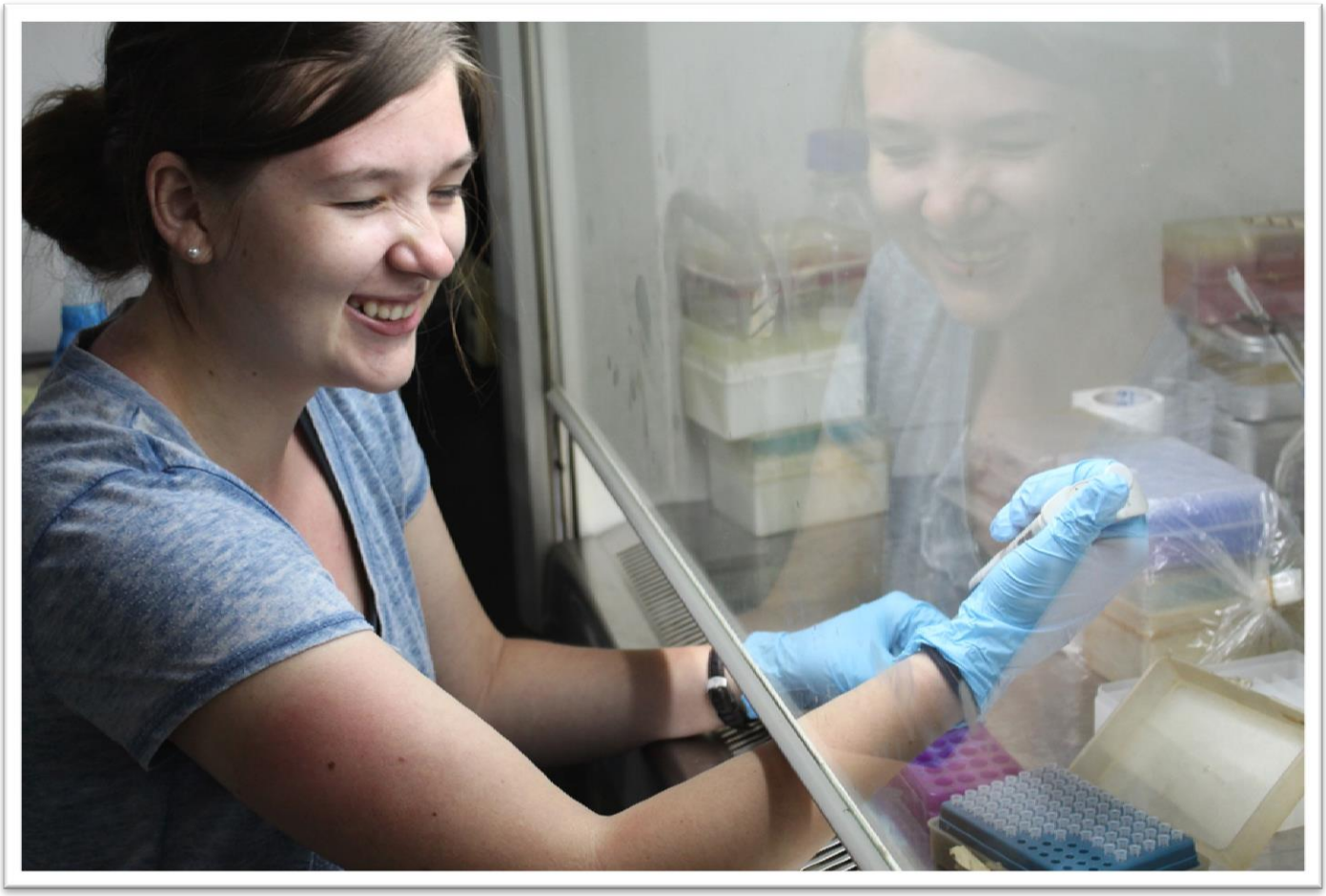
# Iowa Girl to a China World



Morgan Smith

Stuart, Iowa

The World Food Prize

Borlaug-Ruan International Internship

China Agricultural University, Beijing, China

June-August 2016

# Table of Contents

**Acknowledgements**

Finding the courage to go to another country on the other side of the world by yourself for eight weeks requires a lot more outside help than one would think.

