. While speaking with a farmer who also owned and operated a small restaurant (through the translation assistance of Dr. Kang), I learned that many rural Chinese citizens seek additional employment off the farm. The .3-.4 acres allocated by the government to each family in the more populous areas of China does not enable families to garner enough income. Those with two jobs were quick to justify their added workload—approximately 15,000 RMB (US\$ 1800) could be earned per year from a restaurant whereas land plots only produced “a few thousand Yuan” at best per year.

Though the shift of rural citizens from working on the farm to pursuing service-oriented jobs may reduce the number of farmers, this in itself does not necessarily reduce the amount of crops produced. Instead, this trend seems to be leading to higher incomes for rural citizens in general. In the process of abandoning agricultural work, farmers give their land back to the government, which in turn redistributes the land to other farmers—increasing the amount of land available for those left farming. Those

who leave the agricultural sector make more money, and in turn, their leaving creates greater income potential for those who rely solely on the land for their livelihoods.

With the current small land plots, the need for machinery simply doesn't exist. Because each family is responsible for a portion of land that can be tended by manual labor, most farmers do not invest in expensive tractors or other equipment. Those who do own small machinery (such as the farmer/restaurant-owner with whom I talked) often use their equipment as another source of income, renting the machinery out for use during the busiest times of the season.

In the Pinggun area, those with whom I spoke emphasized the importance of cash crops. It seemed as though in that area, the best way to generate income from their land allocation is through the growing of peaches and other non-staple crops. I gathered that this is a trend throughout China—farmers abandoning the staple crops for those that can generate a quick source of income. Therefore, it is only through government regulation of land ownership and production that staple crops have continued to be grown. Those who do grow staple crops make the most of their land by growing different crops during all seasons of the year.

Both of my parents grew up on grain and livestock farms, so in reference to corn, I often have heard the phrase “knee-high by the fourth of July.” Aware of this verse indicative of how tall corn should be by July 4th in order to have a successful harvest, it was interesting to note that the corn in Pinggun was just above knee height—on the 22nd of July! My concerns were placated, however, after learning that the corn I was

seeing would not be harvested until November. Deemed “summer corn,” the corn is not planted until *after* the “winter wheat” has been harvested. Once the corn is

**Photograph 12. Walking
Alongside
Pinggun
Summer Corn**



harvested, the wheat is planted again and left to grow throughout the winter and spring months.

The double-cropping serves to generate more income—agricultural counterparts in the Western regions of China where two harvest seasons are not possible make on average 1,518 RMB (US\$ 182) per year whereas areas where double-cropping is possible see an average income of 3,845 RMB (US\$ 461) per year (9).

The added income, however, comes at a price, as the rate of land degradation increases when the soil is continuously cropped. The

organic fertilizers that farmers in the region apply won't sufficiently protect the land from degradation. In his speech on July 17th, 2004, on the campus of the Chinese Agricultural University, Dr. Borlaug emphasized the importance of using chemical nitrogen fertilizer in addition to organic fertilizer. After viewing the fields in Pinggun, Dr. Borlaug's recommendation took on new meaning. If China's current rate of production is to continue—feeding 21% of the world's population with just 9% of the

world's arable land (9)—then land preservation through the continued and increased use of fertilizer is essential.

My final visit to a rural locale occurred outside the city of Xi'an, the capital of

Photograph 13. Speaking with Xi'an Farmers



Shaanxi province. It was here that the importance of infrastructure development became apparent to me. Although I had noticed on earlier travels that the further one traveled from the paved roads, the less affluent neighborhoods became, it

was outside of Xi'an that the testament to the influence of roads on the ability to generate higher incomes became the topic of discussion. While speaking with a

family who for generations had raised pomegranates, garlic, and

sweet potatoes on the same plot of land, I inquired as to what changes had impacted their farming the most during their lifetime. Without hesitation, the husband and wife

concurred that the construction of a nearby paved road had resulted in increased income. With the thoroughfare, more people were likely to stop by their roadside

Photograph 14. Enjoying Time with the Daughters of a Xi'an Farmer



produce stand, and they now had easier access to fertilizer and pesticide distribution centers. Profit increases aside, the road also allowed the family greater access to educational centers for their two daughters—both of whom have aspirations to one day attend a university .

