Connor Ludwig, Borlaug-Ruan Intern

China Agricultural University

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Acknowledgements

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Introduction

My name is Connor Ludwig. I was born and raised on a family farm near Breda, Iowa a small rural community. Iowa is the heart of agriculture in the US. It is usually the nation's top grower of corn and soybeans. Almost everywhere you go in the state there is a sea of green as far as the eye can see. Since I've grown up in a state where food is grown record amounts, I never have really understood how anyone in the world could starve. Sure I'd see ads and news stories from around the world about food depravity and I've seen some very gruesome images of starving people, but I never fully grasped the size and extent of the problem of hunger on Earth. That is until I went to The Global Youth Institute. I know for a fact that it was at that institute where I finally began to understand hunger, and it sickened me.

I love my life in Iowa. There isn't the cluttered, claustrophobic feeling you get in big cities, but it also isn't like you're an outcast from the world. The people in your community know you and you know them, although since I've grown up with a talkative farmer/ auctioneer as a dad, it seems like usually more people know me. My father owns an average sized farm, and I help out during the summer and when I'm not busy with school, sports, or other activities. I've grown up as the oldest child in a large family with two brothers and two sisters, and go to Catholic school in a nearby town. Looking back, most of my life has been sheltered from the world. Sure we could see terrible things on the news, but they never happened around us and I never watched the news anyway.

My life has always been based in a place of plenty. At my home I can look outside and as far as I can see food is grown. Corn, soybeans, hogs, and cattle were produced almost everywhere I'd been in my life. Some people don't like being around farms or smelly animals, but the smells and all is just home to me. I'm not saying that agriculture is my passion in life, but I understand its importance in the world and I think that is something you don't often get when living in a big city. Actually, I love taking apart, building, and studying machines, computers, and other trinkets. So I've thought for most of my life about being an engineer. Because I have such a career in mind it may seem strange for me to be on an internship focused on solving world hunger, but the Global Youth Institute does that to you.

Beginnings

My journey to the Youth Institute began in a biology class in my sophomore year of high school in 2010. Mrs. Koester, a teacher that I'd had for my sixth grade year of science, came into the classroom and told my class about a program where high schools students could go to an event called the World Food Prize and meet some of the leaders in the fight against hunger. The teacher told us that anyone who was interested could meet with her to discuss it during our study period after lunch. After class I talked to several of my friends about their thoughts, and they

didn't seem to think it was interesting. I couldn't believe that! So after lunch I went to meet with Mrs. Koester and my journey began.

The main requirement to attend the Youth Institute was to choose and research about an issue of food security in a specific country. I chose to write about water scarcity in Kenya. This paper was probably one of the longest and most researched I had written at that point, so that taught me a lot even before I attended the conference. The entire paper took me about a month to complete. It all led up to the Global Youth Institute, which changed my perspective on hunger forever.

When I attended the conference in 2011, I was able to meet and see noted professors from prestigious universities, executives from some of the largest companies in America, and politicians from across the world. That alone made writing the paper totally worth it. I was on cloud nine for most of the ceremonies and events. I remember watching the U.S. Secretary of Agriculture Tom Vilsack and several presidents and leaders of poverty stricken nations discuss issues of global food security. Then it really hit me how poverty in any nation of any size, really has a global effect across various continents. The institute taught me that there is enough food already produced in the world for everyone to have at least three basic meals a day. That alone was the most shocking and important fact I learned. Overall, there was just so much information available it was hard to process, and that was just within the first two days.

On the third day all of the students in the Youth Institute were taken to Pioneer's headquarters in Johnston. It was there that I got to present my findings and listen to other students do the same. It was really amazing because we were all just high school students, but we were discussing problems that the world's top researchers are working on. I think that was what really sparked my interest in continuing on with the program. After all of the students had presented, we all had the opportunity to go into a couple of large conference rooms to listen to presentations by Borlaug-Ruan Interns. Before that point I really didn't have any idea what the Borlaug-Ruan Internship was, but as soon as I saw the amazing places and things that those interns got to do something clicked. At the very end of the institute, all of the students were told that we had the opportunity to be an intern, and I knew I wanted to apply right away.

Arrival

My flight from Omaha left on the 22nd of May. Both of my parents and all of my brothers and sisters came to see me off because this was the longest any one of us had been away from home. We ate in the airport and then said our good-byes. This was a really emotional moment for me because my family is very close, and two months is a long time to be anywhere, let alone half-way across the world. It seemed like my parents were a little reluctant to let me go, and they nearly got their wish when I started hearing my name being called over the intercom for the last call! Luckily I had just gotten through security and proceeded to run to my flight. My parents thought that was pretty funny when I told them about it later, but I wasn't as amused.

When I got onboard my flight I finally got to meet Mohit Jain. I'd known for a while that I would be flying over with Mohit, but had never had much of a chance to talk. He was going to Peking University for his internship so it worked out well for us to fly together. It was very nice to have a companion flyer because this was my first international trip and Mohit had plenty of experience with them. We flew from Omaha to Detroit where we had a four hour layover until our flight to Beijing. We departed Detroit on schedule and everything seemed to be going smoothly. This was obviously the longest flight I'd ever been on so I didn't know what to expect. I now know why the window seat is the worst one to get on a long flight, but it's a good lesson to know.