I'd like to start off thanking **Norman Borlaug** for his vision of the World Food Prize Foundation and all the amazing programs that are associated with it. Countless students have been inspired and recruited to help answer the world's most important question: how will we feed 9 billion people by 2050?

Thank you to **Ambassador Kenneth Quinn and the World Food Prize Foundation** for continuing Dr. Borlaug's legacy, and making this program available to students like myself.

Thank you to **Lisa Fleming and her army** for everything. Sending more than twenty students to foreign countries and keeping track of them for 8 weeks is more work than most humans can handle. Lisa, you are truly amazing and super human. Thank you for reading my e-mails that were more like books.

**Mrs. Dolch**, thank you for believing in me. You helped me take my dream and turn it into a reality. You helped me realize that I can go above and beyond my goals. I am forever thankful to have role model like you.

**Nishanth**, thank you for being a friend while we were a world away from home. Though, we never had the chance to meet up, I'm thankful for our WeChat messages and jokes. I will always remember both of us worrying about the rivers that had developed on campus due to almost two full days of rain.

Thank you, **Dr. Li**, for welcoming me into China like family. I mean this in more ways than one. You made me feel welcome at CAU, and you also took me to Baotou, Inner Mongolia with your family. Because of this, I saw a more personal part of Chinese culture than I had yet seen. I will forever be thankful of you and your family.

**Professor Zhou**, thank you for being somebody to come to whenever I had questions Yu Haiyue or Zhou Li couldn't answer. Thank you for allowing me to be a part of your wedding. I will always remember being your wedding angel.

**Yu Haiyue and Zhou Li**, my roommates and mentors who became my best friends in China, thank you. You were kind and understanding when I was confused, and always made sure I was doing okay. I miss going to hotpots and comparing cultures while we laughed at how I didn't use chopsticks very well. When you make it to the United States to study or just to visit, we will catch up and relive those moments again.

To **my friends** who adjusted their sleep schedules and downloaded WeChat to talk to me, I am so very thankful for you. You're the best friends a girl could ask for.

**Mom, Jasmine, and Jacob**, thank you for being self-less and giving up your last summer with me home for me to follow my dreams. Your support for my crazy endeavors does not go unnoticed. I'm convinced I have the best family in the world. I love you.

**Iowa to China**

*How did I get involved with the World Food Prize?*

As a freshman in high school, I joined our local FFA chapter with the idea that it was the big kid's version of 4-H. Through FFA, I was introduced to a broad range of agriculture, the problems surrounding it, and the World Food Prize. As a sophomore, I wrote my first paper for the World Food Prize Iowa Youth Institute (IYI) about food insecurity in Zimbabwe. I attended FFA's Washington Leadership Conference in Washington, D.C. that following summer. Amongst our days and nights of developing premier leadership, career development and personal growth, I attended my first hunger banquet. This hunger banquet opened my eyes to a problem that was right in front of me, and I knew that night that I had to do more about it. My inspiration and drive to do something meaningful grew. I was lucky enough to be selected to present my paper from IYI at the World Food Prize Global Youth Institute (GYI) in October 2014. Throughout GYI, I was inspired by 1987 World Food Prize Laureate M.S. Swaminathan, and attending another hunger banquet.

Due to my hunger to spread awareness, I had my own hunger banquet at my high school my senior year with the help of my FFA adviser, Mrs. Dolch, and a fellow chapter officer. After that event, I decided to apply for the Borlaug-Ruan international internship hoping to expand my reach even more. I never would have guessed what would happen next.

I was sitting in Hilton Coliseum in Ames, Iowa waiting to receive my Iowa FFA degree when I got the e-mail that informed me I'd be studying plant physiology at China Agricultural University in Beijing, China.

