My Chinese Internship

By Divindy Grant

The seventeen-year-old girl named Divindy Grant who arrived in Beijing was tired, complacent about life, and scared to death. She was scared for many reasons, the first being that when she had landed it had finally hit her that she was alone, didn't speak the language, and had no clue whom she was supposed to meet. She was also afraid that the work would be tedious and boring. On a subtler level, she was wondering if she would like the country of her birth and of her grandfathers, and if it would welcome her back after seventeen years. She was scared of the unknown and the uncontrollable.

That Divindy Grant seems so far away and so different from the person who I am today. That is because last summer I had the amazing opportunity to be the World Food Prize Ruan Borlaug Summer Intern to the Chinese Academy of Agricultural Science in Beijing, China.

I have strong family connections to China. My great-great Grandfather went there as a medical missionary, my great-grandfather was born and raised in China and helped start a hospital and medical school there; my grandfather was born in that hospital and lived in China until it was closed to the west. My parents came to China in the mid-1980's because my father was awarded a Fulbright Lectureship to teach in Beijing. I then was born there in the same room as my grandfather.

We left Beijing when I was six months old to live in the small town of Fairfield in the Southeastern corner of Iowa. Except for the Chinese relics and paintings we have in our house, and the occasional meal at a Chinese restaurant, China became a million miles away. I was comfortable growing up and taking part in small-town Iowa life.

During my Sophmore year (2002-03) I attended the World Food Prize Foundation's Youth Institute, and my eyes began to open. Not until I did the research for my paper and then heard the symposium speakers, did I truly realize how large and how real a problem food and water security really is. Previously, I knew that food and water problems existed, but they were always in far away places concerning people I did not know. Hearing about the problems and solutions currently in progress by brilliant people expanded my thinking and awareness. I knew then that I had to become a Ruan Borlaug Summer Intern, and I wanted to start right away working to help alleviate some of the world's food security problems. Unfortunately, I was too young to apply for an internship that summer so I had to wait.

The summer of 2003, I had what for most people would be a dream summer. I had the opportunity to go to Germany and live with a family there and to travel around with a friend and explore Europe. It was an amazing experience to see all the different countries and cultures. But at the end of the summer, I was somehow dissatisfied. After much reflection I realized that my summer was meaningless and

frivolous in the larger picture. I knew that the following summer I had to do something meaningful. I knew that being a WFPFRBSI would be very meaningful and allow me to do something to help the problem of food security.

Therefore I was delighted when I was selected to be a 2004 intern, and pleased that it would be at the Chinese Academy of Agricultural Sciences (CAAS) in Beijing, China. CAAS is a top ranked, national level research organization and graduate school. It is comprised of a total of 39 research institutes located throughout China, with fifteen of these institutes located in Beijing on a main campus. The institutes deal with plant research, animal husbandry, agricultural economy, environmental studies, agricultural engineering, and new agricultural technology. CAAS has more than 9,000 faculty members and of those, over 6,000 are researchers. CAAS has two key state laboratories, twenty key ministerial laboratories, six state crop improvement centers, twenty-seven quality supervision and test centers, one national crop gene bank, eleven national nurseries of crop germplasms, and 103,000 mu of experimental agriculture and livestock farms.

CAAS's priority is crop research. It has recently refocused its strategy, adjusted its priority, and reformed its mechanics so that it can take in-country agricultural leadership. Its missions are: to be a world advanced institution for innovative science and technology and to be an advanced national incubator of new agricultural technologies.

During my stay in at CAAS, I had the opportunity to work in two different institutes. For my first month I worked in the Soil and Fertilizer Institute. Halfway through my internship, I was transferred to the Institute of Agricultural Biotechnology because the Soil and Fertilizer Institute had finished for the summer all the projects with which I was allowed to help.

In the Soil and Fertilizer Institute's lab I worked under the instruction of Ms. Zhang Wei Li. I worked more directly under Zhang Ren Lian. I also worked with three lab workers Liu Min, Zhang Wei Qing, and Li Guo Xia who have all graduated from Hebei Provence and come to Beijing to work. My wonderful translator was Lin Qinghui. She comes from the Shandong Provence. While I was at CAAS, she graduated with a master's degree in Soil Nutrition.