SECTION IV

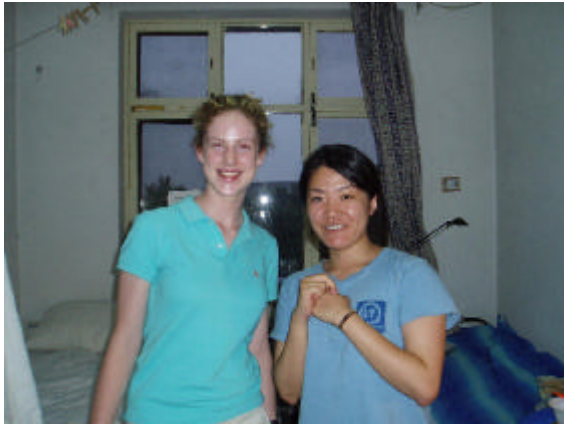
REFLECTIONS ON THE INTERNSHIP EXPERIENCE

While I was one of 46,000 students on the Peking University campus, at times it seemed as though courtesies were extended to me as if I were a Ming dynasty princess. I could not help but notice that service workers would go out of their way to make sure I was comfortable (e.g., while the street sweeper continued to brush sand and debris into the path of an oncoming group of Chinese students, upon seeing me approach, he halted his work until I had passed). In fact, in an effort to take a picture of construction workers breaking up cement with picks, it was necessary to take the photo from behind a bush so as not to interrupt their work. And no sooner had I commented in passing to Dr. Kang that the rainy weather was nice because it made the dormitory rooms cooler, than he disappeared on his bicycle. I later learned that he had set out to look into the availability of air-conditioned living quarters for me. There were none available, and I wasn't disappointed, as by that time in my internship I was comfortably settled into my fan-cooled room.

In the United States, privacy and space are taken for granted, but in a land of more than 1.3 billion people, privacy is rare, and not expected. The room I shared with Tomoko, my roommate from Japan, was only slightly larger than my bathroom at home. Though a tight-squeezed arrangement compared to United States standards, it was with a twinge of guilt that I'd describe my living situation in the Shao Yuan

International Dormitory to colleagues in the lab, because at Peking University (as

Photograph 15. My Roommate, Tomoko, and Me in our Dorm Room



with most universities in China),

Chinese undergraduates are housed six

to a room, graduate students four, and

doctoral students gain the privilege of

having two students per room.

International students studying at

Peking University, however,

regardless of student status, are placed

with a maximum of two students per room.

Try as I might, I found the Mandarin language to be exceedingly difficult to learn. Because scientists from all across China work in the National Laboratory, numerous dialects were represented in the Biotechnology Building. Thus, it seemed as though every person I asked had a different way to say such phrases as “Do you speak English?”, “I like...,” or “Where are you from?” Although my Chinese language skills didn’t advance beyond being able to inquire about prices from vendors, I *was* able to pick up a number of essential nouns—primarily that of food.

Speaking of which, I found the experience in Peking University’s dining halls to resemble the hawkers/tourists experience at the Great Wall; except in the case of the dining halls, it is the hungry people encroaching upon the student workers doling out “jiao zi” (dumplings) as fast as they can. Armed with my mini-notebook (where I’d

recorded numerous terms for food) and my cobalt blue pay card, I was prepared to shout “mi fan he helandou” (rice and snow peas) to receive my evening meal. Besides obtaining the foods desired, the dining halls presented another challenge—where to sit? With rarely a familiar face in sight, my procedure was as follows: stake out an empty seat, smile at whoever is sitting across from you, and before long, conversation will ensue. This plan of action resulted in great conversations with students from all across the globe—Chinese, Japanese, Germans, and Indians. Several Chinese students were quick to pick up on the fact that I am fluent in English, pushing drafts of everything from poetry to legal documents across the table for my review.

Every kindness extended to the Chinese students, even if it was as small as talking with them for a few minutes, was acknowledged with the most sincere graciousness. I found it was not uncommon to receive the “Shaolin salute” (flat left palm pressed tightly over the right fist and held at chest level) simply for acknowledging the presence of the student next to me.