*Before China*

I could not have been more excited. I had a lot of expectations and hopes for my internship as I was told I would come back a totally different person. I expected to interact with farmers directly and talk to them about what problems they were experiencing. I expected to see hunger everywhere and be changed by that. Maybe I would do ground breaking rice research. I would figure out what I want to do with my life. What I got out of this internship was not what I expected, but it is something I would not change for anything.

*My first impressions of China*

What have I got myself into, I thought as I crawled off the plane exhausted from a 13hour flight. There were so many people; I couldn't read the signs; I couldn't message Lisa because the Wi-Fi was in Chinese character. I took a deep breath and followed the herd of people towards customs. When I handed my passport over to the customs officer, I held my breath with the fear of something going wrong. Luckily, I made it through just fine. At the baggage claim, I met a man from Minneapolis named Omar. He calmed my nerves and taught me a lesson I was sure to be reminded of many times throughout this trip: no matter how far away from home you think you may be, you aren't very far at all.

Walking out of the baggage claim, I searched through a crowd of unfamiliar faces, holding up signs with unfamiliar names, trying to find a glimpse of familiarity. After a few minutes, I found a sign with my own name on it being held by two girls who I would come to know as my best friends from China. They told me to come all the way out. As I walked further, I found another sign with my name on it. This time it was held by a gentleman who seemed very excited that he had found me. He motioned for me to stay where I was so he could come get me. Finally, all three of them found me. The man, who I would find out was Mr. Xiao, our driver, took my bag. I learned very quickly that the people of China are very willing to help with almost anything. When we got to the car, it was an hour car ride from the airport to the university. I attempted to stay awake to witness the new world I had been tossed into, but exhaustion took over, and soon I was being woken up at our apartment.

After unpacking, we went to go have dinner. The university's cafeteria was very overwhelming at first. Even though it was the weekend, it was full of students. I did not know what a lot of the food was, but I recognized some fish and decided that would be a good starter. Unfortunately, I did not know that many Chinese meat dishes have bones in them, and this was most commonly found in fish. I took a huge bite, and quickly regretted it. I was a lot more careful with my food after that.

The next day, I went to the lab. Students were in the lab working with solutions, and machines that were unrecognizable to me. It looked like a T.V. show. Doubts began to set in. How will I fit in? How will I know what anything is? Are they going to send me home when they realize I do not have a strong background in the technical research area of science?

No, they did not send me home. A few days later, we were eating at a hotpot restaurant. These restaurants have boiling pots of water in the middle of the table, and you cook your own food at your own leisure. This particular restaurant was on the 6$^{th}$ story of a mall, and I had a seat facing the window. I noticed a tall historical Chinese building in this distance on top of the mountain. I took a chance and asked what is was? It was Baiwang Mountain, and the next day we hiked up it to see a beautiful view of Beijing. That evening when we got back from our hike, Haiyue and Li threw me a welcome to Beijing party. All our lab mates showed up. We had cake, Domino's pizza, and a whole bunch of fruit I was unfamiliar with. They were going to teach me how to play a Chinese version of Poker, but somebody saw the game of Uno I had left out on the coffee table. They demanded I teach them how to play, and so we did. That is when I figured out I would be okay.

After a week of following other students around, I chose my research topic on a subject I was very familiar with since I am from Iowa: corn.

**Abstract**

Maize is grown worldwide, but a common abiotic stress that hinders the growth of maize is drought. Drought affects the growth and development of plants, and can be present in regions with low water availability, or erratic, unpredictable weather conditions. The plant hormone abscisic acid is involved in regulating many vital processes such as seed dormancy, seedling development, and biotic and abiotic stress responses. $B_2$ is used by plants as a functional analogue of abscisic acid. It is more stable, and likely to act like abscisic acid, which is involved in an array of effects on plant abiotic tolerance. This study investigates $B_2$'s role in strengthening drought tolerance in maize. Three different concentrations of $B_2$ (0.001µm, 0.01µm, 0.1µm) were tested on maize treated with 10% PEG6000. Phenotype analysis showed the best concentration to use for the rest of the study. Random samples were tested to show enzyme levels, and the gene expression for ZmDREB2A and Zmvp 14-2. Out of the four enzymes that were tested, it showed only two were assisted by $B_2$ to combat drought stress. However, due to the large margin of error, this study should be repeated multiple times.

