Madeline Geitz

2013 Borlaug-Ruan Intern

State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences, Peking University

CONTENTS

INTRODUCTION

PERSONAL BACKGROUND

In kindergarten, my best friend and I carefully planned out a "junk shop," a way to simultaneously reduce waste and generate money for "charity," a concept I hadn't quite grasped at that point but desperately wanted to be a part of. While this newfound business model ultimately failed, it set the tone for my future pursuits: I wanted to improve lives of people around me. This vague notion of changing lives presented itself in a number of ways throughout my life: in fourth and fifth grade I wanted to become an environmental chemist who designed new ways to create biodegradable products; in middle school I volunteered for a political campaign and lobbied on behalf of microcredit enterprises in Washington, DC; during high school I worked with a local hot meals organization, planted organic vegetables on my school farm, and volunteered for a math club catered towards "at-risk" elementary school students.

These activities were, in no small part, a product of my upbringing. I grew up in Oberlin, Ohio, a progressive college town with a history of social activism. The World Food Prize Global Youth Institute interested me as another opportunity to effect change. During my junior year of high school, I decided I wanted to study science in hope of a futre career, but wanted to utilize this study within the context of social change. When one of my teachers, Ms. Shurr, told me about the Borlaug-Ruan International Internship, I immediately had a new goal.

When I arrived at the Global Youth Institute in the fall of 2012, I was astounded by the community. All of a sudden, I was surrounded by international scientists and policy-makers, all passionate about issues of food security and inequality around the world. Later that week, the 2012 Borlaug-Ruan Interns gave presentations on their summer experiences, and I knew I wanted to apply and follow in their footsteps.

I received the details of my internship placements in April 2013 and couldn't have been more excited. I knew I had a life-changing summer ahead.

PEKING UNIVERSITY

State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences

Peking University is China's oldest comprehensive university, located in the suburban Haidan District of Beijing. Its campus is situated in an area known for academic

institutions, technology companies, and historical sites. Tourists often stop by, wishing to admire the school's traditional landscaping and architecture related to its history as a royal garden during the Qing Dynasty. Peking University students refer to the institution as *Běidà*, short for its official name, *Běijīng Dàxué*.

The State Key Laboratory of Protein and Plant Gene Research was established in 1987, and currently employs 50 full-time faculty and staff members. The laboratory's research focuses on analysis of the structural and functional relationship of bio-macromolecules, protein structure, and gene regulation. The work conducted in the lab applies modern biotechnology research with agriculture and medicine.

RESEARCH

Primary analysis of the biological role of OsWRKYa and genetic transformation of rice using the *Agrobacterium tumefacien*-mediated method

INTRODUCTION

RICE

Over 3.5 billion people globally depend on rice as a staple crop, making it crucial to ensure food security (International Rice Research Institute, n.d.). It is grown in over 100 countries around the world (International Rice Research Institute, n.d.), in Asia representing around 30% of caloric intake.

Genetic alteration of rice produces a crop that that could be more resistant to extreme temperature, grow in soil of all types, or could produce more crop on the same amount of land.

RICE BLAST & WRKY

Rice blast, caused by the rice blast fungus *Magnaporthe oryzae*, poses a threat to food security around the world. Rice blast is one of the most widely-spread diseases affecting rice, estimated to destroy enough rice to feed 60 million people annually and occurring in 85 countries. Thus, clarifying the interaction between rice and the rice blast fungus has great significance for agricultural production.

The WRKY family, so named for the conserved amino acid sequence *WRKYGQK* at the proteins' N-terminal end, is one of the largest families of transcription factors. These genes are known to affect plant resistance to a variety of biotic and abiotic factors. The result of a previous microarray revealed that the expression of *OsWRKYa* (a specific WRKY gene in *Oriza satvia*) was upregulated in rice after inoculated with the rice blast fungi. Studying the biological role of *OsWRKYa* in the rice defense reaction against rice blast may lead to better understanding the regulatory mechanisms of the *WRKYa* gene, allowing rice to be grown more efficiently to feed our world.