Relative to world hunger and food security issues, I have learned that while I was provided the opportunity to work alongside scientists in China on technical solutions, such solutions are only part of the equation (albeit a most important part), as political will and the policies of the respective nations also must be given careful consideration and taken into account. Though idealistic, it would seem that if profit and patent concerns could be removed from the research equation, the distribution of crop-enhancing technologies would more readily occur.

A challenge also exists for researchers and policymakers to remain abreast of the ever-changing agricultural horizon. In addition to easing the economic burden on farmers, policies must be implemented that reflect an awareness that farmers and farming communities are valuable resources, inherent to the success of society—and as such, are resources that must be invested in and nurtured to ensure their continued success.

Over the course of this internship, I came to realize that when it comes to cross-cultural understanding, one cannot underestimate the impact of seeing one's homeland through the eyes of people in another country. United States political practices were harshly criticized in the *China Daily* (the country's English newspaper that I purchased every day from a vendor en route to the dining hall where I would have lunch)—yet the Chinese with whom I spoke did not seem to harbor ill will toward Americans; they simply seemed curious about our government, economy, and lifestyle. Oftentimes, stereotypes of the “typical American” that surfaced in conversations I had with colleagues were quite inaccurate; however, other observations were embarrassingly true.

The flip-side of this held true as well—I found that many of my preconceived notions about China were wholly inaccurate. Though my two months in China have in no way made me an expert on the political and social workings of the country, over the course of the internship, I encountered a land steeped in history and tradition, yet at the forefront of technological advancement, a country where a Starbucks is tucked

inside the walls of the Forbidden City, a country where in one day I could observe devoted Mao Zedong followers place flowers at his mausoleum, and the next, listen as professors praised the current capitalist advancements in China. The China I experienced is a country seemingly negotiating a delicate balance between adapting to Western influence and preserving its past.

It was an honor to represent the United States as a young person with a genuine interest in Chinese culture and a desire to enhance mutual understanding between our two countries. Just as the young calluses with which I was working in the lab could not grow without the proper Phyto-hormones, I have come to believe that the seeds of diplomacy cannot come to fruition until cultural misconceptions are addressed.

The potential of face-to-face relationships relative to cross-cultural understanding became evident through the comments of one of my colleagues in the lab. After a conversation we had been having about our families and common interests she stated, “As we were speaking, I did not think that I am Chinese and that you are American. We are simply two friends.” Though people in different countries may exhibit distinct facial features and participate in diverse customs, we truly are all more alike than different. We all have the same desire to lead the best lives possible, the same yearning to experience the warmth of friendship, and the same goal to ensure that those we love and care about are safe and secure.

I have discovered that while sitting comfortably in the country you call home, it is easy to pass judgment as to the practices and traditions of another nation. When in

that nation, however, one can discover that there is a reason that certain traditions stand as they do. Therefore, it seems that we cannot assume that practices we have become accustomed to in our country ought to be globally applied—each country has unique cultural and historical perspectives that must be respected and given thoughtful consideration.

On July 10th, I had the opportunity to participate in the World Food Prize Foundation joint conference with the Chinese Academy of Agricultural Science. It was inspiring to witness firsthand the admiration for Dr. Borlaug's work that exists throughout the world, and to meet others who share his vision. Just as the Olympic torch passed through the campus of Peking University en route to Athens on my third day in Beijing, my desire to carry the torch of cross-cultural understanding and scientific progress into the next generation has been kindled as a result of my experience as a Borlaug-Ruan International Intern.