## Introduction

*Beta 2010*

$B_2$ stands for Beta 2010, a chemical synthesized by the labs at China Agricultural University. The chemical compound is designed by the structure of abscisic acid and coronatine. Coronatine is a toxin produced by the bacterium pseudomonas syringae; it has also been used in studies to combat drought resistance in soybeans.

*Maize and Drought*

Maize has been a crucial crop among many civilizations throughout time. This is a crop that has faced many abiotic stresses during its existence. Drought is one abiotic stress maize continues to face despite the advances in technology. Maize faces drought everywhere too; in China, in Africa, and even in America. Many studies have been conducted to produce a seed or product that helps maize combat drought, but none have prevailed or made it far enough to be available to the market.

## Materials and Methods

*Experimental Design*

The first step of this study is creating an experimental design. This design begins with maize then moves to the concentration effect which breaks down into three separate parts that have different goals (*Figure 1*). To see the effect of the concentration, the phenotype must be recorded, and the physiological and molecular mechanisms tested. Due to the short amount of time allotted to finish this project, only a portion of physiological and molecular mechanisms could be tested in addition to recording the phenotype.

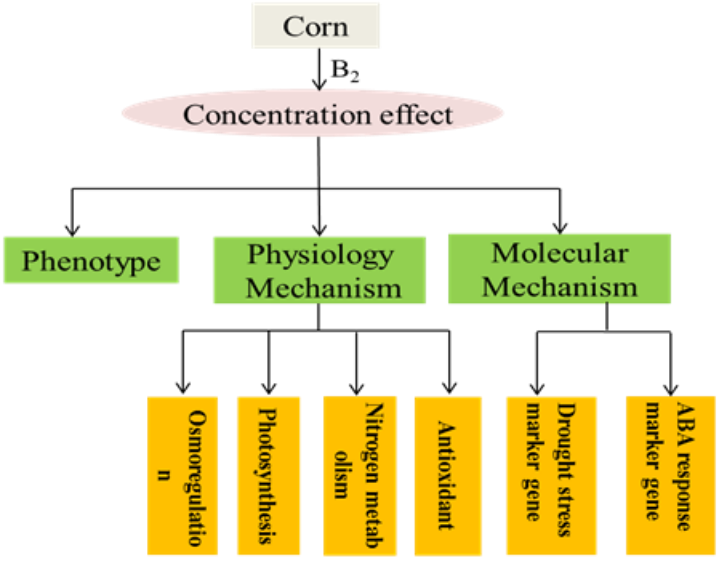



*Figure 1: The experimental Design*

*Phenotype testing*

Maize seeds (Zhengdan 958) will be germinated and grown in quartz sand for 6 days under a 14-hour photoperiod at 22°C after surface sterilizing the seeds in 10% hydrogen peroxide (180mL $H_2O$ and 20mL hydrogen peroxide) for 30 minutes. Then the seedlings will be transplanted into the half-nutrient solution. After 3 days, they will be placed in full nutrient solution containing different concentrations of $B_2$; 0.1 μmol, 0.01 μmol, 0.001 μmol. Five days after treating the plants with $B_2$, PEG6000 will be added. PEG is polyethylene glycol which induces controlled drought stress. This will help us see which concentration of $B_2$ best combats drought stress in corn. To record the phenotype, clear pictures of the roots and leaves must be taken of each concentration (0.1μm, 0.01μm, 0.001μm).