MATERIALS AND METHODOLOGY

The first process I assisted with was to analyze the subcellular localization of WRKYa protein by fusing it with an easily-viewed reporter protein, GFP. I, personally, practiced

the vector construction of fusing *OsWRKYa* and *GFP* genes together. Then, expressed the fused gene transiently into the tobacco leaves by injection using agrobacteria as a vector.

GENETIC AMPLIFICATION

To begin the rice transformation, the desired genetic code was isolated and amplified using Polymerase Chain Reaction (PCR). Using Taq polymerase, dNTPS, forward and reverse primers for the specific genes, and a buffer, the specific gene was amplified and duplicated many times.

The results of this amplification are checked using gel electrophoresis. An agarose gel solution is combined with EtBr (a fluorescent tag) to make sure that the size of PCR product is consistent with the information from the database.

GATEWAY SYSTEM

Once the *WRKYa* gene has been isolated and amplified by the process above, it is modified and inserted into an entry plasmid using TOPO -reaction and Gateway LR reaction. Once the *WRKYa* gene has been inserted into the destination vector, heat shock method is used to transfer said vector into *E. colistrains.*

SCREENING

In order to analyze the success of the transformation, bacterial colonies would be randomly selected for screening. On a new LB + kanamycin plate, colonies from the original plate would be transferred and the plate would be incubated. To screen these colonies, a process similar to the earlier PCR cloning is used called PCR screening.

In a set of microtubes, a small number of cells are transferred from the labelled colonies before and amplified. After the PCR screening is run, gel electrophoresis is used to analyze which colonies show positive for the transformed code. A positive colony will show a bright white band with a base pair number corresponding to the length of the desired gene. A positive colony should be picked up and the plasmid should be extracted.

Once this process is finished, the plasmid is sent for sequencing. Should the sequencing results match the desired gene, the vectors can be used for the transformation into plants by Agrobacterium.

AGROBACTERIUM TRANSFORMATION & CALLUS INDUCTION

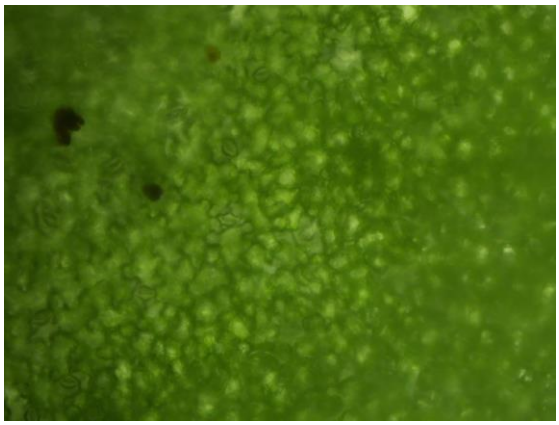
Tobacco, instead of rice plants, were used during my internship because it was a simpler model plant.

A tumor-inducing plasmid, found in *Agrobacterium tumefaciens,* was used to transduce the fused gene into plants.

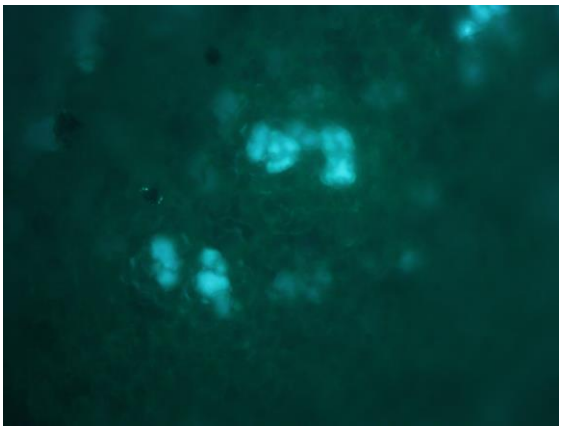
A plant callus is a mass of undifferentiated cells, and is used widely in genetic transformation for its easy manipulation and its secretion of acetosyrigone, which attracts Agrobacterium.