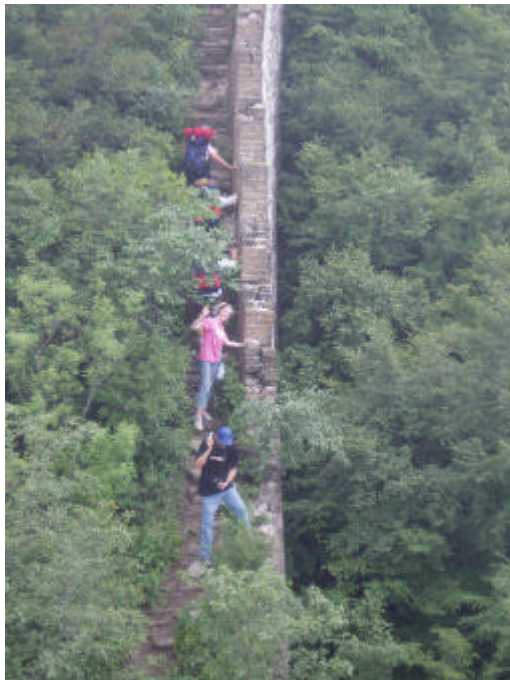
PHOTO COLLAGE



Purchasing My Own Set of Wheels



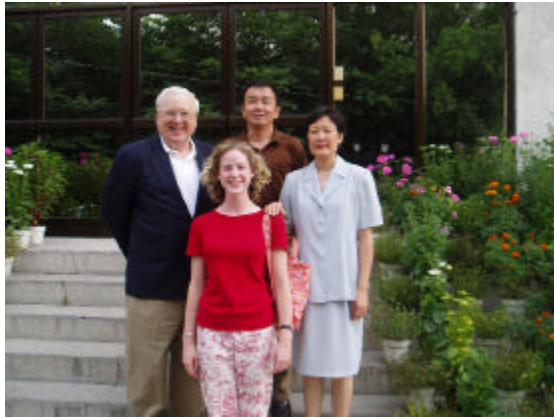
Exploring the Side Streets of Beijing



Climbing the Great Wall with the
Chinese Agricultural University
Mountain Climbing Team



Dr. Kang, Hua, and Me at the Temple
of Heaven



Ambassador Quinn, Dr. Kang, Dr. Gu, and me in front of the National Laboratory of Protein Engineering and Plant Genetic Engineering



Dr. Borlaug, Dr. Chen, and me at the Grand Opening of the Chinese Agricultural University's Center for Food Security



Home Again!



Sharing my Borlaug-Ruan International Internship Experiences on KXIC Radio

APPENDIX A
AGAROSE GEL PREPARATION

Anne Langguth

July 5, 2004

Agarose Gel Preparation

Note: Ethelium Bromide (EB), an extremely toxic chemical, is used in the preparation of an Agarose gel. Gloves should be worn at all times to ensure no contact is made between skin and EB.

1. In the sinks designated for EB use, clean the gel frames.
2. In the flask used for Agarose Gel preparation, combine:
 - a) 100 ml TAE Buffer Solution
 - b) 800-1000 mg of mixed Biowest High-Grade Agarose and Low-Grade Agarose
3. Microwave the mixture for 3-5 minutes to melt the Agarose.
4. Allow the solution to cool for 5-10 minutes.
5. Add 6-8 μ l of EB to the solution. Swirl gently.
6. Pour the solution into the gel frames, and wait 30-45 minutes for the solution to set.

APPENDIX B
GENOMIC DNA EXTRACTION

Anne Langguth

June 30, 2004

Genomic DNA Extraction

1. Prepare the CTAB extraction buffer
 - a) To make 500 ml of 2% CTAB (a component of the CTAB extraction buffer mix), combine:
 - i. 50 ml of 1 M Tris-HCl pH8.0 solution
 - ii. 20 ml of .5 M EDTA pH8.0 solution
 - iii. 140 ml of NaCl solution
 - iv. 290 ml of DDH₂O
 - b) To make sufficient CTAB extraction buffer mix for ten samples, combine:
 - i. 6 ml of CTAB (see instructions above)
 - ii. 12 μ l of β -mercaptoethanol
 - iii. 6 μ l of 100 μ g/ml proteinase K
2. Set incubator to 50° C.

3. While keeping tissue samples frozen in liquid N₂, grind the tissue (keeping samples separate) into a fine powder. Note: the samples MUST NOT be allowed to thaw.
4. Add 200-300 mg of frozen powder to 600µl of CTAB extraction buffer in a labeled Eppendorf tube (one sample per tube).
5. Swirl gently to mix, and incubate 50° C the sample tubes for 45-60 minutes 50° C.
6. After the samples have cooled, insert 2µl of RNase to each tube; allow to sit for 30 minutes at room temperature or 37° C.
7. Add 600µl of 25:24:1 (300µl phenol (25) and 300µl chloroform:iso-amyl alcohol (24:1)).
8. Invert mixture, and set on ice for 5-10 minutes.
9. Centrifuge tubes at 8000 rpm for 10 minutes.
10. Take the supernatant of each sample (discard the precipitate) and add 600µl 24:1 to each tube. Invert tubes several times.
11. Centrifuge tubes at 8000 rpm for 10 minutes.
12. Take the supernatant of each sample (discard the precipitate) and add 1 ml of 100% ethanol to each tube. Invert tubes several times.
13. Centrifuge tubes at 8000 rpm for 10 minutes.
14. Discard the supernatant of each sample, leaving the pellet at the bottom of the tube. Add 200-300µl of 70% ethanol to each tube.. Invert tubes several times.