*Physiological Mechanism: Antioxidants*

After PEG6000 treatment, analysis of the leaf and root phenotype will take place, antioxidant enzyme activity analysis will be done per the following procedure:

Superoxide dismutase (SOD) activity will be determined following the method of Kuk et al (Kuk et al). The activity will be expressed in enzyme units per mg protein. One unit of SOD will be defined as the amount of enzyme which inhibits the reduction rate of nitroblue tetrazolium chloride (NBT) by 50%. Peroxidase activity (POD) will be determined by the guaiacol oxidation method. The reaction mixture will consist of 50 mL of 0.2 M phosphate buffer (pH 6.0), 19 μL guaiacol and 28 μL of 30% $H_2O_2$. The reaction will start after adding 0.5 mL of the supernatant to 1.5 mL of the reaction mixture, and changes in A470 will be recorded for 3 minutes. The enzyme activity will be expressed as an increase in absorbance min-1 mg-1 protein. Catalase (CAT) activity will be estimated by monitoring the disappearance of $H_2O_2$ by recording the decline in absorbance at 240 nm according to Ai et al (Ai et al). The reaction mixture will contain 50 mM sodium phosphate buffer, 100 mM 305 $H_2O_2$ and crude enzyme extract.

*Molecular Mechanism*

The drought stress marker gene and Abscisic acid response marker gene will be analyzed using the RT-PCR. Total RNA will be isolated from leaves using a RNA extracting kit per the manufacturer's instructions. Approximately 2 μg of totally RNA will be reverse transcribed using oligo d(T)18primer and M-MLV reverse transcriptase. Real-time quantitative RT-PCR will be performed on a 7500 real-time PCR system (Applied Biosystems, CA, USA) using SYBR® Premix Ex Taq™.

The data collected will be analyzed statistically according to randomized block design using SPSS statistical software (SPSS Inc., Chicago, IL, USA). The least significant difference will be calculated for the significant data at $P \leq 0.05$. The primer sequence for RT-PCR is as follows:

ZmDREB2A: F primer CCCAACCGCGGAGGATGCAG
R primer GCCTGGGCAGAGGTCGATGC
Zmvp14-2: F primer GCCATCACCGAGAACTTGGT
R primer CAGCATCTCCTGGAGCTTGAA

## Results

*Phenotype testing*

After the previous methods listed above, pictures were taken of the maize. It was shown that 0.001 µm $B_2$ was the best concentration to use because it grew the longest roots as shown in *Figure 2*. Its leaves also had the best water retention.