In order to produce the callus, plant cells are cultured in a medium containing necessarily plant nutrients and a careful balance between auxin and cytokinin. The callus then can be transferred or sustained on the medium. After shelling the grains of rice, they were washed thoroughly in ddH_20 and sodium hypochlorite before being planted in a medium rich with the nutrients needed to ensure proper callus growth. After 5-7 days, the callus was induced.

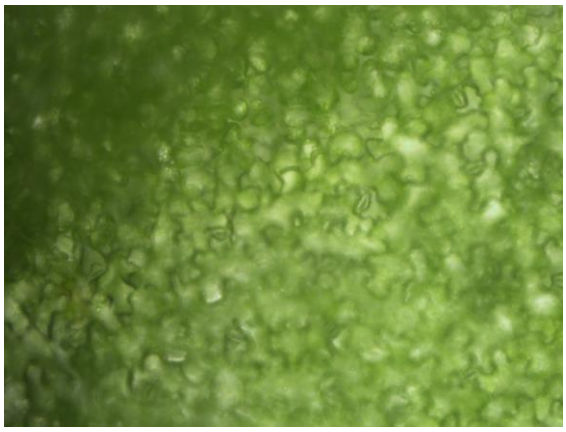
RESULTS



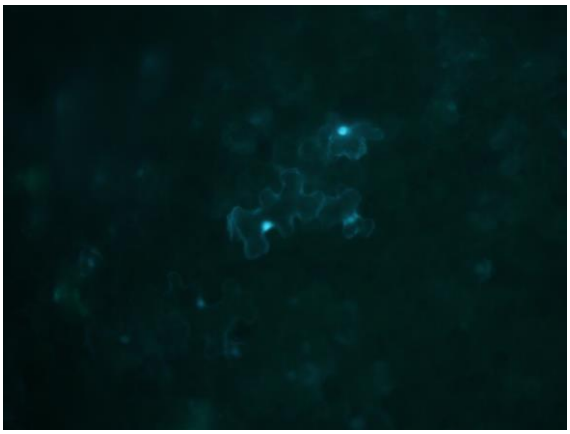
Cells under normal microscope



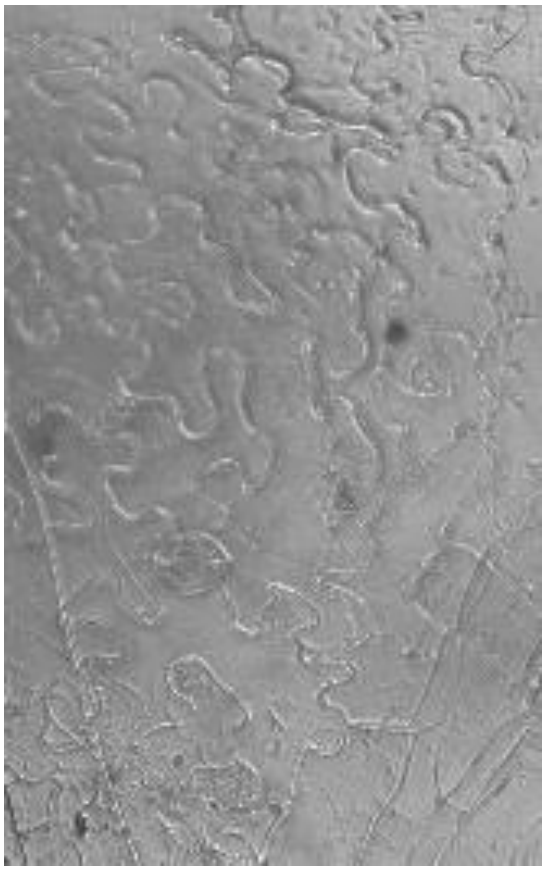
Cells showing intermittent fluorescence – does not identify subcellular localization



