LOCATING TILLER DEVELOPMENT GENES IN RICE




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Abstract:

Successful tiller development is one of the most essential traits observed in high yielding rice varieties. Tillers are the shoots which branch from the main stems of cereal plants, serving as the frame necessary to support the growth of leaves and seed-bearing heads. Improving the number and quality of tillers spawned by each plant enhances their capability to efficiently produce grain.

Unveiling the genes regulating the development of tillers will pave the way for farmers, seed producers and scientists to selectively breed or modify crops to optimize plant productivity. A breakthrough in this area could be considered the next step towards producing more, and higher quality food for the world's rapidly expanding population.

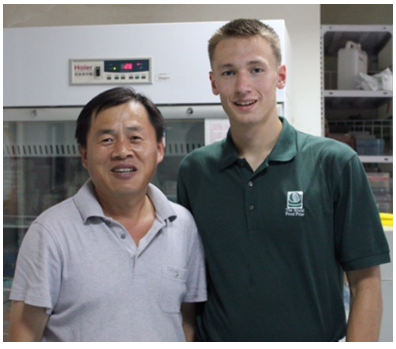
Under the mentorship of program director Dr. Xueyong Li and doctorate student Chun Yan at the Chinese Academy of Agricultural Sciences in Beijing, China, I worked to locate a gene inhibiting the production of tillers in a specific rice hybrid. Given tissue samples of an F2 population bred from the uniform offspring of the varieties Huang-Hua-Zhan and 300192, I was to test the hypothesis: Will breeding a rice varieties exhibiting mutated and unmutated phenotypes lead to discovering the mutated gene's location?

In addition to researching the tiller development gene, I apprenticed under my lab mates; at times assisting them with work on their doctorial projects and partaking in field labor. Furthermore, I accepted an opportunity to travel to a rice breeding facility in the Shandong province. These opportunities augmented my understanding of Chinese culture, strengthened my abilities in the lab and allowed me to make an impact on the future of the CAAS lab for years to come.

Acknowledgements:

First and foremost, I would like to thank The World Food Prize Organization, Norman Borlaug and the Ruan Family for the creating the unparalleled, transformative internship which has greatly impacted my role in agriculture. Furthermore, I wish to thank Ambassador Quinn, Kelsey Tyrrell, Lisa Fleming and all of those who worked behind the scenes to make this opportunity possible. The hard work and dedication of so many has inspired so many to join in the fight against global hunger.

I would also like thank Dr. Li and the Chinese Academy of Agricultural Sciences for hosting me in their lab this summer. Program Director Dr. Li's incredible mentorship, paired with that of my lab mates: Chun Yan, Haiyang, Suyash, Adeel, Uzair, Misan and Lu-Lee, opened my eyes to a swath of unseen possibilities.



In addition, I wish to thank my high school FFA advisor, Mrs. Dillon, for inspiring me to apply to the World Food Prize Iowa Youth Institute. Without her encouragement, I never would have discovered this opportunity which has so greatly impacted my life. Also, 2016 Borlaug-Ruan intern Nicolas Grandstaff, whose incredible presentation drove me to apply. Finally, I want to thank my family for their support through my adjustment and to fellow 2017 interns Jessica and Danny for great friends while we were half a world away.

About Me:

As the sixth generation of a family farm and a student enrolled in Iowa State University's agricultural business and international agriculture programs, I have cultivated an incredible passion for agriculture. From childhood, I had always aspired to take over my family business, but more recently began to discover the need for a global agriculture. As a producer in a land of plenty, I feel it is my mission to use the resources which I have been blessed with to make a positive change in agriculture across the globe.

History and Purpose of CAAS:

The Chinese Academy of Agricultural Sciences (CAAS) was founded in 1957 as a research institute focused on addressing pressing issues facing China's rural and agricultural sectors. The academy has a network of 42 institutes distributed across China, granting researchers the ability to conduct trials and gather data from around the country. The academy is a global player in advanced agricultural research who works in conjunction with 27 joint laboratories managed by foreign countries and organizations pushing to further develop agricultural technologies.

The primary focuses of the academy are to address the needs of farmers, strengthen rural communities and to push for advancements in the resources and methodology available to the agricultural industry. CAAS is renowned for putting cutting-edge technology into the hands of its over 4300 brilliant post graduate researchers from around the globe. Their ability to empower students has yielded incredible advancements in domestic agricultural systems and has made CAAS a symbol of the future of agricultural research.

Institute of crop science: The CAAS institute of crop science was devised during the consolidation of several smaller institutes in the year 2003. The goal of the revised program is to "resolve basic, critical and future key problems for the development of crop science and technology." The crop science institute focuses on germ plasm resource, the discovery of genes, genetic mechanics, the establishment of new varieties and improved cultivation systems.