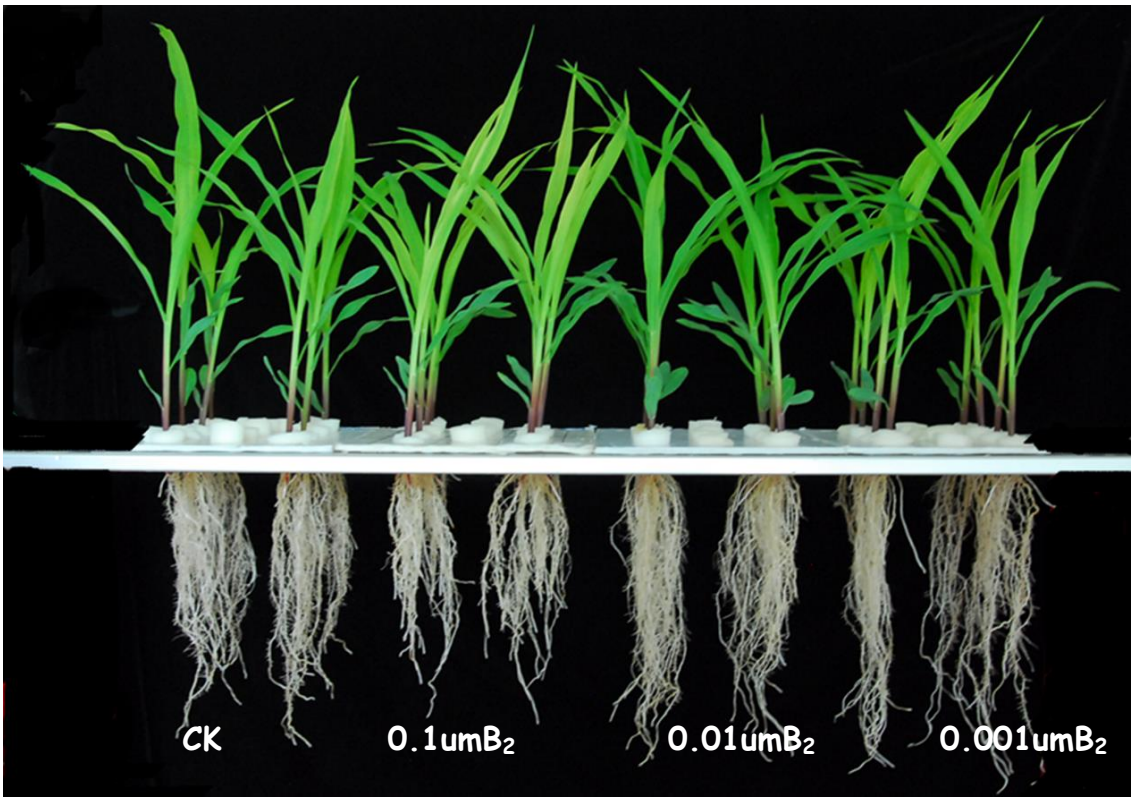



*Figure 1: A visual comparison of the maize phenotypes*

*Physiological Mechanism: Antioxidants*

Superoxide Dismutase (SOD) protects plants against oxidation created by the reactive oxygen species (ROS). Drought plants treated with $B_2$ did not produce more SOD than those plants treated only with drought (*Figure 3*). Peroxidase (POD) increases the process of creating $H_2O$ from peroxide. $B_2$ helped the plants combat stress because they had more POD than plants that were not treated with $B_2$ (*Figure 3*). Figure 3 shows the effect of $B_2$ on catalase (CAT). CAT protects plants against the oxidation created by ROS by turning peroxide into $H_2O$. Plants treated with drought and $B_2$ did not produce more CAT than plants treated with only drought. The ROS is produced in plants in response to stress, in this case, drought stress. Plants treated with drought and $B_2$ had less ROS compared to plants only treated with drought (*Figure 3*).