15. Centrifuge tubes at 8000 rpm for 10 minutes.
16. Discard the supernatant of each sample, leaving the pellet at the bottom of the tube. Add 200-300 μ l of 100% ethanol to each tube. Invert tubes several times.
17. Centrifuge tubes at 8000 rpm for 10 minutes.
18. Discard the supernatant of each sample, leaving the pellet at the bottom of the tube. Allow the tubes to dry in a hood.
19. Add 20 μ l of DDH₂O to each tube.

APPENDIX C

INDUCTION OF THE CALLUS

Anne Langguth

July 9, 2004

Induction of the Callus

Working Procedure of the National Laboratory of Protein Engineering and Plant

Genetic Engineering Peking University

1. Place rice seed (variety No. 11 of Zhonghua) in water, leaving immersed for 4 days at room temperature.
2. After rice seed germination, transplant the seedlings to soil for a period of 2 weeks.
3. When of a sufficient size (approximately 15-25 cm), transfer the seedlings to a larger container for sustained growth until the tassel has emerged for 10 days (a total time of approximately 4 months).
4. Peel the shell of the tassel to gather the new rice seeds (containing the immature embryos). The fresh seeds should be stored at 4°C until the procedure can be continued.
5. For sterilization, soak the seeds in 75% ethanol for 15 seconds, repeating the step 2-3 times.

6. Repeat step 5 using DDH₂O.
7. Soak and shake seeds in NaClO (Hypochlorous Sodium) for 20 minutes.
8. Clean seeds in DDH H₂O 15-20 times, using a filter to dry the seeds between each cleaning.
9. Move seeds to a plate in the flow hood containing 20-25 ml of solidified N6 medium and 2.4 D Phyto-hormone.
 - a) Stocking concentration of medium:
1 L of N6 / 2 ml of 2.4 D
 - b) Stocking solution of 2.4 D Phyto-hormone:
.1 g of 2.4D / 100 ml of ethanol.
10. Cover and seal the plates containing the seeds with Parafilm, and leave in a 28°C dark environment for 1 month.
11. Transfer the calluses to a new plate for regeneration (containing the same medium as in step 9) and allow to grow for 20 days in the 28°C dark environment.
12. Repeat step 11, this time for 15 days.
13. After 15 days, transfer the calluses to a plate containing 25-26 ml of N6S3 medium for three days.

N6S3 medium formula (1 L):

10 ml N6 (Mac) 50x

1 ml B5 (Micr) 1000x

2.5 ml Fe salt 200x

4 ml CaCl_2 200x
1 ml Nicotinic acid
10 ml VB_1
1 ml VB_6
100 mg Inositol
300 mg Casein hydrolate
500 mg Proline
10 g Glucose
9-10 g Agar
10 ml AS medium (added only after the above products have been autoclaved)

14. During the first day of callus growth on the N6S3 plate, prepare the YEB bacteria culture solution (a mixture of YEB medium, Rifampicin antibiotic, and Chlorophenicol antibiotic).

YEB bacteria culture solution formula:

20 ml YEB medium (pH 7.2)

Stocking solution of YEB medium:

1 L H_2O

5 g Beef Extract Powder

1 g Yeast Extract

5 g Tryptone

5 g Sucrose

2 mM MgSO₄·7H₂O (492 mg)

20 µl of Rifampicin antibiotic

Stocking solution of Rifampicin antibiotic:

Dissolve 50 mg of Rifampicin in a small quantity of 100% methanol,

then mix the final quantity with DDH₂O to reach a stocking

concentration of 50 mg Rifampicin / 1 ml H₂O

20 µl of Chloromphenicol antibiotic

15. After the YEB culture solution has been prepared, shake (with the agitation

machine) the bacteria in the solution for 24 hours at 28°C.