Our flight had no problems, and we actually arrived a little earlier than expected. When we left the terminal I immediately saw two people waiting at the gate with a cardboard sign that had my name on it. Needless to say I felt like a celebrity. Mohit and I went to greet them and found out there names were Mingwei Du and Mr. Xiao. Mingwei is a Ph.D candidate at China Agricultural University, and Mr. Xiao is a close friend to Dr. Li (my mentor at the university). Mingwei spoke English very well and I could communicate with him easily. Mr. Xiao on the other hand didn't speak a word of English other than when he gestured with his hands showing how tall I was and said Kobe and basketball. I told Mingwei I wasn't any good at basketball and that I am a little shorter than Kobe Bryant.

As we stood there it became apparent that Mohit's driver wasn't at the airport yet, so we went to some nearby benches and waited. It felt kind of awkward talking to Mingwei at first but that quickly changed and he was soon helping me with my Chinese pronunciations of words as I was reading from a small guide my parents had given to me as a gift. Mingwei told me he would teach me to speak Chinese well in two months and that he was much better than any book. Eventually, we called Dr. Kang who was going to pick up Mohit, and we discovered that he had gone to the wrong airport! He arrived at our airport, T2, very quickly and Mohit and I said good bye and started our separate journeys.

Driving to the university with Mr. Xiao and Mingwei I discovered some differences between driving in China and in the US immediately. For instance in China, seatbelts aren't a big deal and not many people bother with them. Also, turn signals aren't used very often which led to me being somewhat frightened driving around and switching lanes on a highway in Beijing, one of the most populated cities in the world! I started to look out of my window on my way there and was amazed by what I saw. Beijing has a very different look than my rural community of five hundred people.

We finally arrived at the hotel I would be staying in at 2:00 a.m. I had a room on the fifth floor and it was about the size of my room in Iowa. Later on I realized how very spoiled I was to have my own room with two beds and an air conditioner. Most doctorate students only have one roommate, but students working for their Master's degree often have four or five and no air

conditioning. I got settled into my new home fairly quickly, but getting used to the food took a little longer. Thankfully, I grew accustomed to Beijing in a short amount of time.

CAU

China Agricultural University's origins began in the early 20th century when the College of Agriculture was established in Beijing as a place of higher learning for students interested in agriculture. Beijing Agricultural University was established later in 1949 when Peking University's College of Agriculture, Tsinghua University's College of Agriculture, and North China University's College of Agriculture. This college was listed as one of the Top-Six key National Universities and also as one of the Top-Ten Key Universities for further construction and improvement. Later in 1952 BAU's Department of Agricultural Mechanization School, North China College of Agricultural Machinery, and the Ministry of Agriculture's Central Agricultural Mechanization Institute merged together to form the Beijing Mechanized Agricultural College, which was renamed Beijing Agricultural Mechanization Institute in July 1953. This institute was renamed again in 1960 to the Beijing Agricultural Engineering University.

Finally, in 1995 the State Council approved the combining of BAU and BAEU to form the China Agricultural University. Now close to 16,000 undergraduates are enrolled at the university, and 3,000 and 2,000 postgraduate students are registered for MSc and PhD degrees, respectively. Undergraduates now study at CAU's eastern campus, where the gym that housed the wrestling events for the 2008 Olympics is also located and used for important ceremonies. Postgraduate students study at the western campus of CAU where they have access to many top notch research facilities. CAU also has invited many well-known scholars to the university as honorable professors and guest professors, such as Nobel laureate Norman Borlaug. To this day the university is one of the top institutions in China for agricultural studies.

The Lab

On my first day in China I was taken to see Dr. Li's lab. It was Sunday so the lab wasn't particularly busy, but it really impressed me. I hadn't been to too many labs in the US so I didn't know what to compare it with, however it seemed very modern and well-kept to me. I got a brief tour that day and was introduced to several of the students. It was there that I met Maoying Li, who would be one of my closest friends throughout my summer.

The next day I went back to the lab and I was told to follow several students around and get accustomed to the methods and procedures. One of the first students I followed was Xiao Yitao. Xiao was extracting protein from corn the first day I followed him so I was able to see several procedures and methods right away, such as how to grind plant material using liquid nitrogen and a pestle, centrifuging samples, electrolysis, etc. I followed several other students around the lab the first week and got to know many of the student students well and learn a lot about scientific processes. A lot of my time in the lab was spent researching topics I was

assisting in researching. I had very limited knowledge on most of the things I helped with prior to my trip, so I tried my best to educate myself on them. Even with all of this research I never totally understood all of the topics because they are topics that Ph.D. students were researching.

In the lab many different plants were tested and experimented upon. Cotton, soybeans, and corn were probably the main three, but I also saw arabidopsis and some rice being used. I helped a student who was working on trans-genetic varieties of cotton to withstand more salt stress, and another student who was using arabidopsis genes as a way of creating several different genetically modified crops because of its similar genetic structure. That first week really helped me to get introduced to the students, and to learn a lot about what I would be later working on.

So that was how my first two weeks went as I followed many students and took notes on over their experiments. Sometimes if they needed assistance with anything I would help them, but it was mostly just tasks like helping them to carry a large load or move test tubes and samples around. Halfway through my second week I got my first chance to work in the greenhouse that my lab used, with Xiao. The greenhouse was close to three quarters of a mile away from the lab so it was a little bit of a walk. The greenhouse complex was very large because it was used by many of the labs on campus. Xiao and I only used two rooms within the greenhouse. Each of the rooms had a water system and several fans, which allowed us to control the amount of water and moisture content in the air.