Dr. Li's Lab: Under the guidance of Dr. Xeuyong Li, members of lab 513 are focused on rice morphology, or the effects of changing the genetic structure of a plant. They strive to discover genes responsible for the key traits of productive rice plants and how to optimize them. A

staple procedure in the process is called genetic knockout. In genetic knockout, a gene is disabled in a plant which is grown to observe changes in the phenotype. Further tests are conducted to create new genetic alterations to achieve desirable responses from the discovered gene. Once a gene has been idealized, the information is published to be shared amongst other scientists, crop breeder and farmers who can use the advancements in better Chinese agriculture.

Economics of Life in China:

China faces a unique economic situation. In 1978 China commenced a colossal economic reformation, exchanging their centrally owned communist system for the new "socialist market economy." This structure allowed state and privately-owned companies to coexist, leading to a tenfold increase in national GDP and led man people out of poverty via entrepreneurship (CIA.gov, 2017). Although the average income has risen from $156 in 1978 to just short of $8,150 in 2016, a purchasing power parity of $15,500, the income is not very evenly distributed (WorldBank, 2017).

Profile of Chinese Farmers and Demand for Agricultural Development:

Chinese agriculture is a leader in the global initiative to feed the world's rapidly expanding population. From the conception of the People's Republic of China in 1949, the quantity of grain produced by Chinese farmers has increased six-fold; an achievement catalyzed through improvements in breeding practices, rural education and seed genetics (Nunlist, 2017). While they have come a long way towards food security, China has realized that it cannot raise the bar again without seeking to improve the lives of their smallholder farmers.

China is home to 1.4 billion people, of which 550 million will be recognized as middle class by 2022 (Iskyan, 2016). This influx of middle-class citizens is creating a much larger demand for agricultural products. In order to provide the complex diets and renewable fuels the middle class longs for, it is a necessity that they remain on the cutting edge of technology. To complexify the situation, a series of scandals which lead to intense food shortages in years past have made the citizens of China wary of GMOs and the large, foreign agricultural firms, leading the biotech industry.

According a 2015 World Bank study, China has roughly 400 Million workers packed onto only 105 Million Hectares (World Bank, 2015). The average size of a farm in China is one-sixth an acre. These small patches, roughly the area of one and a half football end zones, known as "Mu", are the backbone of China's agricultural production. These plots are owned by the

central government and are leased out to families for generations at a time. Nearly a fifth of this land was deemed contaminated by industrial pollution in a Food and Agriculture Organization study (Bloomberg, 2017).

Chinese farmers face heavy pressure from urban sprawl, limited fertile soil and diminishing water resources, impeding their ability to feed their nation. Most of China's farming is done on the more urbanized eastern side of the country as the western region is primarily arid and mountainous. Of arable acres, roughly 40% are dependent upon aging irrigation infrastructures and inadequate hybrids (Indexmundi.com). Chinese farmers also face severe pressures from disease, locust, and mice. Pest outbreaks in the last few decades have had devastating effects on local farmers and have destroyed millions of acres of crops. (Hayes, 2011)

Life in rural China is often devoid of modern amenities and conveniences, leading a substantial percentage of rural youth to flee toward the cities in search of better lives and more consistent work. Consequently, the average age of the Chinese farmer is now over 50 years old, a serious concern for the nation's agricultural future. To ensure the continuation of smallholder farming, China will need to entice young farmers to return to their family business by improving economic outcomes and quality of rural life.

Arrival:

On the afternoon of June 12th, 2017, I landed in Beijing and was welcomed by my lab mentor Chun Yan. He spent the entire taxi ride to the academy explaining how elated he and the other members of lab 513 were to finally meet me at dinner that night. When we had arrived at the academy and delivering my luggage, we went out for dumplings at a nearby restaurant. Finally, after good night's sleep, I got to meet the rest of my lab mates and started assisting them with their research projects to gain skills I could apply to my own.

Project Background:

Purpose of Gene Function research: The ability to locate genes for optimization is an incredible tool which can be harnessed to help achieve global food security. The technology places the power of understanding into the hands of researchers, breeders and farmers alike, giving each the ability to develop plants which can achieve optimal outcomes in a plethora of unique situations. Understanding genes allows us to manipulate them through genetic engineering or selective breeding or plants to achieve varieties which maximize potential while minimizing inputs.

Process of Locating and Testing Genes: Genes can be tested through a process called knockout. In knockouts genes are located and disabled through the CRISPR/Cas9 process. The affected plants are then grown to observe changes in their phenotypes. Plants which carry the altered genetic sequence are labeled mutant, whereas unaltered plants grown alongside mutants as a control group are referred to as "Wild Type". At maturity, the mutant plants are compared to the wild type plants to determine phenotypic alterations, illustrating the purpose of the altered gene.

Significance of Rice: Rice serves as a model crop for genetic research for many reasons. First, rice has one of the smallest genomes, being only 406mB (megaBases) long, it is nearly a sixth of the size of the corn genome. Second, rice is easily mutated to phenotypic changes, making it ideal for discovering genes and developing knowledge to be translated into other crops. Finally, rice is a staple crop for a large portion of the global population. Any improvements in the characteristics of the plant have the capability to make widespread changes and better the lives of billions of people.