16. During the second day of callus growth on the N6S3 plate, prepare a 1000 ml AB

plate.

AB formula:

900 ml of plate mix:

5 g Glucose

11 g Agar

900 ml DDH₂O

100 ml of AB mix:

50 ml 20x AB Buffer:

(60 g K₂HPO₄ + 20 g NaH₂PO₄) / 1 L DDH₂O

50 ml 20x AB salt:

(20 g NH_4Cl + 6 g MgSO_4 + 3 g KCl + 3 g CaCl_2 + 50 g FeSO_4) / 1 L DDH_2O

17. After AB plate preparation (50 ml of AB solution per plate), transfer 50 μl of YEB bacteria culture to each plate.
18. During the third day of callus growth on the N6S3 plate, harvest the bacteria from the AB plates with a glass bar and dissolve the bacteria in 1/10 MS medium mixed with AS solution.

Mixture formula:

100 ml of 1/10 MS medium:

9 L DDH_2O

20 ml KNO_3

Stocking concentration: 95 g KNO_3 / 1 L DDH_2O

14 ml NH_4NO_3

Stocking concentration: 120 g NH_4NO_3 / 1 L DDH_2O

10 ml $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

Stocking concentration: 37 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ / 1 L DDH_2O

10 ml KH_2PO_4

Stocking concentration: 17 g KH_2PO_4 / 1 L DDH_2O

10 ml $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

Stocking concentration: 44 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ / 1 L DDH_2O

1 ml of AS solution

Stocking concentration of AS solution:

(1 mg AS / 50 ml DMSO) / 450 ml H₂O

19. Move the calluses from the N6S3 plates to new plates containing the 1/10 MS solution. Leave the calluses to be immersed for 30 minutes.
20. Dry each callus with a filter for 3-5 minutes.
21. Allow the callus to grow for 3 days on a fresh plate of 1/10 MS solution
22. Wash the calluses in sterilized DDH₂O 10-15 times.
23. Wash the calluses 5-6 times in sterilized DDH₂O with 500 µl of added Ceftriaxone Sodium antibiotic.

Stocking solution for Ceftriaxone Sodium antibiotic:

250 mg Ceftriaxone Sodium / 1 ml DDH₂O

24. Place the calluses on a filter to dry for 30 minutes.
25. Set the calluses in 25-26 ml of an N6 mixture; formula:
 - 1 L N6 solution
 - 1 ml Hygromycin B
 - 2 ml 2.4D Phyto-hormone
 - 1 ml Ceftriaxone Sodium antibiotic
26. Keep the culture in a dark, 28°C environment for 30 days.
27. Transfer the calluses to 25-26 ml of a new N6 medium; formula:
 - 1 L N6 solution
 - 500 µl Hygromycin B

2 ml 2.4D Phyto-hormone

1 ml Ceftriaxone Sodium antibiotic

28. Keep the culture in a dark, 28°C environment for 15 days.

29. Relocate the calluses to 25-26 ml of HA medium; formula:

1 L N6

1 ml ABA Phyto-hormone

Stocking solution for ABA (Abscisic acid) Phyto-hormone:

Dissolve 50 mg of ABA Phyto-hormone in a small quantity of solid NaOH
(or NaHCO₃), then mix the final quantity with DDH₂O to reach a stocking
concentration of 100 mg ABA / 5-10 ml liquid NaOH (or NaHCO₃).

2 ml 2.4D Phyto-hormone

200 µl Ceftriaxone Sodium antibiotic

30. Allow the calluses to grow in a light environment for 15 days.

31. Transfer the calluses to 25-26 ml of an H6 medium; formula:

1 L N6

2 ml 6BAP Phyto-hormone

Stocking solution for 6BAP Phyto-hormone:

.1 g 6BAP / 100 ml 100% ethanol (or HCl)

32. Leave the calluses in a light environment for 15 days. The seedlings should now
emerge from the callus.

33. Place the seedlings in a new container holding pure N6 medium for sustained growth.

APPENDIX D
RNA EXTRACTION (HEAT PHENOL METHOD)