We went to the greenhouse in order to plant three different varieties of corn grown in China. Xiao's experiment was testing which grew best when exposed to water stress. I didn't realize it at the time, but I was helping to plant the experiment that I would be assisting with for my project. We had to prepare 96 pots with a mixture of soil and fertilizer. Then we planted four of the same variety of corn in each pot so that we would end up with an equal distribution of each type. After we planted the seeds we then had to arrange all of the pots onto a platform within the room.

While I was at the greenhouse I met two German students who were working on a project dealing with winter wheat. It was very interesting talking to them because I have German ancestry, but I've never known much about the country. They were both from the University of Hohenheim which has a transfer program with CAU. These were the first of many foreigners I met at the university. Many transfer students stayed in the same hotel as I did so I was able to meet and converse with many people of different nationalities such as, Egypt, South Africa, Denmark, and numerous others from a variety of cultural backgrounds. I didn't meet any fellow Americans because they were all on summer break, but I did meet a plethora of amazing people.

Around the third week I found out that I would be working with Xiao on his drought tolerance experiment. I was really excited because while I was in China my home state was in a severe drought, so that gave me a connection to home and made it important to me. The only

problem was that we needed to let the corn grow for a couple of weeks so that we could measure the effects of water stress, and Xiao would be leaving the lab for that time period to study for an important English exam that he needed to do well on if he ever wanted to accomplish his dream of studying in the United States. At first I didn't know what I was supposed to do for those two weeks, but soon I was back in the lab assisting anyone who could use an extra hand, which was a lot of people!

A few weeks after I had helped plant the maize samples in the University's greenhouse, we were able to start harvesting samples. This proved to be very tedious work that involved many steps. First, Xiao and I had to select which plants were ready to be tested. Then we would remove all of the dirt from the roots of the plant. Soon after, we would separate the roots and stem, and then perform whatever test was needed. After repeating this several times throughout the week we discovered something had gone awry. The heavy rainstorms that had occurred regularly throughout my stay in China had skewed the result of the experiment, so all of the plants had to be thrown away, and the experiment needed to be repeated. I was able to help replant the samples after this, but I left China before we could begin harvesting again. So Xiao sent me the data I needed to write about our experiment.

Experiencing China

When I found out that I was being sent to Beijing for my internship I was really excited. I have always had a sort of fascination with China and its culture. I suppose what really caught my interest was the fact that China has a history that spans thousands of years and now in recent years its culture has been blended with a western influence, especially in Beijing. It also helped that I love Chinese food. Looking back, I'm certain that Beijing was the perfect place for my internship.

Beijing is full of wonderful landmarks, monuments, and hundreds of other tourist attractions, and I was able to see many of them on my days off. Even at the end of my first week when I was still a little bit jet lagged I went and climbed the Great Wall with two girls from my lab, and the next day I was able to go to the Olympic park and the art district 798. I saw these places and many more, but that wasn't the greatest part about going to see new things in and around Beijing. The absolute best part about exploring Beijing was seeing the Chinese culture first hand. Sure I had read and watched many television shows about the foods, dances, and customs in China, but going there is a completely different experience.

Every location I went revealed a little more of the ancient Chinese culture and the new modern culture that's a blend of east meets west. Take for example the art district I visited called 798. This is an area for contemporary artists from across the world to display their work. It was started shortly after the Cultural Revolution in China in an abandoned factory, as a cheap place for artist to create. Today, it is one of the top tourist destinations in Beijing and famous art is displayed from all over the world. While I was there I saw an exhibit that to me clearly showed a

blend in Chinese culture. The exhibit was called lord and it featured several paintings, but the figurehead of the exhibit was a painting in which the legendary robes of the emperor were nailed onto a cross. It played at both meanings of lord in an eastern and western sense, one as an earthly ruler and one as a spiritual one, respectively. This was just a single example I saw in the district. I was also fortunate enough to be able to see the Forbidden City, Tiananmen Square, the National Museum of China, Yuanmingyuan Park, and the Summer Palace throughout my stay.

While I was at many of these famous locations many people stared at me or asked if they could take a picture with me. I suppose this was to be expected because I am a blonde haired, blue eyed, 6'2" kid from America. You can't be much more different than the typical Chinese citizen. Throughout my stay I heard many comparisons to myself and NBA players, and many people asked me if I was a good player (I'm terrible at basketball). The inquiries and conversations gave me a really unique perspective as a minority that I'd never had in America. That really was one of the most interesting parts of my trip because everywhere I went I stuck out like a sore thumb. Even at some of the most popular tourist attractions I was very different.

On my second night at the university I was able to see another bit of Chinese culture, albeit a modern one. I arrived at the university during graduation week for the PhD and MSc candidates. This was a time for great celebration on the campus and I was invited to a dinner in a restaurant on campus by several of the graduating students from my lab, including Mingwei. I went there and had a great time meeting many new students and made many friendships that would last through my whole stay and continue when I returned to the United States. A typical celebratory dinner was served in which many of dishes were served and everyone picked what they liked out of them. There were many dishes served that I was unfamiliar with and that frankly seemed inedible to me (at least at the beginning of my stay). One these dishes were birds' eggs that had been buried under ash and dirt for a length of time and then dug up and served as a dish that resembled black hard-boiled eggs. I also experienced some of my first tofu in this restaurant, but I would have many more opportunities to eat that. The party lasted well into the night, but I had to leave early due to my feeling a little exhausted.

