Global Food Insecurity: Drought Stress Tolerance in *Zea Mays*

Improving Yields in Arid Regions with Gene Expression

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Chicago, Illinois

2017 Borlaug-Ruan International Internship

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THE WORLD FOOD PRIZE

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Acknowledgements

A life-long dream became a reality when I began the journey of a lifetime 6,585 miles away from home in Beijing, China. Amelia Earhart said it best, "Adventure is worthwhile in it of itself!" I would now like to take the time to thank everyone who had a part in this adventure to China!

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If it were not for the wise words of **Ambassador Kenneth M. Quinn** on the final day of the Global Youth Institute in 2015 about the Borlaug-Ruan International Internship I may not have had this life-changing experience as it propelled me into interests and experiences I never thought I would have as a high school student. **Mrs. Lisa Fleming**, the Director of Educational Programs at the World Food Prize Foundation, has served a critical role ensuring my time in this internship in Beijing, China ran smoothly and with her unwavering support this internship has been one of the most valuable experiences in my life. Special thank you to **Ms. Katherine Walthers, Ms. Libby Crimmings, Mr. Keegan Kautzky,** and the entire **World Food Prize Staff** without your help this internship and my journey with the World Food Prize would not be the same.

I would like to extend my greatest appreciation to all the professors and graduate students at China Agricultural University (CAU) for welcoming me and making this place feel like home. If it were not for your generosity and helpful nature my project would not be at the caliber it is now and for that, I am forever grateful. **Dr. Zhao-Hu Li Ph.D.,** your professional expertise served me well and thank you for once again opening your laboratory for another Borlaug-Ruan Intern to conduct research that will aid in the fight against global food insecurity. I would not have been able to learn as much about molecular biology and agriculture in China if it were not for **Dr. Yuyi Zhou Ph.D.** Your caring nature and knowledge of the topic allowed me to adapt to life in China and gain invaluable experience in a laboratory that will serve me well in college and beyond. I cannot forget graduate students like **Yingru Liu, Nana Zhu, Yuling Guo, Guanmin Huang, Chuanxi Peng,** and **many more** for helping me in the lab and showing me around Beijing on the weekends.

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My World Food Prize Journey

My name is Daniel Vogwill and I am an incoming senior at The Chicago High School for Agricultural Sciences. I began my freshman year with little to no prior knowledge about agricultural but through activities like the National FFA Organization and The World Food Prize my love for agriculture grew.

My journey with the World Food Prize began when I was asked by Ms. Diana Krnjaca, my Honors Human and World Geography to create a paper on a specific country's agricultural issues for the World Food Prize. I immediately ran home and read everything about the organization and Dr. Norman Borlaug's legacy. I stumbled along the Borlaug-Ruan International Internship page and after reading about the internship I was hooked. In the spring of 2015, I attended the Wisconsin Youth Institute at the University of Wisconsin-Madison. This event gave us a chance to pitch our plan and agricultural issue to a panel of advisors from the World Food Prize and receive feedback on how we can improve our reports. While we had not written papers by then I was still determined to impress the panelists with my knowledge on sustainable agriculture in Singapore and perspective on the issue of food insecurity.

As I stood on a dock while vacationing in New York City, I received a call from my teacher. She told me that to be considered for a spot at the Global Youth Institute I would have to submit my paper in ten days. I began my research the moment I got home and after meticulous research, revisions, and planning I finished my paper in time and submitted it for the school to review. I then was told that they loved my paper and that I would be one of the students attending the Global Youth Institute in Iowa in 2015.

On my way to Des Moines, Iowa, I began finalizing my speech I would give to the panelists on the final day of the Global Youth Institute. I read through each word carefully and practiced on strangers the whole way there. When I arrived at the Global Youth Institute and saw all the flags hanging around the lobby and hundreds of kids eager to attend the Borlaug Dialogue, I knew this would be an experience like no other. In my short time at the Global Youth Institute I was able to hear from leading experts in the field, talk with a rural African farmer, meet the World Food Prize Laurette Dr. M.S. Swaminathan, discuss global food insecurity with Simu Naser from Bangladesh, and even package food for those in need while meeting with Her Excellence Ms. Joyce Banda, the former president of Malawi. I still remember the powerful speech she delivered to us on the many assassination attempts during her time in office and when asked why she did not step down her reply was, "I would rather die for my country helping people drastically improve their quality of life than living a life scared and afraid." To this day that speech has been a catalyst in my pursuit to end global food insecurity one seed at a time.

The Global Youth Institute was an experience like no other and when Ambassador Kenneth M. Quinn spoke to us for one final time regarding the Borlaug-Ruan International Internship I was all ready to apply when I was old enough and eagerly waited for my shot. When the time came to apply, I worked for days on end perfecting my resume, essay, cover letter, and I probably answered around one hundred mock interview questions to prepare myself for anything they may ask. After both rounds, I got the email that would change my life forever. "Congratulations! I am pleased to inform you that you have qualified to be a 2017 Borlaug-Ruan International Intern." Later, I found out I would be studying in Beijing, China at The China Agricultural University and my life-long dream of visiting China had arrived; with the chance to study alongside the best in the field to aid in the fight against food insecurity.

From Chicago to Beijing

A journey of a thousand miles begins with a single step, yet in my case, my journey takes me 6,585 miles away from home to Beijing, China. My fascination with Chinese culture and history started when I was very young. This exotic country's rich, dynamic culture was one like no other. I have always imagined traveling to China when I was older, yet I did not realize it would be this soon.

When I applied to be a Borlaug-Ruan International Intern in my cover letter I listed my top three choices all in China. Not only for the chance to visit China but for the fact that the sites here are on the cutting edge of biotechnological and microbiological research with a focus on global sustainability. This fascinated me and made my love for China grow even more.

Coming from a large city like Chicago, I thought I would be well adapted to the urban lifestyle of Beijing but I was sadly mistaken. From the moment I arrived, I quickly learned to expect the unexpected. I followed the signs to customs and after checking in smoothly with the officer I was met by a swarm of people running to catch the train and not know when the next one would arrive I joined the mob and ran as fast as I could. We were then packed into the train car like sardines and lucky I found another couple from America that spoke Mandarin and English. They guided me to the baggage claim and after a long flight to Beijing, I had my belongings and the only thing left to do was find the person waiting with a sign that had my name on it. It did not take long for me to notice the two graduate students holding signs with my name on it eager to meet me. I introduced myself and after a brief conversation, we were off. After a long car ride from the airport to China Agricultural University, I was handed the keys to my apartment and I then was shown my room.

The next few days I was shown around campus and taken to the China Agricultural University Office and Police Station to officially enter me into the database and check my record. Anything I thought I knew about living in an urban city was completely different. This was evident by the traffic alone. The streets were an amalgamation of cars, bikes, buses, trucks, rickshaws, and more all crowding the streets with horns blaring and traffic jams for miles on end. The sheer size of the city was also intimidating. Chicago's population is only two point seven million people and is the third largest city in the U.S. while Beijing's population is around twenty-one point five million people and is the more than the top six U.S. cities combined.