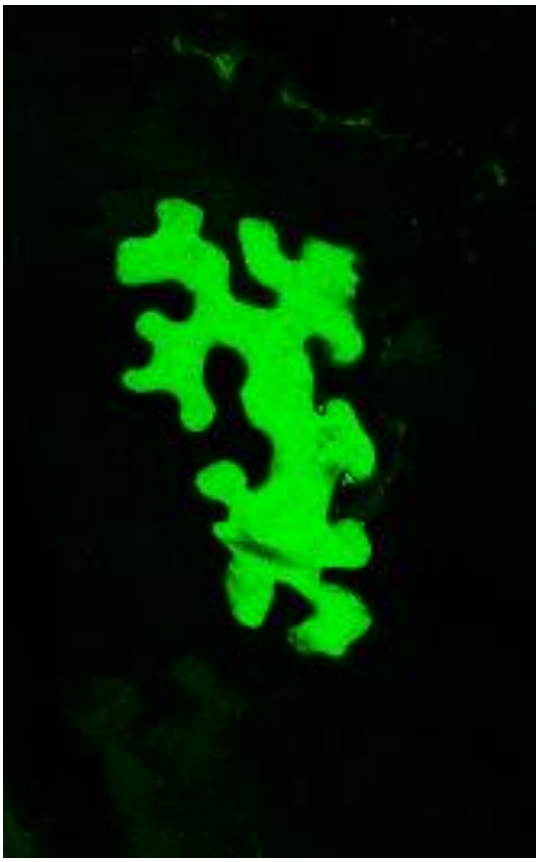
Cells under normal microscope



Shows fluorescent tag in nucleus, showing localization of the protein.



Control group to show fluorescence



This was the result of my first vector, where the localization was very unclear

DISCUSSION AND FURTHER WORK

Analysis of the mechanism of the OsWRKYa gene has particular significance for the World Food Prize's mission of feeding the world. In order to supply a growing population with the nutrition necessary to thrive, crops must be produced as efficiently as possible. Disease prevention thus becomes a top priority for not only consumers, but farmers as well. A bout of rice blast renders a season worthless; improvements made in grain heartiness thus benefit all of society.

The process of studying *OsWRKYa* is a laborious and intricate process, comprised of several major parts. Considering I only had 8 weeks at the lab, it is impossible to finish all the research that needs to be done for this gene. After I leave, the lab will examine this gene further via the loss of function mutant and overexpression, and publish their research.

PERSONAL EXPERIENCE

MADELINE IN CHINA

I was born in a city roughly 300 miles west of Shanghai and always knew I wanted to return to China.

I arrived in Beijing on a Saturday night, terribly tired after a 14 hour flight with a small child crying behind me the entire way. When I arrived at the airport, I met Dr. Kang who immediately remarked upon the size of my luggage: I checked only a single bag and brought my backpack on the flight. Though I was proud of my packing and organization skills, I'd later regret having brought such a small wardrobe. Dr. Kang led me to the international student dorms at PKU, located in the Shao Yuan (勺园) buildings on the south side of campus. To my surprise, my roommate was an American and also from a small town in the Midwest. Miranda Thompson, quickly became a close friend. When I grew tired of campus cafeteria food, Miranda and I ventured out to dinner on weekends. We bonded over our mutual interest in science (she is an aspiring physicist or engineer), geeky TV shows or books, and willingness to try almost anything once.

During my time in Beijing, I managed to meet up with fellow Borlaug-Ruan Intern Rylee McDermott a number of times. She was working at China Agricultural University and we showed each other around our campuses.