On the day after my meal with the graduates the graduation ceremonies took place within the Olympic gymnasium at the east campus. I didn't attend the actual ceremony, but later that night a celebration was held by the school in front of the west campus's administrative building. Many different kinds of food were provided, some I recognized while others were very new to me. At the celebration several events were held, such as karaoke, debates, awards, etc. It was very interesting hearing the song choices for the karaoke because while many were popular Chinese songs, I also heard many popular western songs, such as Adele's *Rolling in The Deep* and Lady Gaga's *Bad Romance.* Most of the speeches and debates went right over my head because my translator for the evening, a student from the lab, couldn't translate quickly enough. The festivity was a great way for me to see and experience modern culture in China.

During the duration of my internship I was able to also learn a lot about the lifestyle in China. Much of this I learned from the students I spent time with. One of the most shocking things I learned from talking to my friends in China was the difficulty of receiving education. I was told that only fifteen percent of the graduating high school students in China are able to continue their education in college. If these students don't receive a college education it is extremely difficult to find a job to support even a single person let alone a whole family. This is due in large part to living in the world's most populous country. I also heard many stories of how much many of my friends' parents had to sacrifice to get them to college. Often times their parents would send most of their monthly income to their children, an income that is so little it stunned me.

During my internship, the 2012 London Olympics occurred, and this gave me a unique vantage point to see how China had changed in four years since it hosted the 2008 Olympics in Beijing. I was able to see during my internship was the effects of the Beijing Olympics in 2008. This year being the next Olympics since Beijing was a great time to judge how the Olympics affected the city. I believe that those Olympics were an overwhelming success. There were countless times I would ask about when and why a new looking building or a tourist attraction was built and my answer was usually for the Olympics. Even four years after the event I saw skyscrapers with the Olympics emblem emblazoned across them. Most of the subway system in Beijing was renovated or built before the games, and it all really makes the city reflect the countries rise from a problematic past. I also toured the Olympic park in Beijing, which was one of the highlights of my stay in Beijing because the games were just so amazing to me when I watched them. Seeing the places where countless records were shattered and the world's greatest athletes performed was just fantastic! Many of the students themselves told me how much it meant to them to be part of the country that hosted such a memorable event, and the many positive changes they've seen as a result from it.

Overall it's hard to put my experiences in China into words let alone a short section of my paper. I loved meeting all of the wonderful people and seeing some of the most famous sights in the world. The experience also really gave me a sense of how lucky I am. My friends told me many stories of the difficulties of living in China, and this really gave me a sense of gratitude for all that I have been given and life, and a sense of obligation to those who aren't as fortunate as myself. My trip to China has taught me so much from science to politics to charity. I will never be able to repay the World Food Prize for this experience.

Pictures

Experiment: Plant Response to Water Stress

Xiao Yitao, MSc student, CAU

Connor Ludwig, Intern, CAU

Abstract:

In this experiment three varieties of corn, which are all commonly grown in in China, were planted within a mixture of nutrient soil and vermiculite. These pots were then placed in a greenhouse where the humidity, temperature, and water were all controlled. The samples were grown for several weeks. The three sample of each variety were each harvested five times at different time periods after the initial growth of the plants, the first of these samples were collected 16 days after planting. The samples biomass, ABA content, AO content, and RNA were all examined. The data collected shows a much stronger relation to increased levels of AO and the triggering of stress induced mechanisms within a plant.

Introduction:

Abiotic stresses, especially salinity and drought, are the primary causes of crop loss worldwide. (Vinocur, 2005). As the world population increases, more and more people are dependent on crops that are grown on less and less land, and when something interrupts the growth of these crops the effects can be noticed world-wide. Therefore the development of drought tolerant plants is imperative to the goal of global food security. Abscisic acid (ABA) is an important plant growth regulating hormone. It has been proven to be involved in the regulation of various plant developments such as; seed dormancy, germination, senescence, and adaptive responses to environmental stresses including freezing, drought, and salinity. When a plant is exposed to drought stress ABA levels are increased within the plant. These increased levels cause the plant's stomata close to limit water loss. In the process of ABA synthesis, there are some important enzymes, including zeaxanthin epoxidase (ZEP) and epoxycarotenoid dioxygenase (NCED) in the early steps and aldehyde oxidase (AO) in the last step. Aldehyde oxidase (AO) belongs to the family of molybdenum hydroxylases and catalyzes the oxidative hydroxylation of a number of diverse aldehydes and aromatic heterocyclic compounds in reactions that necessarily involve the cleavage of a C-H bond. Inside a plant's cytosol, the enzyme catalyzes the oxidation of indole-3-acetaldehyde (IAAId) to IAA and abscisic aldehyde (ABAId) to ABA. So AO is related to the synthesis of ABA and the adaptive stress-induced mechanisms in plants.

Hypothesis:

ABA is commonly thought to be the main plant hormone responsible for triggering plant defensive mechanisms, but we believe that AO may have a connection to it also. We aim to distinguish the differences between the two.