As time went on, I began to adapt to life in China. My love for Chinese food and adventurous mindset opened me up to new experiences and allowed me to adjust and adapt quicker than others. My background in genetics, molecular biology, and biotechnology was slim but after much hard work and hours spent reading through Ph.D. paper one after the other, I began to develop a new skill set that will come in handy in college and beyond. My passion for scientific research has led me to compete in the Chicago Public School Science Fairs over the past decade of my life but up until now I have never conducted research in a laboratory setting and I immediately fell in love. My project allows me to combine my love of agriculture and scientific research in a way that aids in the fight against global food insecurity while allowing me to gain that skills necessary for a job in the global sustainability. My time in the lab is invaluable and when I arrived I knew that there was no better place than China Agricultural University to conduct my research, see China, and make a difference in the world one seed at a time.

Abstract

Maize (*Zea mays. L*) is a staple crop grown internationally and its applications range from biofuel to high fructose corn syrup and a medium for growing penicillin to animal fodder and so much more. However, drought is one of the biggest factors that threaten the crops' yield. This research project focuses on addressing this key issue by increasing drought stress tolerance. By analyzing the effects Coronatine (COR - $C_{18}H_{25}NO_4$), a phytotoxin which at low concentrations can actually increase the drought stress tolerance in transgenic maize varieties (Zheng-58 and B-73) it can possibly lead to a better understanding of the gene or genes responsible for drought stress tolerance and root elongation.

Arid climates such as that of the northern province of Inner Mongolia in China face a constant battle with the lack of water. By locating the gene or genes responsible for drought stress tolerance in Maize varieties, they can be cross-bred with other plants to create a plant that in turn is better suited for the arid climate of that region.

If in fact, the application of Coronatine at low concentrations does, in fact, yield greater root elongation, then the plants sprayed and treated with Coronatine will be able to more successfully combat drought. One hundred and fifty seeds of both Zheng-58 and B-73 Maize will be treated with various concentrations of Coronatine (0.001μm, 0.01μm, and 0.1μm) and compared to a control treated with dihydrogen monoxide (H_2O). From here the treatment that has the greatest root elongation, shoot health, and overall well-being will be selected for its strong phenotype and put through further genetic testing to locate the gene or genes responsible for drought stress tolerance and root elongation.

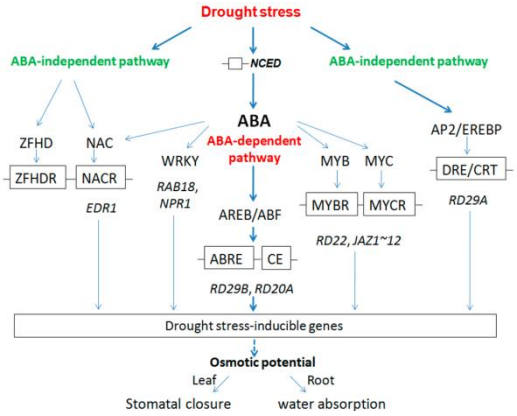

Figure 1: Gene pathways through Abscisic Acid (ABA) signaling for gene expression under osmotic stresses.

Through RNA extraction and reverse transcription polymerase chain reaction or RT-PCR, the specific gene expression in the crop was examined and analyzed. By comparing those with the strongest phenotypes, the gene or genes responsible for drought stress tolerance were located. From this data, conclusions can be drawn on how the gene or genes responsible for drought stress tolerance and root elongations interact and use them to crossbreed and create stronger more drought-tolerant varieties of Maize to use in arid and drought-ridden places globally. Until then, more testing must be done to further justify the current findings through experimental plots, more treatments, and genetic testing.

Introduction

Research Introduction

By the year 2050 there will be nearly nine billion people in the world and with this comes a dilemma facing agriculturalists around the world. Drought continues to be an issue many nations around the world face and those with arid climates struggle to grow crops at all. However, a crop that was tolerant to drought could be the answer to this problem. With geneticist and agronomists alike working on drought stress tolerance research a better variety of maize could be closer than ever before.

When locating the gene or genes responsible for drought stress tolerance could they lay in those genes that improve drought tolerance through enhanced photosynthetic efficiency and root development and together create a better use of the water the plant does collect? In addition to this, the information provides important genetic insights into the natural variation of maize drought tolerance and the identified genes can serve as direct targets for genetic engineering and for maize trait improvement.[1]

The ability to grow crops in an arid environment would raise food production by a significant margin due to the fact that over forty-one percent of the planet's surface is an arid region.[2] With improvements to drought stress tolerance in crops, we could begin farming in these areas and thus provide more food for the growing population.

China Agricultural University Introduction

China Agricultural University (CAU) was founded in 1905 and was the result of a merger between The Beijing Agricultural University (BAU) and The Beijing Agricultural Engineering University (BAEU). The university has twenty colleges and over sixty departments. The campus is also divided into an east and west campus with shuttles that run between both. Each campus is different because the west campus is where the seven thousand graduate students are and the east campus for the twelve thousand undergraduate students. China Agricultural University ranks among one of the top three universities in the world for agricultural research.[3]

The Center for Life Sciences (CLS) on the western campus was finished in 2007 it became home to offices and labs with a focus on agricultural research in fields like molecular biology, genetics, crop science, etc. Dr. Zhao-Hu Li Ph.D. who runs the lab I completed my research in received his doctorate degree in agronomy from Auburn University (Alabama) and since worked on research that he hopes to use to aid in the fight against global food insecurity.[4]

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[1] Wang, Xianglan, and Hongwei Wang. "Genetic variation in ZmVPP1 contributes to drought tolerance in maize seedlings." *Nature Genetics*. Nature Genetics, 15 Aug. 2016. Web. 11 Aug. 2017.

[2] ICARDA. "World's Dry Areas." *Research on Dry Land Systems – CGIAR.* ICARDA, n.d. Web. 11 Aug. 2017.

[3] "Brief History." *About China Agricultural University.* China Agricultural University. 12 Dec. 2012. Web. 11 Aug. 2017.

[4] Creamer, Jamie. "Beijing University VP, Auburn Ag Alumni's 'Sweet Home Alabama' Memories." *The Season.* Auburn University Magazine, 05 Oct. 2016. Web. 11 Aug. 2017.

Procedure | Methods

Seedling Germination Procedure

Step 1: Seed sterilization - (Maize [Zheng-58 and B73] The Zheng-58 variety of plant is more resistant to most stresses while the B-73 variety of maize is very sensitive to stresses.) Add 60mL of distilled water into the Erlenmeyer flask and then add 30mL of 30% Hydrogen Peroxide (H_2O_2). Add a cover to prevent spill and place in a temperature controlled incubating shaker for 30 minutes.