Status of Research: While the rice genome has already been mapped and joins many other organisms in having been sequenced, there is still much to learn about function. In the words of my lab mate Suyash, "Why is there so much 'junk' material between genes? What more is there for us to know?" Having mapped the genome, researchers are working diligently to identify the purpose filler DNA material.

Definitions/Key Concepts:

DNA Structure: Two chains of nitrogenous bases attached with hydrogen bonds come together to form a molecule of DNA. Each individual strand is a series of the bases Cytosine, Guanine, Adenine and Thymine bound to each other via phosphodiester bonds. The chains wind around each other to create a double helix structure where each base can associate with its counterpart on the opposite strand. The associations form pairs of the counterparts Cytosine and Guanine, which share two ßhydrogen bonds, or Adenine and Thymine, which share three.

DNA Replication and the Function of Primers: A DNA molecule can be thought of as a zipper. Exposure to heat or enzymes hydrogen bonds fastening the two strands together dissipate, the two-individual strands are released. Primers, which are sequences of bases complimentary to the strands, latch on to the unzipped DNA chains and stimulate the rebuilding of the strands. If the temperature falls below the threshold, the primer will fasten itself to the DNA in a process called annealing. After the primer has annealed, an enzyme named DNA polymerase reads the DNA strand and rebuilds the missing side using stray CGAT bases.

Optimal Genes: Optimal Genes (interchangeable with traits/expressions) refer to the most suited expression of a trait in a particular environment. This commonly refers to an expression which maximizes yields and quality while reducing inputs and labor.

INDELs: INDEL, short for insertions and deletions, are small differences in the length of a string of nucleotides in a DNA sequence. Occasionally when genes are passed on during breeding the two copies of a gene are not similar in length. Often caused by improper copying of the genetic material, these uneven strings mean the affected region will not code properly, resulting a in a mutation.

PAGE: Polyacrylamide Gel Electrophoresis, is a test used in labs to compare the size of INDELs. To perform a test, a thin gel with small pockets for DNA samples was prepared once PCR is completed. The samples are then loaded into the pockets to and the tray holding the gel is flooded with a buffer solution to prevent changes in the pH of the gel or DNA. The gel is then exposed to a procedure called electrophoresis. In electrophoresis, the tray is hooked up to a power source which allows electricity to flow through the gel from the anode towards the cathode; dragging along with it the negatively charged DNA.

Intro To varieties:

Mother 300192: The rice variety on the right, labeled 300192 is a mutant variety known for its lack of tillers. There is no commercial application for 300192, but it is used in research to breed observable alterations into the wildtype.

Father HHZ: The variety on the left, labeled Huan-Hua-Zuan, Mandarin for Miraculous-Transformed-Breakthrough, is a common variety of rice used by researchers in China. The plant exhibits a strong capability to produce tillers and is known for its fair yields. HHZ serves as a demonstration of the standard rice hybrids used by farmers.

Methodology:

To locate the gene responsible for the observed tiller deficiency, DNA from the parent generation and F2 generation of the 300192 and HHZ hybrid needed to be extracted and mapped. To do this, I was given twenty-four plant samples; one of each parent and twenty-two from members of the F2 population expressing the phenotype of abnormally low tillers development.

The F1 population of the HHZ and 300192 plants demonstrated a uniform, desirable phenotype. However, the F2, the offspring of the F1 generation, only expressed a desirable phenotype in three quarters of the population, evincing the undesirable trait from 300192 in the remaining quarter. Plants from the mutated quarter were gathered and prepared for DNA extraction.

DNA Extraction:

The DNA extraction process began by folding a strip of plant material placing it into an Eppendorf Tube. Two metal BB's were placed on top of the leaf and the tube was closed and marked. The sealed tubes were then submerged in liquid nitrogen for 30 seconds to flash freeze the plant material so that it would become brittle for grinding.

Frozen samples were then loaded into a genogrinder, a machine designed to pulverize samples. The grinder was a tray capable of oscillating 20 times a second, shaking the material and BB's at such a speed that the material was ground into a fine powder, improving it's reactivity. The samples were shaken in the grinder for 35 seconds, or until a fine powder was achieved. When the tubes were removed, a buffer solution, CTAB, was added to the powder to mitigating the effects of particles in the atmosphere.

Buffer solutions like CTAB are made up of an equal amount of weak acids and weak bases which cancel each other out, functioning to reduce pH shock to an experiment. When more powerful acids or bases are added to the solution, the weak varieties abandon their weak counterparts and bind to the stronger products, reducing the effect on pH.

In addition to functioning as a buffer, CTAB also works as detergent which, in the words of Paul Winnard of John Hopkins School of Medicine, "simultaneously solubilizes the plant cell wall and lipid membranes of internal organelles and denatures proteins." Preparing the cell for the removal of genetic material. (Winnard, 2017)

After CTAB was added to the solution, the sample tubes were incubated in a water bath for up to an hour at 65^{o} C. When incubation was complete, the tubes were removed from the bath and taken to a ventilated hood where a solution of 24:1 Chloroform: Isoamyl Alcohol was added to denature the remaining proteins from the DNA.