Materials and Methods:

We began our experiment with three different kinds of maize called 80, 4967, and 368, respectively. The different seeds of maize were sown into pots containing nutrient soil and vermiculite. These pots were watered every five to seven days to field capacity. If the nutrition was not enough, the necessary amount of nutrient solution was added. All of the pots were grown within a greenhouse set to its highest temperature of 28°C, under a photoperiod of 16 hours. Water was withheld from half of the pots after 15 DAS (days after sowing).

Samples from the plants were taken five times; one day, ten days, fourteen days, and seventeen days after the application of drought stress treatment, which varied accordingly to the water status change. The last sample was taken after re-watering all the plants. Plant samples were separated into groups of roots and leaves, and whole plants. The wholes plants were collected for biomass determination. Fresh samples were frozen in liquid N_2 and stored at -80°C for later analysis.

Biomass determination

The samples that contained whole plants were washed several times with water, and then cut and divided into roots and leaves. The roots and shoots were weighed and then dried within an oven at 85°C. If the weight didn't change between the original weight and the dried weight, they were weighed again.

ABA determination

The quantitative determination of ABA was carried out via an enzyme-linked immuno-sorbent assay (ELISA). Plant tissues (1.0g) were extracted with 15mL of a cold mixture of 100mM NaHCO3 and methanol (80/20 v/v) containing 1mg of butylated hydroxytoluene in a volume of 100mL. The samples were extracted twice at 4°C for 24 hours each, and then evaporated. The assay utilizes the monoclonal antibody for ABA, and the determination of (+)-cis-ABA in the plant extract is based on the competitive binding of ABA and the tracer (alkaline phosphatase-labeled ABA) to the antibody-coated microwell. In total, 100 mL of standard ABA or plant extract and then 100 mL of diluted tracer were added to each well. After incubation for 3 h at 4 ℃, the wells were washed three times. The alkaline phosphatase reaction was started by the addition of 200 mL of substrate solution. After 60 minutes at 37°C, the absorbance was determined at 405 nm, using an MR 4000 microplate reader.

Extraction and Detection of AO proteins

Tissue samples were ground into a powder with liquid nitrogen and homogenized in ice-cold extraction buffer (250 mM TRIS-HCl, pH7.5, 1 mM EDTA, 10 mM GSH, and 2 mM DTT,10uM FAD, 50 uM leupetin, 80uM sodium molybdate). A ratio of 1 g tissue to 2 ml buffer

(1:2 w/v) was used for leaves and 1 g tissue to 1 ml buffer (1:1w/v) for roots. The homogenized plant material was centrifuged at 18 000 g and 4 °C for 25 min. The resulting supernatant was subjected to native polyacrylamide gel electrophoresis (PAGE) on 7.5% polyacrylamide gels in a Laemmli buffer system (Laemmli, 1970) in the absence of SDS at 4 °C. Each lane in the gel was loaded with the same proteins. After electrophoresis, the gel was immersed in 0.2 M phosphate buffer (pH 7.5) for 10 minutes, then AO activity staining was developed at room temperature in a mixture containing 0.1M TRIS-HCl, pH 7.5, 0.1mM phenazine methosulphate, 0.5mM MTT (3[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium-bromide),and 1mM substrate (1-naphthaldehyde or indole-3-aldehyde) in the dark for above 1 h. Native PAGE was carried out with a Protein Ⅱxi Cell(JunYi, China).

RNA extraction and Quantitative real-time PCR analysis

RNA was extracted from frozen root and leaf tissue (0.1g)using Quick RNA Islation Kit (Hua Yue yang Biotechnology, Beijing). For quantitative real-time PCR analysis (qRT-PCR), purified total RNA was transcribed using a Promega SuperScript FirstStrand Synthesis System for RT-PCR (Promega Corporation, Madison, USA) according to the manufacturers' instructions. The cDNA was then used for quantitative real-time PCR. Primer Express Program 3.0(Applied Biosystem, Foster, CA,USA) was used to design the primers of chosen genes: NCED(vp14/NCED 5'-GGCTTC CAC GGC ACC TTC ATC AC- GGG C-3' (forward) and 5'-CGG GGA ACT GAT CTG GGC TCC CTC TGG-3'(reverse); zmAO1 5'-GGGAGGCTGTGTACGTTGAT-3'(forward) and 5'-TCTCCACCGCTTGGAATATC-3'(reverse); zmAO2 5'-GGCCTCCTCTCCAGAATACC-3' (forward) and 5'-ACCTCAACAGCAATGGAACC-3'(reverse); molybdenum cofactor (Moco) sulphurase 5'-CGGCAGGTGTACTTTGGGCAAA-3'(forward)and5'CGGGGTCCTGATTCGGTCACTCAG-3'(reverse); β -tubulin5'GTACCGCGCTCTCACCGTGC3'(forward)and5' ACGGAACATAGCAGATGCCGTGA-3' (reverse).The β -tubulin gene was chosen as an internal control to normalize all data. Real-time quantitative RT-PCR was performed on a 7500 real-time PCR system（Applied Biosystems using SYBR® Premix Ex Taq^{TM} (Perfect Real Time) (TaKaRa Code: DRR041A). According to the manufacturer's protocol, 1.5μL cDNA,0.4μL PCR forward/reverse primer(10μmol), 10μL 2×SYBR® Premix Ex Taq^{TM} and 0.4μL ROX Reference Dye II (50X) were suspended in a final volume of 20μL with ddH_2O.RT-QPCR cycling conditions consisted of an initial polymerase activation step at 95°C for 30 sec, 40 cycles of 5 sec at 95°C, and 35 sec at 60°C. Melt-curve analysis was performed to monitor primer-dimer formation and the amplification of gene specific products. The relative quantification method ($_{\triangle\triangle}C_T$) was used to evaluate quantitative variation between replicates examined.