Step 2: Then soak the seeds in 100mL of water. Firstly, rinse the seeds with water 3 times or until there are no longer bubbles in the Erlenmeyer flask. Then soak the seeds for 24 hours in the distilled water (H_2O) and place a black bag over the seeds. Place the seeds in the artificial climate box for 24 hours.

Step 3: Now transfer the seeds to the silica sand to germinate and place them one centimeter deep and one centimeter apart from each seed. Then I will cover them with silica sand and then place atop over the bucket so they will germinate in the dark. This is because allowing the seeds to germinate in the dark aids in the hypocotyledonary axis elongation process. Also, transfer twelve seedlings to the Phytotc™ Root Observation Kits and note the length every three days and water with 20mL of distilled water when dry. This helps to observe Coronatine's effects on root elongation a factor critical to drought tolerance.

Step 4: When in the hydroponics boxes observe the differences between the plants and use some of the following characteristics to determine the strong from the weak seedlings. All of these observations will be logged in your observation charts in your lab notebook and later transferred to your final report for analysis. The Chlorophyll in the leaves that were stronger would emulate a more intense green color. Other factors could be the color of the roots and whether or not they have rotted and/or grown as strong as those in the control.

Hydroponics Procedure

Step 1: After seeds have reached maturity or it has been one week since planting in the silica sand, transfer the seeds to the hydroponics boxes but first, you must prepare the Hoagland Nutrient Solution. Begin by adding 100mL of Calcium Chloride ($CaCl_2$), 100mL of Magnesium Sulfate ($MgSO_4$), 100mL of Monopotassium Phosphate (KH_2PO_4), 100mL of Ethylenediaminetetraacetic Acid Ferric-Sodium Salt (FeNa-EDTA), 100mL of assorted Microelements necessary for a plant's growth and overall health, and 200mL of Potassium Nitrate (KNO_3). Add these chemical compounds above to a bucket of Hoagland Nutrient Solution and mix well for five minutes.

Step 2: Then add five liters (5,000mL) of the Hoagland Nutrient Solution to each of the ten hydroponic boxes. Label the boxes with the concentrations of the treatments (0.001μm,0.01μm, 0.1μm, 0.0μm [control]) and the variety of maize (Zheng-58 & B-73). For each concentration add (0.001μm - 5μL, 0.01μm - 50μL, and 0.1μm - 500μL). Remembering to change the pipette tip each time.

Step 3: The next step you will add sponges around the bottom of the plants and then place sponges or stickers on/in the middle as a spacer. After the eight plants are in the hydroponics boxes then add the air

hose with the oxygen stone at the end to allow air to go to the roots of the plants and the oxygen stone allows for the air be so aggressive as to push the water over the edge of the hydroponics box.

Step 4: Take samples from the tip of the root, the lateral roots, the branch, and two of the leaves for a total of five samples and place them in the tin foil and/or the pre-labeled micro-centrifuge tubes. Collect samples from one plant at 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, 96 hours, 144 hours, and 192 hours. However, for the 0.1μm Coronatine treatment, it stops collections after 24 hours.

Step 5: Once you have collected the samples move them into a styrofoam container that has RNA Liquid Nitrogen in it due to its finer quality. After collecting all of the samples move them into the eighty degrees Celsius below zero cooler and leave the samples there until you perform RNA extraction.

Step 6: Compare the root growth of the concentrations along with the overall health of the plant to determine which concentration of Coronatine is best for the root elongation and in turn the drought stress tolerance. Then prepare the next set of seeds to test that concentration for a second time.

Step 7: Once the final collection at one hundred and ninety-six hours is taken, take professional pictures or each of the concentrations and varieties of maize. This will give a visual aid to the root and shoot growth under each of the treatments.

Step 8: Analyze the data and decide which concentration of Coronatine is the best and prepare another eight hydroponics boxes for the concentration and control for both varieties of maize. Five days after being treated with Coronatine place four of the hydroponics boxes under drought stress by simulating drought with PEG-6000 (Polyethylene Glycol - $C_{2n}H_{4n+2}O_{n+1}$) at twenty percent and monitor the leaves for how they respond to it. Periodically, you will also collect samples at 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 24 hours, 48 hours, and 96 hours. Then test for physiological data such as the water content in the leaves by weighing them after you collect them and then after they have dried in the blast oven dryer for 72 hours then weigh the plants again and the difference in weight is the water content.

RNA Extraction with Lysis Solution B Procedure

Step 1: Begin by thawing the Lysis B Solution in fifty-degree Celsius water and leave there for five minutes or until it is done thawing and in this time, collect the remaining materials.

Step 2: Quickly weigh no more than 100mg of fresh tissue, or frozen or RNAfter™ stabilized tissue, and homogenize it under liquid nitrogen. (Do NOT allow the frozen sample to thaw during weighing before adding the Plant RNA Lysis B Solution.)

Step 3: Immediately transfer the homogenate (<100mg) to a micro-centrifuge tube. Add 450μL of Plant RNA Lysis Solution B/2-ME, vortex vigorously and incubate at sixty degrees Celsius for five to ten minutes. Mix by inverting the tubes at regular intervals.

Step 4: Add 150μL of Protein Precipitation Solution into the lysate. Mix well and incubate on ice for five minutes. Then spin in a centrifuge at top speed (12,000 rpm) for five minutes at room temperature. (The solution will become cloudy due to precipitation of detergent, proteins, polysaccharides, and secondary metabolites.)

Step 5: Transfer the supernatant to an RNA Spin Filter (with yellow ring) inserted in a clean 2mL Collection Tube. Spin in a centrifuge at top speed (12,000 rpm) for two minutes. (Most tissue clumps and cell debris can be removed by the spin filter, but a small amount will pass through and form a pellet in the collection tube. Do NOT disturb the pellet.)

Step 6: Transfer the flow-through from the collection tube to a new 1.5mL micro-centrifuge tube, avoiding disturbing and pipetting the pellet. (675µL transferred)

Step 7: Add 1.5 volumes of RNA Binding Solution/Ethanol Mixture to the lysate (flow-through), and mix well by vortexing or pipetting 675µL. (For example: Add 675µL of RNA Binding Solution/Ethanol Mixture to the 500µL lysate. A stringy precipitate may form after the addition of RNA Binding/Ethanol Mixture. This does NOT affect RNA isolation. Load the solution and precipitate into the RNA spin column in the next step.)

Step 8: Load the Lysate/Binding/Ethanol Mixture (<675µL) to the RNA Spin Column inserted in a 2mL collection tube, spin in a centrifuge at top speed (12,000 rpm) for one minute and discard the flow-through. (If the volume of Lysate/Binding/Ethanol Mixture is greater than 675µL, transfer any remaining Lysate/Binding/Ethanol Mixture to the RNA Spin Column and repeat the above step.)

Step 9: Transfer the RNA Spin Column into the original collection tube and add 500µL of RNA Wash Solution I, spin in a centrifuge at top speed (12,000 rpm) for one minute and discard the flow-through.