The most exhilarating times I had in Beijing were travelling about the city from site to site – navigating chaotic public transport, and, memorably, biking fourteen kilometers through streets without biking lanes. Growing up in a relatively rural community, I experienced a great deal of culture shock being constantly surrounded and pushed about by so many people. The custom of letting people out before entering is not practiced here: on elevators, on buses, on subways. I quickly learned that the most effective way to guarantee a spot is to make yourself as large as possible and barge through crowds. In the last couple weeks, I confidentially navigated the all-Chinese bus system by myself and had no issues finding my way through the city via subway.

There were many unexpected events during my last couple weeks in China. Dongshu, my mentor, departed suddenly because a family member had taken ill. Sadly, I did not have a chance to say goodbye or to thank her. Then one of the lab professors died unexpectedly in the last week of my internship and most students left campus to attend his funeral.

Before this summer, I had very little experience with independence. I had never even been to camp, and here I was on the opposite side of the world. Coming from a small college town where I recognized everyone to a city of 20 million was a disorienting, to say the least. The subway system is a normal part of people's lives! I don't see the same faces every day! I can actually get lost!

Though I didn't mention it to my parents or those in the lab for fear they would worry about me, I didn't eat my first few days because I was too nervous to interact with students in the dining hall. In the last two weeks, however, I independently went out to restaurants and food stalls and took the bus around the city, trusting my very rudimentary Chinese. I also maintained a very clean room the entire time I was at PKU! I thought I'd eternally need my parents' allusions to natural disasters to summon the motivation to pick my socks up off the floor.

This newfound self-reliance is one of the most valuable things I took away from the trip. While it was fascinating to have a greater understanding of plant defense mechanisms, knowledge is useless without the confidence and determination to use it, something that comes from being "on your own."

I must mention Miranda and Rylee one last time. Without these two now dear friends, the long stretches of free time would not have been interrupted by impromptu nighttime walks, trips around the city, or wonderful shared meals and conversation. I wish them the absolute best in their futures and hope that we'll stay in touch.

Finally, there's my lab experience to reflect upon. I realized I haven't written as much about my lab work as I expected to at the outset. This is largely because actual activity in the lab was sporadic. This was no one's fault – the students are busy with many projects that don't concern me. Also, because I'm an intern, I couldn't work independently. Because I didn't work quite as many hours as I would have liked to, my project was not completed to the extent I had hoped. That being said, the experiments and techniques I performed here were immensely rewarding. I am incredibly privileged to have done research at such a prestigious institution.

My work here has also greatly reinforced my interest in academia. I love learning and research and plan to continue it as long as I can. Because the State Key Laboratory of Protein and Plant Gene Research focuses on research easily applied to current scientific needs, I am excited that such work can bring about positive change in others' lives. In my college essays, I wrote vaguely about wanting to study science in the context of social responsibility; I now have been given a glimpse of what that actually involves.

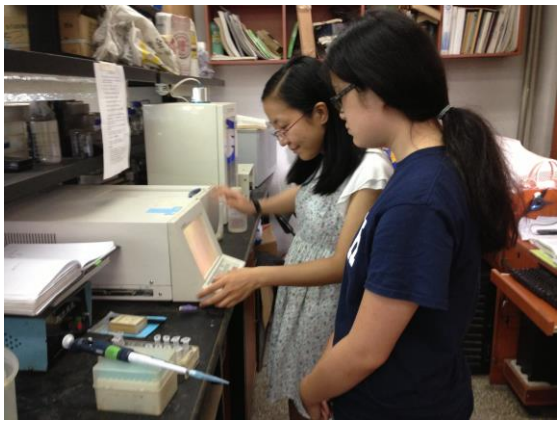
The ultimate lesson I learned via this internship is this: life is incredible. It's a broad, naïve, and slightly silly statement, but it truly reflects my beliefs. I was granted a view of

a world radically different from what I've seen before, and from this, I've gained an intense desire to continue to experience educational and life lessons for the rest of my life.