Results:

Biomass accumulation in response to drought treatment.

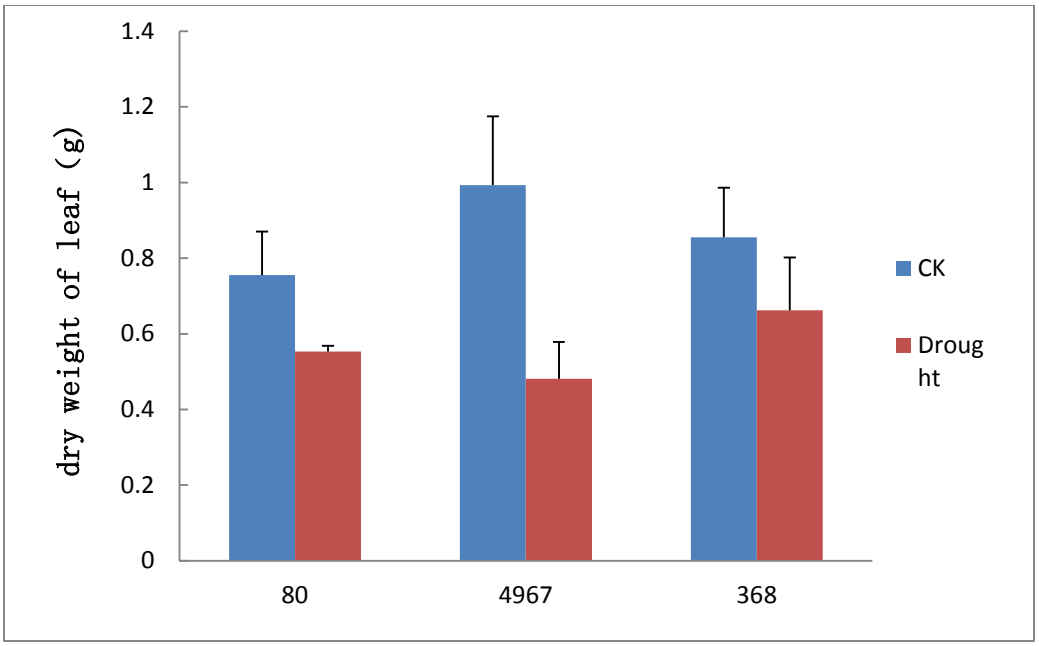


Fig.1. This graph displays the effect of drought on the leaf weight of maize.

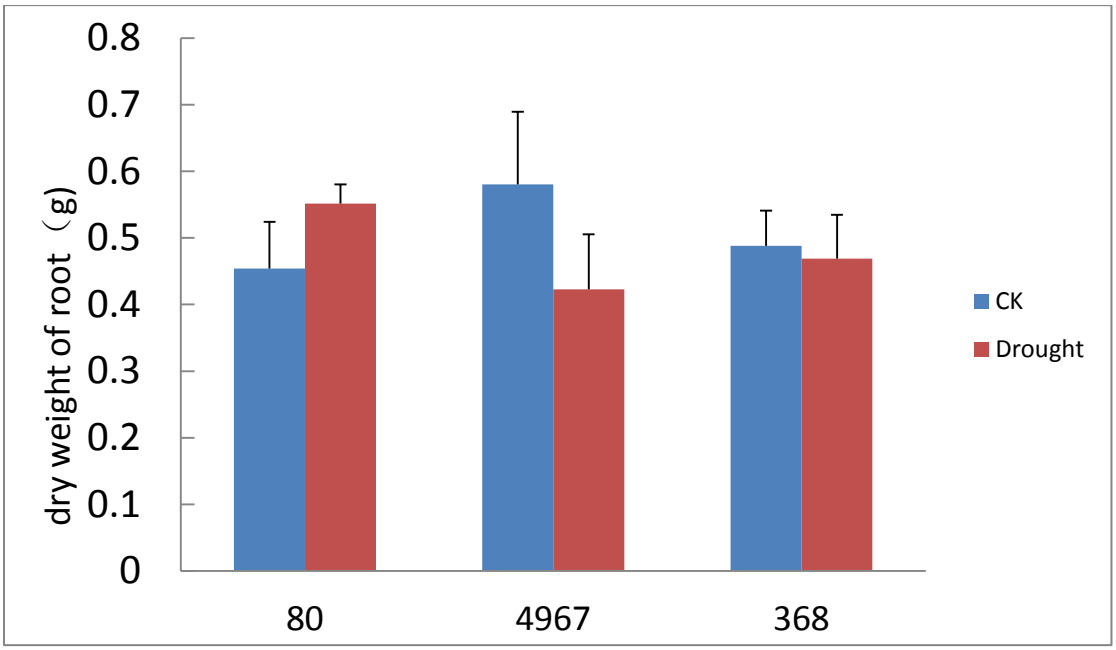


Fig.2. This chart shows the effect of drought on the root weight of maize.