Step 10: DNase I Digestion. For each isolation reaction, premix 80µL of DNase I Incubation Buffer with 2µL DNase I in a new sterile tube (Mix by flicking or inverting the tube, do NOT vortex!). Add 82µL of the solution to the center of the RNA Spin Column membrane, and incubate at room temperature for twenty minutes. (If multiple extractions are performed, prepare a fresh mixture of DNase I Solution before use; do NOT store a premix of DNase I Solution.)

Step 11: Add 500µL of RNA Wash Solution I to the RNA Spin Column, spin in a centrifuge at top speed (12,000 rpm) for one minute, and discard the flow-through.

Step 12: Transfer the RNA Spin Column to the original collection tube. Add 600µL of RNA Wash Solution II, spin in a centrifuge at top speed (12,000 rpm) for one minute and then discard the flow-through. Repeat this step once more.

Step 13: Place the RNA Spin Column into the original collection tube and spin in a centrifuge at top speed (12,000 rpm) for three minutes to remove any residual ethanol. Transfer the RNA Spin Column to a clean 500µL (1.5mL) micro-centrifuge tube. (If centrifugation speed is lower than 10,000 rpm or residual ethanol from RNA Wash Solution II must be removed completely, incubate the RNA Spin Column at sixty degrees Celsius in a dry oven for five minutes to evaporate all remaining ethanol.)

Step 14: Add 40µL of Nuclease-Free Water into the center of the RNA Spin Column membrane and let stand for one minute. Then place in the centrifuge at top speed (12,000 rpm) for one minute and store the RNA sample at negative seventy-five degrees Celsius.

Step 15: Transfer 1μL of the RNA you just extracted onto the Nanodrop spectrophotometer machine. Remember to clean the surface before and after using the machine to avoid cross-contamination. This machine will read the concentrations of the RNA you have extracted and mark down in your notes the data you receive from this. Any concentration whose graph follows a polynomial function's path, then it is a good phenotype and strong RNA.

RNA Purification Procedure

Step 1: To purify you RNA samples after they have been in the negative eighty-degree cooler for four weeks or more, begin by adding diethyl pyrocarbonate ($C_6H_{10}O_5$ - DEPC water) to the RNA so that it is now equal to 200μL.

Step 2: Then, add 200μL of Chloroform ($CHCl_3$ - 25:24:1) and mix well by vortexing and then transfer the 1.5mL micro-centrifuge tubes into the centrifuge and spin at top speed (12,000 rpm) for ten minutes.

Step 3: Transfer 170μL of supernatant to a new 1.5mL micro-centrifuge tube and add another 170μL of Chloroform ($CHCl_3$ - 24:1) and mix well by vortexing. Transfer the 1.5mL micro-centrifuge tubes into the centrifuge and spin at top speed (12,000 rpm) for ten minutes.

Step 4: Transfer 130μL of supernatant to a new 1.5mL micro-centrifuge tube and then add 500μL of Ethanol (C_2H_6O - 100%) and 4μL of Sodium Acetate ($C_2H_3NaO_2$). Then leave overnight or for twelve hours in the negative eighty-degree cooler. After, spin at top speed (12,000 rpm) in the centrifuge for thirty minutes. Discard the solution and spin at top speed (12,000 rpm) in the centrifuge for one more minute to remove excess ethanol and then add 8-20μL of diethyl pyrocarbonate ($C_6H_{10}O_5$ DEPC water)

Reverse Transcription Procedure

Step 1: Remove the samples of RNA that you previously extracted from the subzero negative eighty degrees Celsius freezer and incubate on ice.

Step 2: Begin by calculating the volume of in microliters (μL) of the RNA. Simply take the concentration you received from the Nanodrop spectrophotometer and convert nanograms (ng) to micrograms (μg) by multiplying the nanograms by $1x10^{-3}$ and then divide by two. Then you must solve for the volume of water needed to create 10μL of the solution. To do this, take 10μL minus the 2μL for the 5xgDNA Buffer and then subtract 'x' with 'x' being the volume of RNA you solved for above.
Step 3: Begin by adding 2μL of 5xgDNA Buffer to the 200μL micro-centrifuge tube and then the volume of water you solved for using the 10μL limit and volume of RNA you solved for above. Finally, add your volume of RNA sample to the 200μL micro-centrifuge tube.

Step 4: Place the 200μL micro-centrifuge tubes on a Digital Dry Bath Incubator set to forty-two degrees Celsius for three minutes and when complete, incubate on ice while you prepare the next step.

Step 5: In a separate mixing 200μL micro-centrifuge tube, prepare the next 10μL solution. Firstly, begin by adding 2μL of 10xFast RT Buffer for each one of your samples you have plus one more. For

example: If you have ten samples add 22μL of 10xFast RT Buffer for the ten samples plus one extra if you use too much one time. Then, add the same amount of FQ-RT Primer Mix as the 10xFast RT Buffer since this also calls for 2μL of FQ-RT Primer Mix. Next, add 5μL of RNase-Free ddH_2O for each sample you have plus one extra like above. Then add 1μL of RT Enzyme Mix for each of sample you have plus one extra again like you did above. Finally, mix the solution in the centrifuge at top speed (12,000 rpm) for ten seconds.

Step 6: Add 10μL of the mixed solution to the 200μL micro-centrifuge tubes that are currently incubating on ice.

Step 7: Transfer the 200μL micro-centrifuge tubes back to the Digital Dry Bath Incubator set to forty-two degrees Celsius for fifteen minutes.

Step 8: Then transfer the 200μL micro-centrifuge tubes to a new Digital Dry Bath Incubator set to ninety-five degrees Celsius for three minutes.

Step 9: Remove the 200μL micro-centrifuge tubes from the Digital Dry Bath Incubator and incubate on ice. Then add 80μL of RNase-Free ddH_2O to each of the 200μL micro-centrifuge tubes and then place in the twenty degrees below zero cooler to store until you are ready to test with RT-PCR.

RT-PCR Procedure

Step 1: Begin by preparing the 10μL enzyme mix. To do this, begin by adding 3.6μL of RNase-Free ddH_2O and then adding the primers. This whole process it all specific to the certain gene that you are trying to isolate in the RT-PCR. For example: If you wanted to isolate gene V-167829 you would use two primers (V-167829 MYBF Primer and V-167829 MYBR Primer) and at this point you would add 0.2μL of both primers and then this would give me a combined total of 0.4μL of primers and this quantity is determined by the length and specific gene you are looking to isolate. Finally, add 1μL of the cDNA you previously created from the reverse transcription process that is incubating in the negative twenty degrees Celsius cooler.

Step 2: Load your 200μL micro-centrifuge tubes that you just mixed into the Gene Amplification Mastercycler and then set the cycling conditions to the specific temperatures and durations depending on your gene you wish to isolate. For example: For the V-167829 gene set the initial denaturation to ninety-four degrees Celsius for two minutes. The for thirty cycles the denature is at ninety-four degrees Celsius for thirty seconds and then the annealing is at fifty-seven degrees Celsius (this temperature is determined by the length of the gene) for another thirty seconds. Next, the extension is set to seventy-two degrees Celsius for 1rb per minute and this time depends on the length of the gene. Then it is on to the final extension at seventy-two degrees Celsius for ten minutes, and this repeats for thirty cycles. Finally, it is held at twelve degrees Celsius.