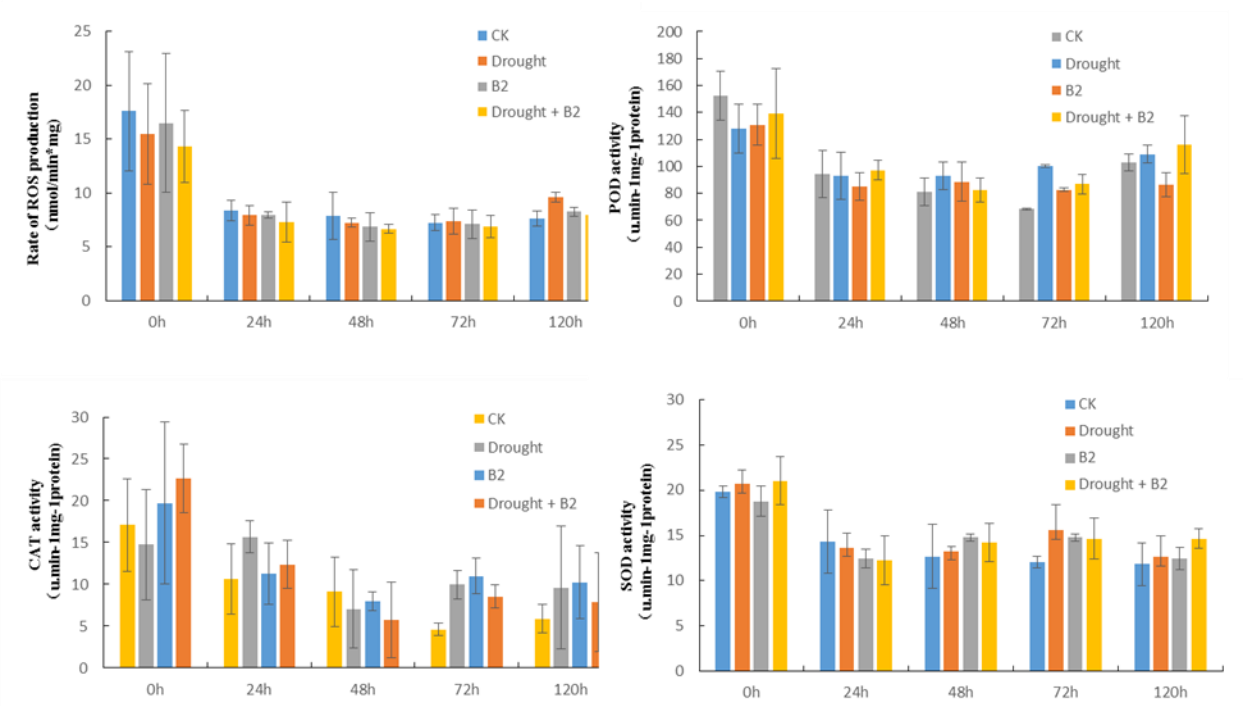
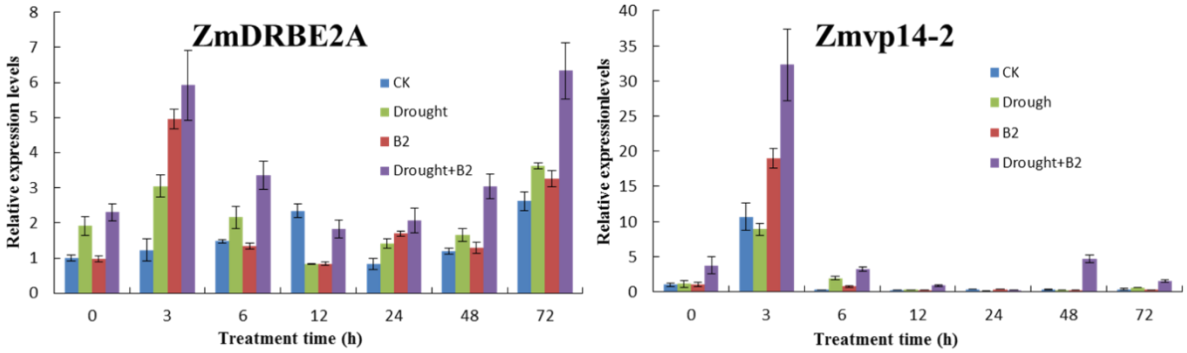



*Figure 3: Effect of PEG6000 treatment on the ROS, POD, CAT, and SOD activity in maize leaves.*

*Molecular Mechanism*

Finally, the induction of drought- responsive gene ZmDREB2A and ABA- responsive gene Zmvp14-2 was assayed using qRT-PCR technique. There was no significant difference in expression at 0h. PEG6000 treatment significantly enhanced leaf expression of ZmDREB2A for all the treatments. PEG6000 treatment rapidly induced foliar ZmDREB2A expression which peaked at 3h and then decreased thereafter and peaked again at 72h. Expression which treated with $B_2$ was always higher compared with control at different time spot. After PEG6000 treatment, expression which treated with $B_2$ is much higher than PEG6000 itself.

The data (*Figure 4)* suggests that $B_2$ can induce the expression of ZmDREB2A which represents the higher drought tolerance. The same tendency was observed in Zmvp14-2. PEG6000 treatment strongly induced leaf Zmvp14-2 expression which peaked at 3h and then decreased




thereafter. Altogether, this data demonstrates that both ZmDREB2A and Zmvp14-2 are induced by $B_2$. The higher gene expression represents higher drought tolerance which means $B_2$ can significantly increase drought tolerance through modulating drought-responsive and ABA-responsive genes.

*Figure 4: Effect of PEG6000 treatment on the gene expression for ZmBREBA2A and Zmvp14-2 in maize leaves. The gene expression at 0h is control.*

**Discussion/Conclusion**

The data that was collected supports and refutes our hypothesis that $B_2$ will help combat drought in maize. The plants treated with PEG6000 and $B_2$ had higher amounts of peroxide, and less amount of reactive oxygen species which support the hypothesis. However, the same plants did not produce more catalase or superoxide dismutase than the control group which does not support our hypothesis. Due to the large margin of error in the data, this study should be repeated multiple times.