The resulting mixture was placed in a centrifuge at 12000 rpm for ten minutes to separate the contents by phase density. When centrifugation was completed the less dense material, an aqueous phase known as supernatant was removed and relocated to a new Eppendorf Tube. The remaining dense phase, consisting mostly of plant debris, was discarded.

The removed aqueous phase contained dissolved DNA was cleaned through the addition of absolute ethanol in an amount twice the volume of the supernatant. The ethanol-supernatant mixture was placed in the refrigerator for an hour to allow time for DNA purification.

Following the purification step, samples were centrifuged a second time to separate the DNA molecules from the supernatant. The liquid was drained, leaving a small pellet of DNA in the bottom of the Eppendorf Tube. The pellet was cleaned again with through an ethanol flush, either absolute or diluted to 75% solution. Deviating from the listed protocol, the solution was centrifuged a final time at 12000 rpm for 5 minutes before the ethanol was dumped and a suction machine was used to remove any lingering droplets.

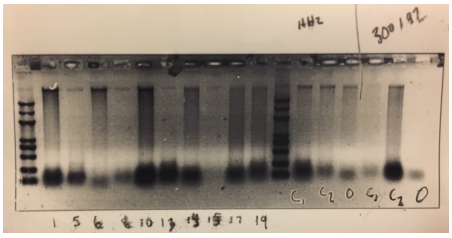
The tubes were then placed under the ventilated hood to hasten the drying of the DNA pellet. The pellet was then dissolved into water to create a solution of DNA which could be tested for concentration and purity.

Agarose Gel:

Agarose served as the perfect medium for experiments. Its gelatinous, porous structure is derived from galactose, a sugar indigestible for most bacteria and fungi. The medium was created by adding one gram of agarose powder to 110ml of TAE buffer solution and heating the beaker in a microwave until the powder completely dissolved. The new solution was then poured into an open tray to solidify.

Pockets were prepared in the gel by inserting templates into the liquid agarose. When the solution had solidified at room temperature, the templates were removed. The pockets would then house up to 10 µL of DNA which was blended with a coloring agent. The agent used in our lab was Ethidium Bromide, a fluorescent compound known to glow intensely under UV light when bound to DNA. (Liu, 2013)

The DNA-Ethidium Bromide mixture was then pipetted into the agarose gel for testing. A current ran through the gel from the tray's built in anode to its cathode, pulling with it the negatively charged DNA molecules. The porosity of the agarose granted the DNA molecules the ability to pass through the material, acting as a thick filter.



When electrophoresis was completed, the DNA was strung out into bands by varying concentration, gauged by the electronegativity of DNA segment sizes. Any material which did not advance under electrophoresis was regarded as impure waste materials.

Pool Samples:

Agarose gel tests were conducted until ten samples of pure, similarly concentrated DNA molecules were acquired. These samples were combined to make a pool to resemble the entire population for the PAGE gel process. DNA from the pool was placed next to DNA from each parent plant and a simulated F1 generation in a PCR template for amplification.

Polymerase Chain Reaction:

Polymerase Chain Reaction, or PCR, amplifies sections of DNA strands by facilitating natural regeneration. The process of regeneration is induced when a PCR machine cycles through ideal temperatures for denaturation, annealing and extension; effectively doubling the quantity of samples with each cycle. The simple procedure yields a colossal quantity of identical strands, providing an incredible advantage for labs which would otherwise have a limited quantity of genetic material for experimentation.

To prepare samples for PCR, a solution of DNA, MasterMix, (a purchased solution of free nucleotides and enzymes) and primers were added to a 96-well template tray. The tray was placed into a PCR machine where a replication program was selected. The program used in this experiment was a simple PCR program which heated the DNA to 95 degrees Celsius, dissolving the DNA's phosphodiester bonds and releasing the individual DNA strands.

The plate was temporarily cooled to 50 degrees Celsius to encourage the annealing of primers. As DNA only extends from the 5' to the 3' direction, forward and reverse primers were used simultaneously to rebuild the antiparallel strands of the original double helix. When primers had annealed to the DNA sequence, the samples were brought back up to 72 degrees Celsius for primer extension; where time was allotted for the DNA polymerase to rebuild and reattach the strands for the next cycle.

To restrict amplification to only a specific region of the DNA sequence, the time available for strand extension was reduced to 30 seconds. The reduction in time allowed the forward and backward primers to extend in the direction of each other on opposite strands. The area between the two markers was replicated exponentially, while not enough time was provided to replicate the entire sequence. When the PCR process was completed, samples were primary mapped in a PAGE gel.

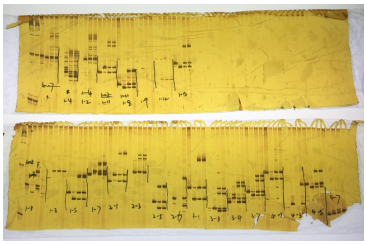
Bulked Segregation Analysis:

Bulked Segregation Analysis, or BSA, was a process of differentiating between mutated an unmutated chromosomes through Polyacrylamide Gel Electrophoresis, the lab's staple procedure for identifying and comparing INDEL mutations.