Step 3: Now you must create your gel that you will use to analyze the cDNA for the specific gene you are isolating. To do so you can use gel electrophoresis and do this by taking your plastic gel plate and adding the premixed gel from the Regular Agarose G-10 Gel Mix. If you do not have this gel premixed

simply begin by adding 6g of Regular Agarose G-10 Gel Mix and adding it to a clear 500mL laboratory bottle with a cap. Then add 400mL of TAE (Tris base, acetic acid, and EDTA) Buffer and microwave for five to ten minutes. Next, add the remaining gel to the bottle that is left and then add 20µL of Ethidium Bromide ($C_{21}H_{20}BrN_3$). Remember to wear a mask and gloves during this process of creating more gel. Once the gel is created, clean the plastic gel plate with ddH_2O. Then you can add the 1.5% gel into the plastic gel plate to create the mold and let it sit for fifteen minutes. Also, remember to place the gel back into the blast oven dryer when you are done creating the mold.

Step 4: Take the gel mold out on the plastic gel plate and transfer it to the electrophoresis machine and add TAE (Tris base, acetic acid, and EDTA) Buffer until the gel is completely submerged. Leave the first notch in the gel open for the DMarker Dye - BL2000 DNA alone. In the notches begin to add 5µL of the DNA enzyme mix solution. Then add 3µL of DMarker Dye - BL2000 DNA to each notch.

Step 5: Run the electrophoresis machine fifteen to twenty minutes all depending on the length of the gene you are isolating. For example: The longer the gene the longer the time between fifteen and twenty minutes and vis versa for smaller genes.

Step 6: Transfer the gel plate to the Gel Imaging Analyzer but remember to drain the top of the gel of the TAE (Tris base, acetic acid, and EDTA) Buffer by tilting the plate a little first. (Do NOT invert the plate or the gel will be ruined.) Now, begin the Genesnap from Syngene™ software on the computer and place the gel plate in the Gel Imaging Analyzer. Finally, run the program and use the key for your specific cDNA marker to determine the length of the gene. Once this is done, save your results and discard of the gel in a special waste bin due to the fact that the gel contains Ethidium Bromide ($C_{21}H_{20}BrN_3$) and that is a toxic compound.

qRT-PCR Procedure

Step 1: Begin by labeling three 1.5mL micro-centrifuge tubes with the primer names you will use for this qRT-PCR. In the 1.5mL micro-centrifuge tubes pre-mix 7.5µL of SYBR Pre-mix, 5.1µL of ddH_2O, 0.3µL of ZmJAZ1 R Primer, 0.3µL of ZmJAZ1 F Primer, and 0.3µL of ROX Reference Dye II.

Step 2: To determine the quantity multiple the initial value by the number of samples and add a few more just in case. Then add 13.5µL of the mix to each of the ninety-six wells. Next, add 1.5µL of cDNA. Make sure to change pipette tips each time with the cDNA.

Step 3: Now cover the ninety-six wells with a seal and smooth out the air bubbles to make sure it is very tight and move the samples to the qRT-PCR instrument and run the 7500 Fast Software.

Step 4: To set up the software first change the setting to your specific project. For example: Beginning with the Experimental Properties change the settings to 7500 Fast (96 wells), Quantitation - Comparative $C_T(\Delta\Delta C_T)$, and SYBR® Green Reagents for two hours. Then, fill out the plate set up with the samples and primers you used and next, adjust the run method based on your primers you used. This will determine the temperature and time you will have the run method run for. Start the program. Save the data to analyze later and run the final ninety-six wells through a gel in electrophorese & RT-PCR.

Materials

Seedling Germination

- 4 Erlenmeyer Flasks
- 100mL Graduated Cylinder
- TH2-C-1 Thermal Shaker set to 28.1 degrees Celsius
- Sodium Hypochlorite (6.7% - NaClO)
- Coronatine ($C_{18}H_{25}NO_4$)
- 2 Clear Containers

Root Observation

- 3 Zheng-58 COR seeds
- 3 Zheng-58 Control seeds
- 3 B-73 COR uniform seeds
- 3 B-73 Control uniform seeds
- Phytotc™ Root Growth Kit
- Germination Paper
- 100mL Graduated Cylinder
- Artificial Climate Box
- Rack for Root Observation

Hydroponics

- Temperature and Light Controlled Lab Room
- Plant Lights (Industrial)
- 14 Hydroponics Containers with Lids
- 2 Large Containers
- Silica Sand
- Hoagland Nutrient Solution
- 100mL of Calcium Chloride ($CaCl_2$)
- 100mL of Magnesium Sulfate ($MgSO_4$)
- 100mL of Monopotassium Phosphate (KH_2PO_4)
- 100mL of Ethylenediaminetetraacetic Acid Ferric-Sodium Salt (FeNa-EDTA)
- 100mL of Microelements
- 200mL of Potassium Nitrate (KNO_3)

Large Jug (50 L)
Distilled Water (H_2O)
Large Mixing Spoon
5L Pitcher
Coronatine ($C_{18}H_{25}NO_4$)
14 Oxygen Stones
14 Air Tubes and Air Pump
Foam Strips
1.5mL Micro-centrifuge Tubes (224 Labeled)
Micro-centrifuge Rack(s)

RNA Extraction[q]RT-PCR

- Plant RNA Lysis B Solution B12-ME
- Protein Precipitation Solution
- RNA Binding Solution/Ethanol Mixture
- RNA Wash Solution I & II
- DNase I
- Nuclease-Free Water
- DNase I Incubation Buffer
- Digital Dry Bath Micro-centrifuge Tube Incubator
- Beckman-Coulter Micro-centrifuge 20 R Centrifuge
- Vortex Kylin-Bell 5
- 24 Mortars and Pestles
- Thermo Scientific Nanodrop 2000 Spectrophotometer
- Thermo Scientific Sub-Zero Cooler set to negative eighty degrees Celsius
- Distilled Water (H_2O) heated to fifty degrees Celsius
- 1.5mL Micro-centrifuge Tubes (224 Labeled)
- RNA Spin Columns (with Yellow Ring with 2mL tube)
- RNA Spin Columns (with Blue Ring - with 2mL tube)
- Micro-centrifuge Rack(s)
- Isopropyl Alcohol (C_3H_8O)
- Dixie Cups Dixie Cups
- 5 x gDNA Buffer
- RNase-Free ddH_2O
- 10 x Fast RT Buffer
- RT Enzyme Mix
- FQ-RT Primer Mix
- Specific Primers for genes
- 2xTaq Master Mix Dye
- Gene Amplification Cycler
- Blast Oven Dryer
- Regular Agarose G-10 Gel
- Electrophoresis Machine
- Ethidium Bromide ($C_{21}H_{20}BrN_3$)
- Gel Imaging Analyzer
- DMarker - BL2000 DNA
- Plastic Gel Plates with molds
- TAE Buffer (Tris base, acetic acid, and EDTA)
- ZmJAZ1&2 R&F Primers
- MicroAmp® Fast Optical 96-Well Reaction Plate
- ZmActin and ROX Dye II
- SYBR® Pre-Mix
- 7500 Fast Software
- qRT-PCR machine
- PEG-6000 (Polyethylene Glycol - $C_{2n}H_{4n+2}O_{n+1}$)
- Chlorophyll Meter SPAD 502+
- Sodium Hydroxide (NaOH)
- Diethyl Pyrocarbonate ($C_6H_{10}O_5$ - DEPC water)
- Chloroform ($CHCl_3$ - 25:24:1 & 24:1)
- Ethanol (C_2H_6O - 100%)