## Works Cited

L. Ai, Z.H. Li, Z.X Xie, X.L. Tian, A.E. Eneji, L.S. Duan, Coronatine alleviates polyethylene glycol-induced water stress in two rice (Oryza sativa L.) cultivars, J. agron. Crop Sci 194 (2008) 360-368.

Y.I. Kuk, J.S. Shin, N.R. Burgos, T.E. Hwang, O. Han, B.H. Cho, S. Jung, J.O. Guh, Antioxidative enzymes offer protection from chilling damage in rice plants, Crop Sci. 43 (2003) 2109-2117.

**China to Iowa State University**

I could easily explain all the difficulties and frustrations I encountered during my eight weeks in China. However, there is no way I could even begin to explain every bit of knowledge and experience I was blessed with due to those frustrations.

Coming from a small high school, our science program was not the most developed and as a result, I was very worried about not knowing how to do anything. Luckily, this worrying was for nothing. Everybody at China Agricultural University was very welcoming and happy to explain and show me what to do. By my last two weeks, I was very accustomed to what I was supposed to be doing and how to do it. I learned to ask questions more openly instead of being afraid to ask questions. When I came to China, I thought I would love working in the lab with plants. I learned that I was not passionate about it, but I did find what I am passionate about. When I went to Baotou with Dr. Li and his family, I attended the China-Baotou International Beef and Mutton Industry convention. While we were there, I got a fair amount of interaction with the mutton that was being raised, and the meat that was harvested from the animals. I was completely engaged the whole time, and when we returned to Beijing, it clicked that livestock is what I am passionate about.

Though the food was hard to get used to in the beginning, after about three weeks, I had figured out my favorite foods, and how to avoid bones in meat. I only disliked eating the food in China because it ruined American Chinese food for me. At meals, I learned a lot about the Chinese culture. Anytime we went out to eat, there were always toasts to somebody at the table, or for the event they were celebrating. When we were not cooking our own food at restaurants, we sharing food made for an army on a spinning table. In Beijing, I never went hungry.

A lot within me changed while I was in China. Looking through my journal, I realize the entries from my first few weeks contradict my last few weeks. As I adjusted to culture shock, my first weeks were rough. I had to adapt to the amount of people, the language barrier, and the way of which things worked. For the first time in my life, I was a minority. I'm so thankful for this because it taught me what people visiting the United States must feel like. I have gone out of my way to make foreigners feel more welcome after returning from this internship.

As the final weeks of my internship wound down, I needed an extra push to finish out strong. That extra push came in the form of a family from Oklahoma who lived across the hall from me. A professor from Oklahoma State University had come to Beijing for work and brought his wife, children, and a nanny. His wife is from a town that my school played against in sports. They had both been in FFA during high school. I realized the world is a very small place, and as Omar taught me earlier, no matter how far away from home you think you may be, you are not very far at all.

The final week was bittersweet. I was so ready to come home, but I did not want to leave what had become familiar to me. I made a small home in Beijing and I had to leave it for a new one in Ames, Iowa. I wanted time to slow down so I could savor the food, admire the beauty of the campus around me, and take in everything I had taken advantage of while I was there. When I left the airport, I had tears in my eyes. I know a part of me will always remain in Beijing.

The most important lesson I believe I learned while I was in China is that there are so many differences between the United States and China; from how our government works, to our food, to our religions, and even the variety of pets. But one thing is the same throughout, no matter the color, the race, the nationality, the religious beliefs, or the goals, we are all trying to find a way to live this life that makes us happy.

## Photos


My welcome party cake


Badaling Great Wall


Professor Zhou and her husband at their wedding


Dr. Li supervising my work


Summer Palace


Zhou Li and Yu Haiyue sending me off at the airport

Zhou Li and I at Tiananmen Square


Baiwang Mountain