Anne Langguth

July 5, 2004

RNA Extraction (Heat Phenol Method)

Composition: .1 M LiCl

100 mM Tris-HCL pH 8.0

100 mM EDTA

1% SDS

50% Phenol

1. Prepare the RNA extraction solution using the following formula to make 10 ml of solution:

3.125 H₂O

5 ml phenol

.25 ml 20% SDS

.125 ml 4 M LiCl

.5 ml 1 M Tris

1 ml .5 M EDTA

2. Set incubator to 80° C.
3. While keeping the tissue samples frozen in liquid N₂, grind the tissue (keeping the samples separate) into a fine powder. Note: the samples MUST NOT be allowed to thaw.
4. Add 100-200 mg of frozen powder to 600 µl of RNA extraction buffer in a labeled Eppendorf tube (one sample per tube).
5. Swirl gently to mix, and incubate the sample tubes for 5 minutes at 80°C.
6. Set incubator to 4° C.
7. Add 600 µl of 24:1 (chloroform:iso-amyl alcohol). Invert tubes several times.
8. Centrifuge tubes at 12,000 rpm for 5 minutes.
9. Take the supernatant of each sample (discard the precipitate) and add 600 µl of 4 M LiCl to each tube.
10. Swirl gently to mix, and incubate the sample tubes for 30-60 minutes at 4° C.
11. Centrifuge tubes at 12,000 rpm at 4° C for 20 minutes.
12. Discard the supernatant of each sample, leaving the pellet at the bottom of the tube. Add .5 ml of 100% ethanol to each tube. Invert tubes several times.
13. Centrifuge tubes at 12,000 rpm at 4° C for 5 minutes.
14. Discard the supernatant of each sample, leaving the pellet at the bottom of the tube. Allow the tubes to dry at room temperature for 5 minutes.
15. Resuspend the pellet in 400 µl of DEPC H₂O.
16. Centrifuge tubes at 12,000 rpm at 4° C for 10 minutes.

17. Take the supernatant of each sample (discard the precipitate) and add 40 μ l of 3 M NaAc pH 5.2 and 1.2 ml ethanol to each tube.
18. Allow tubes to cool for 30 minutes in the refrigerator at -70°C .
19. Centrifuge tubes at 14,000 rpm at 4°C for 20 minutes.
20. Discard the supernatant of each sample, leaving the pellet at the bottom of the tube. Add .2 ml of 85% ethanol to each tube. Invert tubes several times.
21. Centrifuge tubes at 12,000 rpm at 4°C for 10 minutes.
22. Repeat steps 20 and 21 using 100% ethanol.
23. After discarding the supernatant, allow the tubes to dry in a hood.
24. Add 10 μ l of DEPC H_2O to each tube.

WORKS CITED

1. "Introduction to the China Rice Functional Genomics Program." Rice Functional Genomics Program. 24 June 2004 <<http://www.rifgp.ac.cn/ricehomeen.asp>>.
2. Goff, Stephen A. "A Draft Sequence of the Rice Genome." Science 5 Apr. 2002: 92-100.
3. Xu, Yongbiao. "An Introduction to the China Rice Functional Genomics Program." Comparative and Functional Genomics **3** 12 Mar. 2002: 161-163.
4. Kang, Dr. Dingming. Personal Interview. 24 June 2004.
5. "Polymerase Chain Reaction (PCR)." National Human Genome Research Institute. 27 June 2004 <<http://www.genome.gov/glossary>>.
6. Powledge, Tabitha M. "The Polymerase Chain Reaction." Breakthroughs In Bioscience. 28 June 2004 <<http://www.faseb.org/opar/bloodsupply/pcr.html>>.
7. "Electrophoresis." National Human Genome Research Institute. 29 June 2004 <<http://www.genome.gov/glossary>>.
8. Liu, Yao -Guang, and Robert F. Whittier. "Thermal Asymmetric Interlaced PCR: Automatable Amplification and Sequencing of Insert End Fragments from P1 and YAC Clones for Chromosome Walking." Genomics **25**: 674-681.
9. Tso, T.C, and He Kang, eds. Dare to Dream: Vision of 2050 Agriculture in China. China Agricultural University Press: Beijing, 2004.