Multiple Uses

- Zheng-58 Maize seeds
- B-73 Maize seeds
- 2.5-1000µL Pipette with Tips
- Timer & Scale
- Markers/Pens/Pencils
- Notebook & Labels
- Tweezers/Scissors/Wipes
- Ice & Styrofoam Cooler
- RNA Liquid Nitrogen
- Nikon/Professional Camera
- Black Velvet Photo Sheet

- Surgical Mask & Gloves
- Distilled Water (H_2O)
- Tinfoil/Black Plastic Bag

Results | Data | Analysis

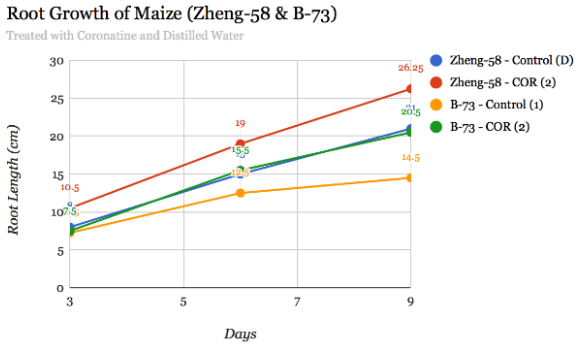


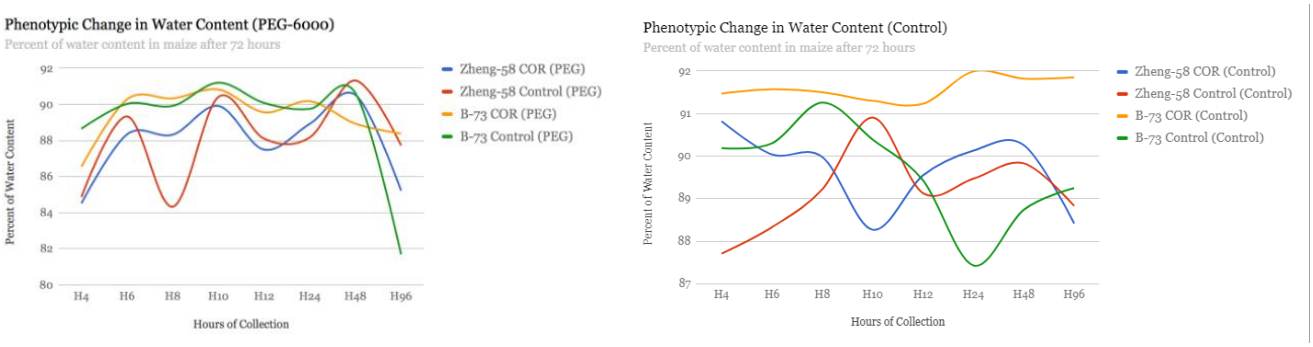
Figure 2: Root growth of transgenic maize with data from the table below.

Maize Variety	Treatment	Day of Observation	Root Length (cm)	Root Count
Zheng-58	Distilled H_2O - D	Day 3	8.00 cm	5
Zheng-58	Distilled H_2O - D	Day 6	15.00 cm	7
Zheng-58	Distilled H_2O - D	Day 9	21.00 cm	8
B-73	Distilled H_2O - 1	Day 3	7.25 cm	3
B-73	Distilled H_2O - 1	Day 6	12.50 cm	4
B-73	Distilled H_2O - 1	Day 9	14.50 cm	4
Zheng-58	Coronatine - 2	Day 3	10.50 cm	4
Zheng-58	Coronatine - 2	Day 6	19.00 cm	5
Zheng-58	Coronatine - 2	Day 9	26.25 cm	7
B-73	Coronatine - 2	Day 3	7.50 cm	4
B-73	Coronatine - 2	Day 6	15.50 cm	5
B-73	Coronatine - 2	Day 9	20.50 cm	5

Maize Variety & Treatment	Final Length with Root Count	Differences
Zheng-58 Control (Distilled H_2O - D)	21.00 cm	--
Zheng-58 Coronatine - 2	26.25 cm	5.25 cm
B-73 Control (Distilled H_2O - D)	14.50 cm	--
B-73 Coronatine - 2	20.50cm	6.00 cm

Figures 3-4: Data on the root length and root count over nine days and the differences in root elongation due to Coronatine.

This chart and graph represent the root growth observations of the set of seedlings grown in the Phytotc™ Root Growth Observation Kit. The seedlings treated with Coronatine fared better with a margin of 5.25 cm in the Zheng-58 seedlings and 6.00 cm in the B-73 seedlings. Due to this, at low concentrations, Coronatine can cause greater root elongation which is a key factor in drought tolerance.



Figures 5-6: The visual representation of the effects drought, simulated by PEG-6000, had on the water content in the maize.

	H4	H6	H8	H10	H12	H24	H48	H96
Zheng-58 COR (PEG)	84.52	88.34	88.32	89.91	87.49	88.92	90.52	85.22
Zheng-58 Control (PEG)	84.87	89.33	84.33	90.41	88.10	88.17	91.32	87.72
B-73 COR (PEG)	86.55	90.28	90.33	90.82	89.56	90.17	88.93	88.38
B-73 Control (PEG)	88.65	90.03	89.92	91.20	90.08	89.75	90.62	81.69
	H4	H6	H8	H10	H12	H24	H48	H96
Zheng-58 COR (Control)	90.82	90.04	89.98	88.27	89.55	90.13	90.26	88.42
Zheng-58 Control (Control)	87.71	88.33	89.22	90.91	89.13	89.47	89.83	88.83
B-73 COR (Control)	91.47	91.57	91.50	91.30	91.23	91.99	91.82	91.85
B-73 Control (Control)	90.19	90.3	91.26	90.39	89.43	87.43	88.74	89.25

Figure 7: Data belonging to the graphs above on water content in maize after simulated drought test with PEG-6000.

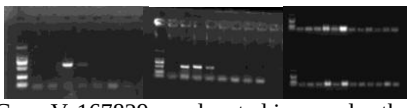
In these graphs above, the water content in the maize was measured after collection times and then after seventy-two hours in the blast oven dryer and the difference in weight was the water content and while the control did not have much of a change at the ninety-six hour collection there is a visible drop in water content due to the simulated drought from the PEG-6000.