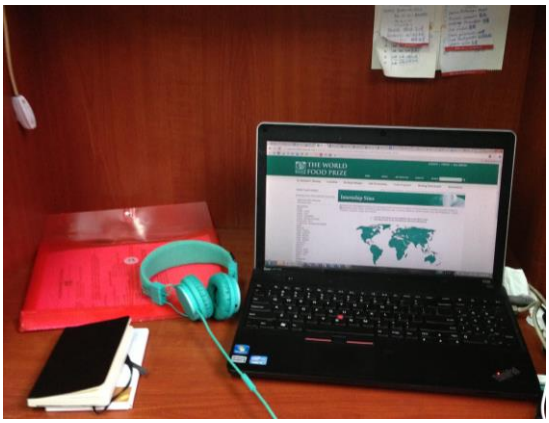
PHOTOS

My dorm room.

The lab.



Me working with my mentor, Dongshu.



My desk.

REFERENCES

Green, M. R., & Sambrook, J. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed., Vol. I). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

International Rice Research Institute . (n.d.). *Rice Blast*. Retrieved from Rice Knowledge Bank.

International Rice Research Institute. (n.d.). *About Rice*. Retrieved from International Rice Research Institute.

Pandey, S. P., & Somssich, I. E. (2009, August). The Role of WRKY Transcription Factors in Plant Immunity. *Plant Physiology, 150*(4), 1648-1655.

Patton, A. J. (n.d.). *Gateway Cloning Technology Overview*. Retrieved from Molecular Biology Switchboard.

Prelich, G. (2012, March 1). Gene Overexpression: Uses, Mechanisms, and Interpretation. *Genetics, 190*(3), 841-854.

Rushton, P. J., Somssich, I. E., Ringler, P., & Shen, Q. J. (2010, May). WRKY transcription factors. *Trends in Plant Science, 15*(5), 247-258.

TeBeest, D. O., Guerber, C., & Ditmore, M. (2007). Rice blast. *The Plant Health Instructor*.

Zhu , Y., Genji, Q., Wei, T., Qu, L.-J., Guo, D., Chen, Z., . . . Gu, H. (2013, 27 March). Transcriptional Profiling of Rice Early Response to Magnaporthe oryzae Identified OsWRKYs as Important Regulators in Rice Blast Resistance. *PLOS ONE, 8*(3).

ACKNOWLEDGEMENTS

My deepest gratitude goes out to **The World Food Prize Foundation** and all those involved. Thank you to the late **Dr. Norman Borlaug**, whose humanitarian work improved the lives of millions and whose vision has inspired many to work towards the end of world hunger. Thank you to current World Food Prize President, **Ambassador Kenneth Quinn**, for heading the organization that granted me this experience. Thank you to **Lisa Fleming**, whose tireless work as Director of Global Education Programs led me to the greatest summer of my life. Thank you to **Keegan Kautzky**, Director of National Education Programs, who was one of the first to read my report back in Ohio and somehow deemed me worthy of being sent to Iowa for the Global Youth Institute. Thank you to **Micki Zartman**, Ohio coordinator of the Youth Institute, for sending me to Iowa.

My experience at Peking University would not have been complete without the involvement of some very important people. Thank you to **Dongshu Guo**, my mentor, for including me in her research and showing me the ropes for living on campus. Thank you to **Dr. Hongya Gu** for hosting me in her department. Thank you to **Dr. Dingming Kang** for showing me around campus and providing me with all I needed. Thank you to everyone else in the lab for being welcoming and allowing a lowly intern near your research projects and equipment.

Thank you to **Rylee McDermott** and **Miranda Thompson**, two incredible friends.

Back in Oberlin, I want to thank **Ms. Donna Shurr**, my teacher at Oberlin High School, for introducing me to the Global Youth Institute/Borlaug-Ruan International Internship.

I, of course, could not have done any of this without the support of my family. Thank you to my parents, **Robert Geitz** and **Kerry Langan**, for their encouragement of my academic and social pursuits and to my sister, **Anna Geitz**, for being the driving force behind my desire to change the world.

I am indebted to you all. Much love.