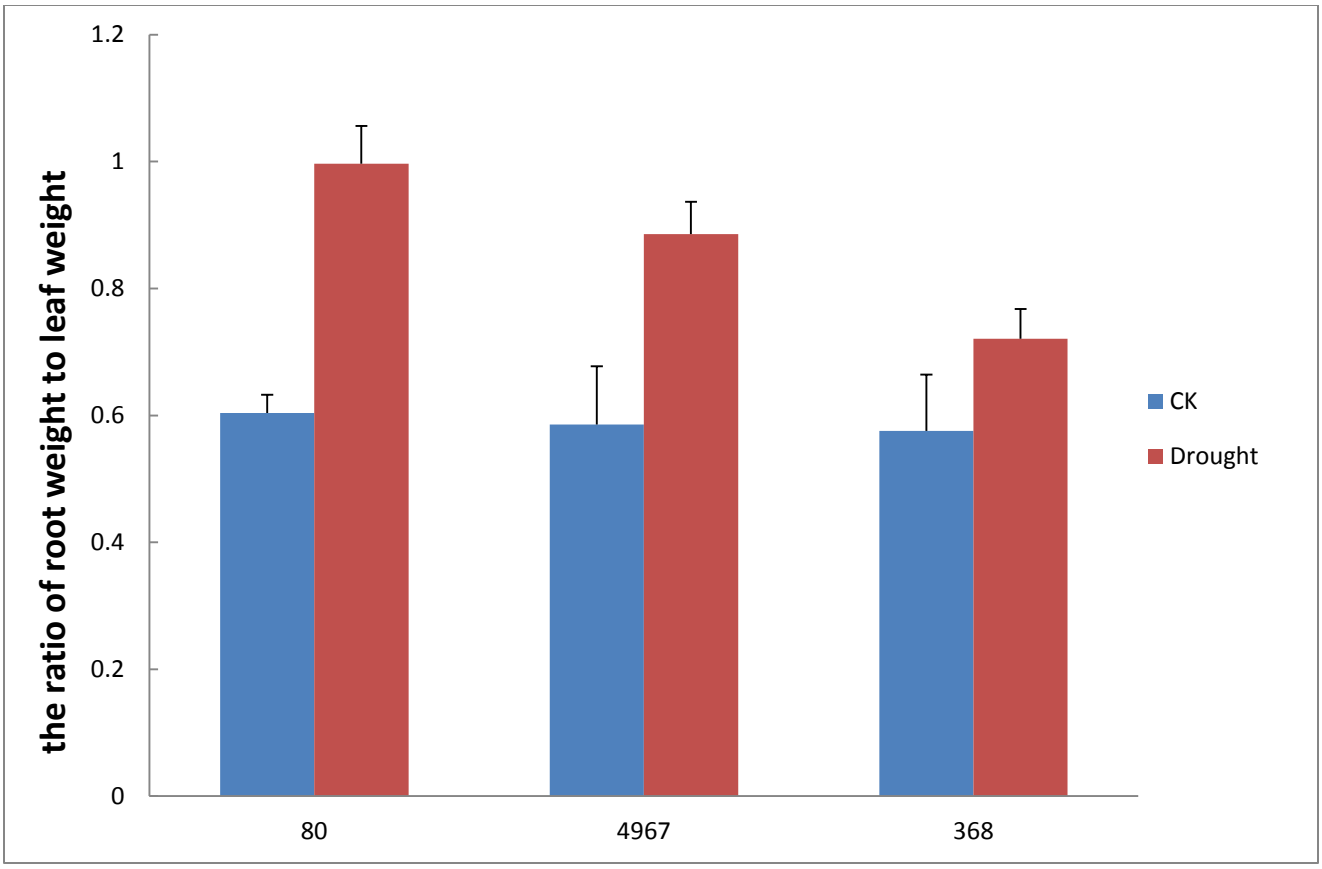


Fig.3. The above chart illustrates the effect of drought on the ratio of root weight to leaf weight.

4967 368 80 4967 368 80

Fig.4. The above picture shows the growth of the three maize varieties. The left picture displays maize under drought conditions. The right picture is of plants under normal conditions.

Drought reduced the leaf weight of all the plants and the root weight of maize variety 4967 and 368. This shows that drought inhibited the development of the plants. However, the root weight of 80 increased a little, this was probably due to 80's higher resistance to drought, and because a certain degree of drought stress can stimulate the growth of roots. Under drought condition, the root to leaf weight ratio of all varieties was increased. This shows that drought may affect the development of leaf more seriously than the development of root. Of all of the root to leaf weight ratios, 80's was the highest. Variety 80 also had greatest amount of growth throughout the experiment. By combining the root weight, the root to leaf weight ratio, and the total growth, it was proven that variety 80 had the greatest drought tolerance.