Chlorophyll Levels in the Leaves

	Sample #1	Sample #2	Sample #3	Sample #4	Average
B-73 Control (Control)	29.6 µmol/m^{-2}	22.6 µmol/m^{-2}	29.0 µmol/m^{-2}	28.4 µmol/m^{-2}	27.4 µmol/m^{-2}
B-73 Control (PEG)	25.8 µmol/m^{-2}	26.7 µmol/m^{-2}	25.4 µmol/m^{-2}	23.8 µmol/m^{-2}	25.4 µmol/m^{-2}
B-73 COR (Control)	31.1 µmol/m^{-2}	27.7 µmol/m^{-2}	25.0 µmol/m^{-2}	25.9 µmol/m^{-2}	27.4 µmol/m^{-2}
B-73 COR (PEG)	26.6 µmol/m^{-2}	26.3 µmol/m^{-2}	20.3 µmol/m^{-2}	30.3 µmol/m^{-2}	25.9 µmol/m^{-2}
Zheng-58 Control (Control)	29.2 µmol/m^{-2}	26.0 µmol/m^{-2}	27.9 µmol/m^{-2}	26.5 µmol/m^{-2}	27.4 µmol/m^{-2}
Zheng-58 Control (PEG)	27.6 µmol/m^{-2}	23.0 µmol/m^{-2}	26.2 µmol/m^{-2}	29.7 µmol/m^{-2}	26.6 µmol/m^{-2}
Zheng-58 COR (Control)	21.5 µmol/m^{-2}	24.5 µmol/m^{-2}	24.9 µmol/m^{-2}	30.3 µmol/m^{-2}	25.3 µmol/m^{-2}
Zheng-58 COR (PEG)	18.0 µmol/m^{-2}	28.4 µmol/m^{-2}	28.8 µmol/m^{-2}	30.7 µmol/m^{-2}	26.5 µmol/m^{-2}

Figure 7: Chlorophyll levels indicating a change in appearance and production of Chlorophyll due to Coronatine and PEG-6000

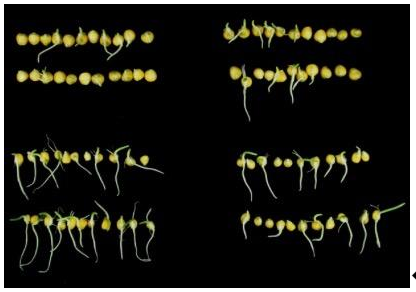
In this data table, the Chlorophyll content shows after ninety-six hours not much has changed with the Chlorophyll in the leaves like predicted and without much of a change between the control and PEG possibly a longer timeframe may show more significant differences in the Chlorophyll levels.



Figures 8-10: (From left to right) Gene V-167829 was located in samples through RT-PCR in the Gel Analyzer

In the gel analysis above, gene V-167829 was located in sample number three and four in the first image and two in the second image (Zheng-58 Leaf Control H6 (2), Zheng-58 Leaf Control H12 (3), & Zheng-58 Leaf 0.001 COR (4) - 500bp-750bp). Sample number thirty-six, thirty-eight, forty-eight, & fifty in the third image is the purified set of RNA for samples thirty-three to fifty-six. (B-73 Leaf Control H6 (36), B-73 Leaf Control H12 (38), B-73 Leaf 0.001 COR H6 (48), B-73 Leaf 0.001 COR (50)). This shows that more genetic testing will need to be done because there was not enough time to cross analyze the samples for gene variation.

Photographs

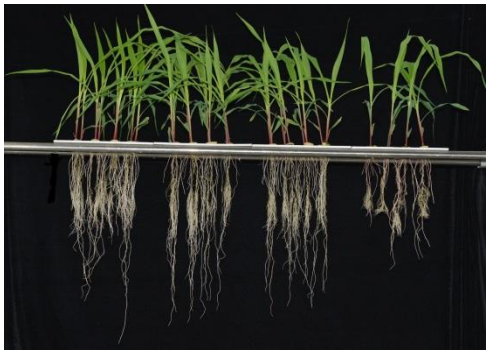


← Seed Germination (left: Control Z-58/B-73 right: COR Z-58/B-73)

← Root Growth (left to right: Control Z-58/B-73 COR Z-58/B-73)

← Drought Stress Trial (left to right: treated with COR(3) - $H_2O(3)$)

← Zheng-58 (left to right: 0.0μm, 0.001μm, 0.01μm, and 0.1μm)



← B-73 (left to right: 0.0μm, 0.001μm, 0.01μm, and 0.1μm)

Conclusion

Having hypothesized that if the transgenic maize was treated with 0.001 µm of Coronatine it would harbor greater drought tolerance due to its increased root elongation and photosynthetic processes, I can now conclude that this hypothesis generates an inconclusive result from the research. In conclusion, more data will have to be collected mostly from genetic comparisons and gene mapping. Due to the short period of time in China, I had with a project of this length, I would need more time and test plots. While my results may be inconclusive at the moment, I have begun the work that could pave the way to a more drought tolerant variety of maize in the future and for now with the data I do have I can conclude that there are prominent physiological changes in the water content of maize after ninety-six hours under simulated drought stress and that Coronatine at 0.001 µm generates root elongation which is one of the properties needed to create the drought-tolerant maize variety in the future.

If I were to do this experiment again in the future, I would complete this experiment over a longer period of time. My internship at China Agricultural University with the World Food Prize Foundation was only set for eight weeks and my project is supposed to take one to three years. I knew I would have to work efficiently and quickly to get it all done but by the time I left there were so many more things I wanted to test, research, and analyze but I just did not have the time to do so. Also with more time, I am now more familiar with the lab equipment and procedure and this would allow me to work through more samples and tests at a faster rate. In addition to more time, I would have tested more transgenic varieties of maize under more than just drought stress and branch off into other abiotic stresses. Also, I would have wanted to grow the maize to harvest and test the corn quality and then take it a step further and plant the next generation to see how this process would look down the line and the differences between the maize under drought or other abiotic stresses versus the control maize. If it were also possible, test the Coronatine on a test plot in a field across both arid regions and a control field that could show the applications of this research in real-world scenarios.

Hypothetically, if a drought-tolerant variety of maize were to be created then the applications could be worldwide. This would mean that farmers in arid regions could begin to grow crops with very minimal water and over time more advancements could be made with other crops giving way to a new line of drought-tolerant crops for arid region farmers. For families in these arid regions, it would mean jobs and food for their families and a way to provide for them in times of need. While this technology and advancements may be a few years or even decades away, this research and many more just like it, is paving the way to an end to global food insecurity one seed, one advancement, and one person at a time.