A solution of the reagents in the adjacent chart were prepared in a beaker and poured between two glass panes. Unlike agarose gel, which dries into gel form, PAGE gels form via chemical reaction. This reaction takes seconds and gives PAGE gels a much more homogenous porosity, providing a more accurate band reading.

Double Distilled Water	35 ml
40% Polyacrylamide	10ml
10x TBE Buffer	5ml
TEMED	50 µl
10% Ammonium Persulphate	500 µl

Gels were given time to solidify before being locked into place on a PAGE tray. DNA was pipetted into the gel for comparison and the tray was hooked up to a power source. Gels were charged for 70 minutes at 190V before being removed from the tray for cleaning.



When cleaning was complete, the gel was placed in a solution of water and silver nitrate ($AgNo_3$) to stain the DNA molecules. The gel was left to cure in solution for seven minutes on a rocking tray. When the timer expired the solution was dumped into a retaining so the process was repeated with a new solution of water, sodium hydroxide (NaOH) and formaldehyde to fix the silver stain.

When fixation was completed, the gel was wrapped in plastic to protect the researcher from DNA binding compounds. The gel was placed on a lighted table to illuminate the strands of DNA material which had sorted out by length between primer sections. The larger strand sections having a higher negative charge, reacted more dramatically to the electrical current, drawing them further down the gel than their shorter counterparts.

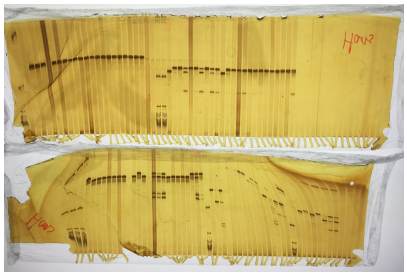
The PAGE gel was loaded so each parent was displayed separately and could be compared to the F1 and F2 populations. If the gel displayed no changes between the father and F2 population's DNA, it could be assumed that the primer amplified region was not the region responsible for a change in phenotype. If the F2 band demonstrated a similarity to the mother's band, the affected sections could have caused the stunted phenotype.

Polymorphism Mapping:

See Appendix I for Recombinants Chart.

Interpretation of the page gels showed that much of the F2 generation's genome was identical to that of the parent generation. This similarity was observed by the expression of double bands in the F2 column, meaning the amplified region demonstrated characteristics similar to both the mother and father plants. However, a region in the 10th chromosome exhibited a single band, showing resemblance to the mutant, dwarf mother plant.

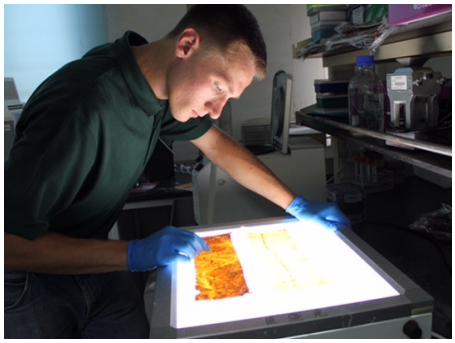
Upon discovery of mutations in the 10th chromosome, new tests were prepared to observe the genotype of each rice sample at eight separate regions in the chromosome. The regions were in series, representing the length of each section and the situation of INDELs. Each new PAGE gel compared the size of the wild type, mutant, F1 and each of the 22 F2 samples to determine to number of recombinants each section expressed.



Recombinants, also known as polymorphisms, are when some plants exhibit genotypes which are inconsistent with the predictions of a researcher and can be harnessed as a gauge to measure the distance to the center of a mutation. Gels which have completed electrophoresis are scored according the quantity and style of recombinants present. The scoring process made locating mutations simple by allotting 0 points to the expression of a single, mutant band, 1 point for the expression of two bands and 2 points for the expression of a single wild type band.

Gels with lower scores demonstrated less differentiation between samples at specific locations of the genome, meaning they were closer to the location of total mutation. After scoring many gels, an area between primers 10-2 and 10-8 was identified as having a long region with very few recombinants, demonstrating a nearly homogenous similarity to the mother parent's DNA.

Key Observations



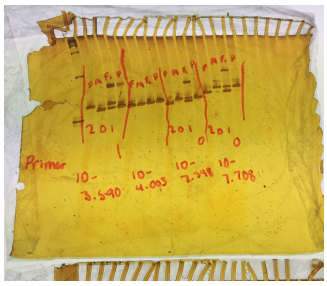
While testing PAGE gels for polymorphisms and sub-primers I began to notice an outlier sample. The sample number 9 was exhibiting an abnormally high number of recombinants when amplified by particular primers. This phenomenon led me to believe that DNA sample nine was misphenotyped. If mispheontyped, it may simply have been a genetically normal tillering plant exhibiting fewer tillers due to environmental or external factors rather than due to mutation. This theory was debunked after further testing primers when fewer recombinants occurred in adjacent areas of the genome, demonstrating that the plant was genetically similar but not identical to the other mutants

Results/Applications:

This particular project was to the introductory the introductory researcher a CAAS student completes at the beginning of their Masters or Ph. D degree. My research concluded at the end of my internship when I had successfully located the region containing the gene which coded for tiller development. My research was then adopted by a lab mate who would fine map the samples to narrow down the coding region even further and begin experimenting to find an optimal expression.