3.2 Effects of drought on root and leaf ABA contents (unmeasured)

3.3 Effects of drought on root and leaf AO activities

3.3.1 Effect of drought

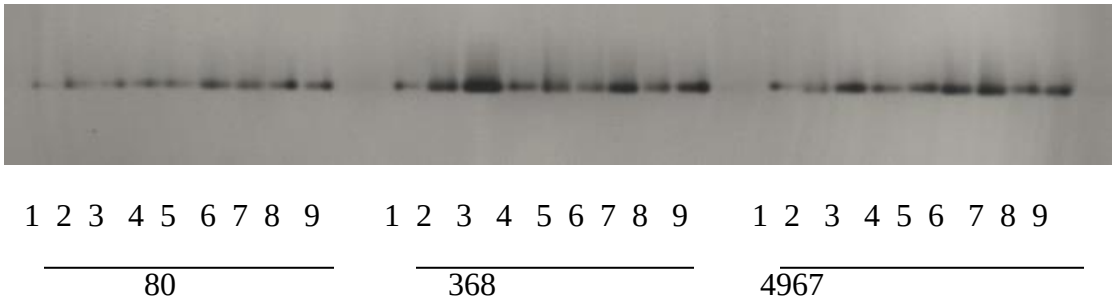
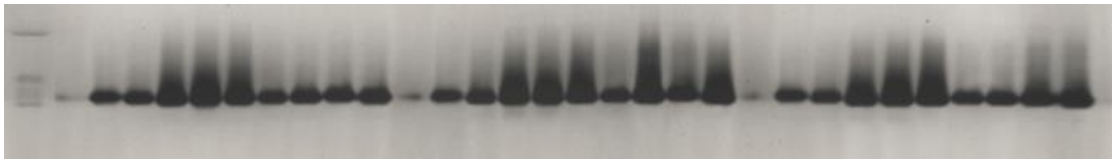


Figure 5. Effect of drought on the activities of AO in maize leaves. 1, 2, 3, 4, and 5 are under normal condition and are respectively sampled 1 day, 10 days, 14 days, 17days, and 19 days after application of drought stress treatment, and 6, 7, 8, and 9 are under drought condition and are respectively sampled 10 days, 14 days, 17days, and 19 days after application of drought stress treatment.

Under drought condition, almost all the AO activities of leaves increased except the 368's 6 and 7. However, the tendency is not very obvious; this may be because the time of sampling is not very appropriate. Among the three varieties, the AO activity of 368 and 4967 increased most, and 80 faintest. This may show that variety 80 has the best drought tolerance.



1 2

3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9

80 368 4967

Figure6. Effect of drought on the activities of AO in maize roots. The leftmost is Arabidopsis's AO. 1, 2, 3, 4, and 5 are under normal condition and are respectively sampled 1 day, 10 days, 14 days, 17days, and 19days after application of drought stress treatment, and 6, 7, 8, and 9 are under drought condition and are respectively sampled 10 days, 14 days, 17days, and 19days after application of drought stress treatment.

Under normal condition, the activities of AO on 14 days, 17days, and 19 days increased significantly. It was because of that the small trays under pots stopped redundant water flowing out of pots, which flooded the roots, made them decay, and leaded to the increase of ABA. But in general, drought could increase the activity of AO (6, 7, 8, 9). Among the three varieties, the AO activity of 368 increased most, 4967 second, and 80 faintest. This may show that 80 has the best drought tolerance, and the 368 lowest.

3.4 ABA biosynthesis gene expression in leaves subjected to increasing water stress

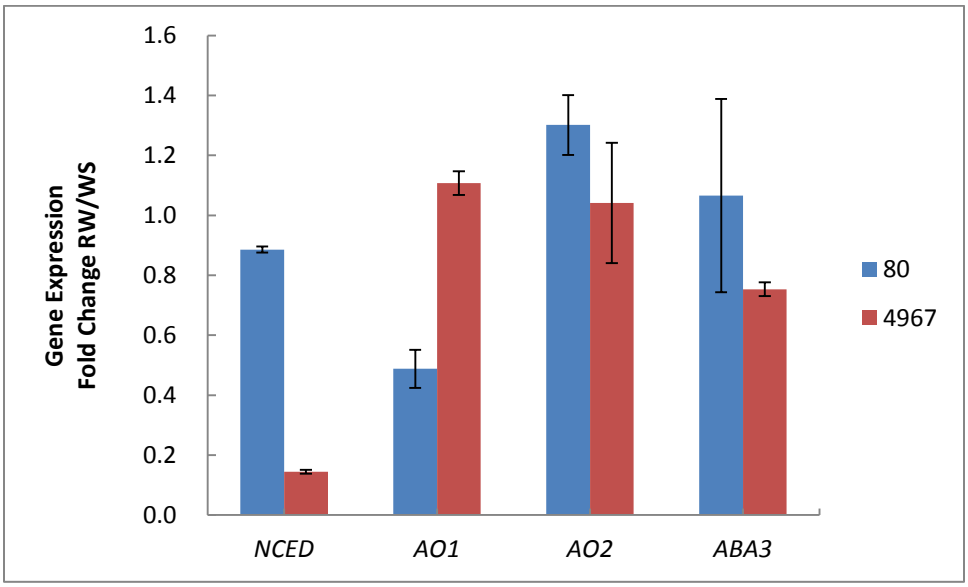


Figure 7. Fold change in ABA biosynthesis gene expression for re-watered *Zea mays* leaves relative to water-stressed controls.

Rewatering after a long period of drought, *NCED* and *ABA3* expression reduced, AO1 and AO2 expression almost didn't change in 4967. In 80, the NCED and AO1 expression reduced, AO2 expression increased, and ABA3 expression almost didn't change. Among the four genes, only NCED expression reduced both in the two varieties. This may show that NCED are closely related to drought and drought can increase its expression. Other genes' expressions were not consistent, so more experiments are needed to be done to pinpoint their role in drought tolerance.

Resources

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