My greatest take away from this whole experience has been the growth I have made as a scientist and the inquisitive mindset to keep searching for the answer. In the words of Dr. Norman Borlaug himself, "Civilization, as it is known today, could not have evolved, nor can it survive, without an adequate food supply."[5] While I may only be one person, Dr. Norman Borlaug's legacy served as my motivation to make a difference in the world and help aid in the fight against global food insecurity one seed at a time.

[5] Borlaug, Norman E. "The Green Revolution, Peace, and Humanity." Nobel Lecture. 11 Dec. 1970. Web. 11 Aug. 2017.

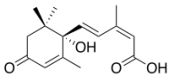
Implication on Global Food Insecurity

Based on this research it is quite evident that drought tolerance could not only be the key to improving global food security but could open new abilities for people to farm and cultivate crops in arid regions around the world. In order to beat the rising environmental challenges and the growing population we will need to grow even more food with less space and by developing more drought tolerant crops we can begin to utilize arid regions land that previously went untouched.

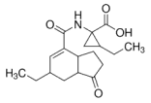
In these arid regions, the majority of the population is living in poverty and nearly sixteen percent of this population is in chronic poverty. Those four hundred million people are living off one dollar and twenty-five cents a day! In addition to this, over twenty-three hectares are lost per minute due to drought and that results is over twenty million tons of potential grain production each year.[6] If a crop were to be more tolerant to drought then twenty million tons of grain could be used to feed the families in poverty, offer them a job as a farmer, and create a stable source of income and nourishment for their families.

The key to drought stress tolerance is if the photosynthetic effects and root elongation rise, it will allow for more optimal use of the water it receives and utilizes effectively and this leads to higher yields.

In the recent research scientists at China Agricultural University (CAU) have been conducting on Coronatine's (COR) ability to alleviate drought stress tolerance at low concentrations on winter wheat they have found that leaves of plants treated with Coronatine under drought produced less abscisic acid (ABA) than those not treated. Thus, Coronatine might alleviate drought effects on wheat by reducing active oxygen species production, activating antioxidant enzymes and changing the abscisic acid level.[7]



← Abscisic Acid ($C_{15}H_{20}O_4$ - ABA) ~ Coronatine ($C_{18}H_{25}NO_4$ - COR)[8] →



The results of this study and more show that when administered at low concentrations Coronatine can have positive effects on drought stress tolerance and brings us one step closer to creating a more drought-tolerant crop. In addition to this study, China Agricultural University's Dr. Yuyi Zhou and colleagues, worked on a project that also tested Coronatine's effects at enhancing heat tolerance in wheat through photosynthetic performance and found that these results demonstrated that Coronatine enhanced heat tolerance by regulating nitrogenous metabolism and chloroplast ultrastructure to maintain photosynthetic performance and reduce yield loss under heat stress.[9] Agricultural scientists are one step closer to creating drought-tolerant crops to drastically improve global food security one seed at a time.

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[6] ICARDA. "World's Dry Areas." *Research on Dry Land Systems – CGIAR.* ICARDA, n.d. Web. 11 Aug. 2017

[7] Li, Xiangwen, and Xuefeng Shen. "Coronatine Alleviates Water Deficiency Stress on Winter Wheat Seedlings." *Journal of Integrated Plant Biology* 52.7 (2010): 616-25. Web. 11 Aug. 2017.

[8] "Pub Chem Structure Search." *National Center for Biotechnological Information,* United States National Library for Medicine, n.d. Web. 11 Aug. 2017.

[9] Zhou, Yuyi. "Phytotoxin coronatine enhances heat tolerance via maintaining photosynthetic performance in wheat based on Electrophoresis and TOF-MS analysis." *Scientific Reports* 5.13 (2015): 1-13. *National Center for Biotechnological Information.* Web. 11 Aug. 2017.

Cultural Immersion | Personal Growth

Stepping onto the plane to go to Beijing was an emotional time for me and one I will never forget. The moment they called my zone I burst into tears. I had never been away from home for two months, alone, and in a foreign country and the idea was more overwhelming than before. In true fashion, my mom gave me advice for the trip. I was thinking it would be something along the lines of homes is where you make it, or you are amazing you can do this and we will see you in no time, but I was wrong. She told me, "Do not swim in Beijing rivers." I was so perplexed at her statement but she was quoting my grandma who gave her similar advice going to camp. Absurd advice to reinforce that I had all I needed!

After landing in Beijing I was definitely overwhelmed with all the people, signs I could not read, and the idea of being on my own for the next two months. I took a minute and decided to sit down, breathe, and let the mob of people pass me by as I collected my thoughts and then worked my way to customs, baggage claim, and then out to a swarm of unfamiliar faces in search of a sign with my name on it.

I was immediately greeted by friendly faces of two graduate students by the name of Leo Yi Liu Yingru and Zhu Nana. After being ushered to the car, I was on the road to the university and unsure what was ahead for me. Over the next several days, I got settled into my apartment and began work in the lab.

The lab was like a scene out of a movie with students hard at work, using machines, compounds, solutions, and tools I had never even seen before and I immediately felt like a fish out of the water. I met with Dr. Yuyi Zhou, the head professor and supervisor I would work with, on a daily basis to discuss topics and after one week I was on my own. She emailed me a group of Ph.D. papers for me to read that was an amalgamation of topics from molecular biology to genetics and biotechnology to microbiology. After reading through them and taking notes I had a better understanding of the topics than before and she told me that, "To truly learn something you must suffer through difficult topics on your own to learn from your mistakes and make solving the problem that much more of an achievement."

My time in the lab has been truly invaluable and the amount of growth I have made as a scientist has been remarkable. I can walk into college and already know how to use the tools and machines properly and have a basis of knowledge on the topics and procedures and for that, I am forever grateful.

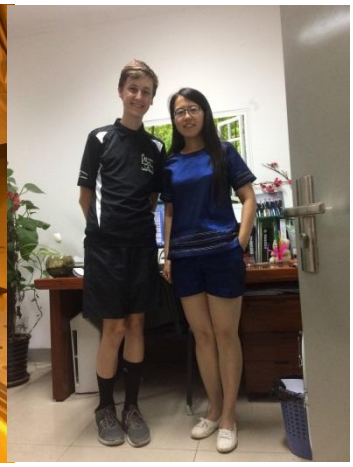
When the twenty-four interns and their parents made their way to Des Moines, Iowa for the orientation they told us we would not be the same person when we arrived back home and at first I thought they meant we would learn more and be smarter in our field of study but after being here in China for two months I can see what they meant by the growth we would experience. I have learned not to take things for granted and some luxuries we have at home we might not even think about are a rarity for people outside of the United States and even things as basic as education are a struggle. With Beijing's population at twenty-one and a half million people getting into high school or university requires top grades and test scores and seats are limited so some students could end up without a college education. For some they do not have a choice of schools either merely based on its proximity to their home and even their major is determined because even if they do not love a subject they select it because it is the smarter choice. This experience has opened my eyes to life outside of the United States and it allowed me to grow as a scientist, leader, and overall human being and my time in China is one I will not forget.

Photographs | Experiences





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