	Wild Type		Mutant	
Plant Number	**Plant Height**	**Number of Tillers**	**Plant Height**	**Number of Tillers**
1	67.2	12	59.5	3
2	71.0	16	50.2	4
3	83.2	10	50.0	4
4	87.0	14	62.6	5
5	87.0	15	60.6	4
6	80.2	15	43.3	3
7	79.1	11	51.3	3
8	83.8	16	32.0	2
9	75.5	12	38.1	2
10	94.4	13	52.5	4
11	78.1	11	45.2	3
12	79.6	9	53.5	2
13	82.3	14	41.3	3
14	79.0	23	53.1	3
15	79.8	15	45.4	2
16	65.0	11	51.0	2
17	81.1	20	60.2	2
18	75.2	11	49.8	2
19	78.2	14	40.0	2
20	77.3	16	55.2	2
Average	79.2	13.9	49.74	2.85

Gene function research has the capacity to change the outlook of agriculture in the developing world. Using optimal gene expression to improve the yield of rice crops will help to bring financial stability to farmers across the country, slowing the encroachment of corporate agriculture by enticing more young people to remain in the agricultural industry. Furthermore, over half of the world's population depends on rice consumption as a primary source for calories and another fifth depends on the crop for income (IRINNews, 2015). Even the slightest improvements in the strength of available rice varieties, or the quality/composition of grain produced will have colossal impacts on rice consumers across the globe.



Field Work/External Projects

In addition to my lab research I worked alongside my lab mates to prepare trial plots for experimental crops. These trial plots were used to observe phenotypic changes and to collect seeds to expand research populations. Varieties were planted in a field to the north east of Beijing, segregated from commercial production to prevent the spread of GM pollen.

Growing Rice: Rice is grown in marshy, irrigated plots. The soil north of Beijing was mostly a dry, sandy clay which displayed muck-like qualities when plots were flooded. Plants were bundled by variety and started in dense plots to consolidate nutrient consumption before being transplanted by hand to more fertile plots to begin their reproductive stages.

When rice is transplanted it is critical to remove all soil from the roots to ensure proper function in its new habitat. Samples were plucked and planted at a distance of one half meter from each other in straight rows guided by twine. Plants were allowed time to grow before data and samples were collected for use in lab research.

With the exception of tillage, our lab mimicked farmers by using entirely hand labor. Planting, transplanting, fertilizing and extractions were completed by students or one of the employees of the academy farm. When rice fields were harvested they were drained and seeded to wheat over the winter to maximize plot efficiency.

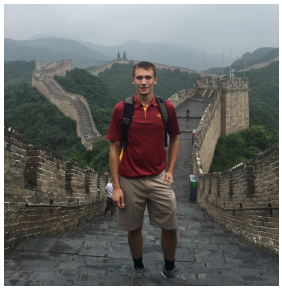
Shandong Province: In order to grow and test long season rice varieties, CAAS partners with the Shandong Rice Research Institute in the city of Jining. This research center, located roughly 375 miles south of Beijing, sits in one of China's most productive agricultural region. In exchange for plots to grow samples in, Dr. Li, Lu-Lee and I traveled to the Jining facility to assist with their planting season. While at this facility I had to opportunity to toil alongside China's working class, living out the life CAAS strives to improve.

Cultural Experience

I was blessed with the opportunity to experience many cultures during my stay in Beijing. Coming from a small, quiet town in Northeastern Iowa, acclimating to a foreign culture was difficult at times, but allowed me to objectively analyze my surroundings and my home environment alike. I quickly picked up on segments of the language which would guide prove vital.

Through my time in Beijing, I learned to admire the grit and determination of the nation's people who work diligently to improve their lives and those of their families. There is a great divide between the culture of urban and rural China. With the influx of workers from the countryside filling unskilled jobs, there is a larger opportunity for skilled workers to move up in the social ladder. The opening of higher paying jobs has brought wealth to the streets of the nation's capital as it seeks to renew its perception in the eyes of the world. These new jobs have led to a massive wage gaps between citizens.

On the contrary, people from the rural areas of China exhibit the meaning of perseverance. Many come from economically unstable backgrounds and have grown up sharing their resources with those around them. My interactions with rural Chinese laborers reiterated the importance of collaboration and to tend to the needs of others. They displayed incredible humility while working long into their lives to provide for loved ones and create better futures for their children.



Hidden among Beijing's colossal buildings and modern infrastructure are fragments of a very rich history. During short breaks from lab work I was able to explore historical sites and learn about the nation's extensive past. I was able visit The Great Wall, Confucius' Temple, The Forbidden City and many other temples, museums, and national symbols during my stay. In addition, I was able to experience life in many parts of country and observe cultural nuances while working in conjunction with other institutes.

Dorm culture:

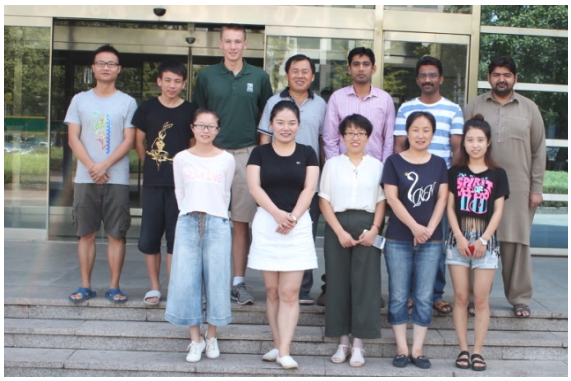
This summer, my dorm mates became a family I could depend on and trust in any situation. I lived in a dorm of international students pursuing their Masters or Ph. D degrees and was roomed with the only other person from the Americas, vet student named Juan from Uruguay. The majority of students in the dorm came from Central and West African countries or Subcontinent Asia. A friend of mine in invited me to attend church with fellow dorm mates and to join the dorm soccer and basketball leagues.

The large population from India and Pakistan were incredibly inviting. When I arrived, the majority of students from Subcontinent Asia were Muslims approaching the end of Ramadan, a holiday where they would fast during daylight hours for 30 days. My dorm/lab mates Adeel and Uzair invited me to a meal to get to know each other better. Living with people from people of so many backgrounds, beliefs and ethnicities has greatly increased my appreciation for people and ideas around the globe.

Lab Culture and Experience

My lab was the perfect posterchild of workplace diversity. Amongst our members were Christians, Atheists, Hindus, Buddhists and Muslims. Researchers came from all across China and Subcontinent Asia, transcending all differences, to pursue the common goal of achieving an impact on food security while pursuing education.

Coming from a production agricultural background, I was very honored to have the opportunity to work in Dr. Li's lab. Spending this summer in the lab augmented my understanding and appreciation for the rigorous studies and procedures develop the crop varieties used on farms around the world, including my own family farm. Spending time learning how to identify genes and modify plants has opened my eyes to a field of opportunities I had never considered before.



Being new to the lab environment, it took me a while to become adjusted to the work schedule. Between the jet lag and being on the eastern side of China's only time zone, I found myself waking up around 4:30 AM for the first week. To fill the time before my lab mates arrives, I took walks around campus and began familiarizing myself with equipment. As the internship continued, I finally adjusted to a normal schedule; some nights even working until midnight without realizing the amount of time which had passed.

As I got to know my lab mates better, I learned incredible things about their backstories and ambitions. Being a novice, they guided me through me through complex procedures and taught me the principles used in the lab which are imperative to become successful. Outside of work they taught me so much about parts of the world I knew so little about. I began to see the skills and perspectives each person brought to the table and how they utilized them to make the work environment incredibly efficient and cohesive. I am thankful for irreplaceable cultural experiences I had and the priceless lessons I learned throughout the course of the internship.

I am thankful for my lab mates who treated me as their equal. My lab mates have inspired me to study agriculture science in addition to business this year at Iowa State University and have cultivated a respect for the work required to develop new agricultural technologies.

Personal Growth and Development/World Food Prize Story

The sun's scalding rays beat down on the backs of our necks as Lu-Lee and I strolled through an alleyway in Qufu. We had planned to cut across an industrialized suburb in an attempt to avoid the tourist traffic on the main roads, unaware of what lay ahead. What seemed to be an ordinary moment would mark the beginning of an incredible journey.

Down the lane ahead of us, we noticed people had stopped walking. On all sides, people who were more phantom-like than human with ghastly eyes and gaunt faces, were taking in a momentary distraction from their simple, yet demanding, lives. Mesmerized by my surroundings, I nearly stumbled over a crowd of older men sitting around a table. The men

immediately hunched like rabbits over their game of cards and pause to watch me. I gazed out over them, trying my hardest to blend back in and avoid causing a commotion. As this scene unfolded, it suddenly became became very clear. The stories I had heard for so long had now become my own. No longer was I just a student - I was a hunger fighter.

Looking back, it was February of my Junior year in high school when my Agricultural Education Advisor, Mrs. Dillon, set the Iowa Youth Institute application on my desk. My eyes glanced over the sheet briefly as I raced to put together a valid excuse to get out of writing a research paper. Game plan in place, I looked up and began to explain that my time was consumed between sports and my family farming operation. Her signature, enthusiastic grin refuted my argument and she baited me saying, “Hans, I’ve never found an opportunity more tailored to you.”

I got to work the next day on conducting research over developing an affordable agricultural education system for the citizens of Haiti. Through my research, I realized the need for quality agricultural and basic education to help promote farming practices which preserved the island’s fragile soil. Delving deeper into my research, I began to realize my passion for making a difference in the lives of others.

Weeks flew felt like seconds until the Iowa Youth Institute was right around the corner. At the conference, I presented my research and had the opportunity to network with other people who shared my interest in food security. Later, I was informed I would advance on to the Global Youth Institute in October, I was absolutely ecstatic to continue my journey in the organization. I put time in over the summer to revise my paper while I worked on several farms across the United State to grow in my understanding of domestic agriculture’s global impacts.

I presented my updated research at the Global Youth Institute and gained a better understanding of the hunger crisis. At the final meal of the conference, I noticed a man sitting alone at a table and asked to take a seat next to him. We made small talk for a few minutes before he revealed that he was Dr. Gurdev Khush of India, the 1996 World Food Prize Laureate. He explained to me how he and Henry Beachell bred short-stemmed rice with excellent disease resistance and yield, which they distributed across southeast Asia. In awe, I listened to his incredible story until he broke my gaze with a question that has forever remained with me, “Now, what will you do to make a difference?”

As we packed up to leave the institute, Mrs. Dillion once again pulled me aside and said, “Hans, I found the next thing.” Fully trusting her, I followed her to a lecture hall where the 2016 Borlaug-Ruan interns were presenting about their research internships from the prior summer. Astonished by the caliber of the interns’ presentations, I sat silently in the audience; contemplating applying for a position. When the final Intern finished at the podium he pulled a card from the prior night’s hunger banquet from his pocket and read it aloud to. The card read about a young boy from India, and that he had met that exact boy during his internship.

Touched by the incredible work of those who walked before me, I applied for the Borlaug-Ruan internship. A few months later I received word that I had been accepted and assigned to the Chinese Academy of Agricultural Sciences in Beijing. Thrilled to finally have the chance to use my life as a vessel to improve the lives of others, I immediately began researching the nation and language, looking for any way to get a head start on my journey.

Upon my arrival to Beijing I was impressed by the city's modern infrastructure and efficient transportation system. I was introduced to my lab and dorm mates and invited to partake in many unique cultural observances. My lab mates also helped me find places to purchase food, clothing and supplies to supplement the materials I had brought from home.

Early on, I had extra time in the evenings to become accustomed to the neighborhood and get involved in dorm activities. As we got further into our research I became increasingly involved in the lab so much that I often found myself working late into the night. On occasion my lab mates had to remind me to go back to the dorm to rest when I had become completely engrossed in experiments.

Prior to my experience in the lab, I was skeptical of the acclaimed impact reported from genetic function research and optimization. Despite having read claims of the incredible breakthrough and discoveries CAAS had made recent in years, I craved a tangible, personal project where results could be seen at the end of the day. However, my placement in molecular biology proved to be an incredible blessing. My time spent in the lab has forever shaped my perspective of science in the role of achieving global food security.

Outside of the lab, I became a far more independent and self-starting person. My submersion into a foreign culture endowed me with incredible insight into the lives of people around the world, and a new perspective on the life I had always lived. Furthermore, having the ability to work in the fields alongside members of China's working-class cultivated a new appreciation for the simple things in life we take for granted. Hearing their stories and experiencing their lives has influenced me to work diligently to bring agricultural advancements to their communities.

Today, standing amongst the workers in the street, I realized that it was now my mission to harness the summer's experiences as a tool to catalyze a positive impact on their lives. I no longer felt powerless in the battle for food security and knew that had it not been for this internship I would not have developed a new appreciation for the field of agricultural research, witnessed the need for global agricultural cohesion in its truest form, or have found my role in putting an end to world hunger.

Appendix I

Polymorphisms Expressed at Each Primer Section

Plant Number:	Primer 10-1	Primer 10-2	Primer 10-3.590	Primer 10-7.348	Primer 10-7.708	Primer 10-4	Primer 10-5	Primer 10-6	Primer 10-7	Primer 10-8
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	1	0	1	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	1	0
5	0	0	0	0	0	0	1	0	0	1
6	1	0	1	0	0	0	0	0	0	0
7	1	0	0	0	0	0	1	X	0	0
8	1	2	0	0	0	0	1	0	1	0
9	0	0	0	1	1	2	2	2	0	X
10	0	0	0	0	0	0	1	X	0	0
11	0	0	0	0	0	0	0	1	1	1
12	0	0	0	0	0	0	0	1	0	0
13	0	1	0	0	0	0	0	1	1	1
14	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	1	1
16	0	0	0	0	0	0	0	0	2	1
17	0	0	0	0	0	0	0	0	2	2
18	0	0	0	0	0	0	0	0	2	2
19	0	0	0	0	0	0	0	0	2	2
20	0	0	0	0	0	0	0	2	0	2
21	0	0	0	0	0	0	0	1	2	0
22	0	0	0	0	0	0	1	1	2	1
Recombinants Total:	**3**	**3**	**1**	**1**	**1**	**2**	**8**	**9**	**18**	**14**
Plant Number	Primer 10-1	Primer 10-2	Primer 10-3.590	Primer 10-7.348	Primer 10-7.708	Primer 10-4	Primer 10-5	Primer 10-6	Primer 10-7	Primer 10-8

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