I worked on Ms. Zhang Ren Lian's doctoral research project. The purpose of this experiment was to help determine whether more Nitrogen and Phosphorus non-point source pollution occurred by water leaching from the depths of the soil or by surface water runoff. This experiment was a preliminary lab test, designed to simulate conditions in the highly polluted Tai Hu region. The results will be used to help pinpoint areas that should be tested in the field. By determining how the most pollution is reaching the water, methods can then be implemented to reduce pollution and farmers can be educated about how to fertilize their crops with the least polluting

effects on the environment.

My project had three hypotheses that were designed by Ms. Zhang Ren Lian. They were:

1. The Nitrogen and Phosphorus content will be higher in leached water than in runoff water.
2. Both the fast test method and the standard method will have similar trends with regards to the concentration of Nitrate.
3. The concentration of Nitrogen and Phosphorus in the water samples will be less in samples taken after more time has passed.

Leaching is a process by which chemicals in the soil (especially the plant nutrient nitrogen) are moved out of the soil by water. Because of the depth the water must penetrate, it usually takes time from when the rain begins until the water flows out. Water runoff is water that runs off the surface of the land, and flows into waterways. It usually starts happening as soon as the rain starts, and ends soon after the rain ends.

We were looking at the run off and absorbance levels of five different chemical nutrient areas. They are Olsen-P, Total Phosphorous, Nitrate (NO3), Ammonium (NH4), and Total Nitrogen. These were all tested using the standard test method, and then the Nitrate level was also measured using the fast test method.

A comparison between the fast and standard test methods was done because we were curious about the correlation between the two methods. The fast test method is the one used while working in the field, and the standard method is the more accurate, but more time consuming lab method. Because the fast test method is used so much in the field, we wanted to know what the correlation between the two methods was so that we could get an idea about how accurate the results we got from the fields were. The fast test method measures the Nitrate count by using the test strips of a Nitrate test kit. For this experiment, Merck's Nitrat-Test strips were used. The standard method is measured by using a spectrophotometer to measure the absorbance of light by the different water samples taken during the experiment. First controls were needed. These were formed by adding different amounts of whatever the chemical was being tested to pure water. Then the absorbance was found for the controls. Then the samples were tested and their absorbance was found. The absorbance of the samples was compared with those of the controls, and their chemical content was able to be deduced. We had to test for one chemical nutrient at a time. We did this by adding certain chemicals to the samples, depending on what we wanted to test for to isolate a specific chemical nutrient.

To perform this experiment, soil was gathered from Shun Yi suburb of Beijing because the soil was very similar to that of the Tai Hu region, and was easily available and accessible. The soil was then placed in three 60cm x 30cm x 30cm boxes each with a soil weight of 32.7 Kg. Fertilizer was then added to two of the boxes so that we now had three different nutrient levels of soil. Nitrogen was added to the soil in

the form of KNO3 fertilizer, and the Phosphorus was added using KH2PO4. (See table 1)

Table 1

	Olsen-P	NO3	NH4
Box 1 N1P1 (Grain Nutrient Content)	30mg/kg	53.8mg/kg	3mg/kg
Box 2 N2P2 (Mid range nutrient content)	80mg/kg	100mg/kg	3mg/kg
Box 3 N3P3 (Vegetable Nutrient Content)	160mg/kg	200mg/kg	3mg/kg

Two simulated rainfalls were performed over the boxes. Each rainfall had a strength of 80mm/ hour. The first rainfall had a 120mm accumulation and the second rainfall had 6o mm of accumulation. Using glass tubes that were attached to holes in the sides of the boxes, the runoff water could easily be channeled into storage containers. Samples were taken initially at five-minute intervals, but then for the leaching only, after twenty minutes in ten-minute intervals.

My job was basically that of a lab assistant/ worker. I filled test tubes with water samples, mixed chemicals, measured the absorbance on the spectrophotometer, took the fast Nitrate test, washed the dirty test tubes, ground and weighed soil for soil tests, and was the lab's official English teacher. Whenever we would have a long rest while someone was testing the absorbance of the already made samples, we would have language lessons. The girls would teach me Chinese, and I would teach the girls English and tell them about America.

Results

Due to publication rights of the data, I am only allowed to share and specifically analyze the N1P1 Nitrate data in this paper. But in general Hypothesis 1 and 3 were found to be true in almost all cases, and hypothesis 2 was found to be true if the concentration of the Nitrate in the sample was high, but not if the Nitrate concentration was low.

In Table 2, the accumulated amount of Nitrate content is much higher in leached water than in runoff water. With the fast test method the leached waters Nitrate content was 55 times greater and for the standard method the leached water's Nitrate content was 144 times greater. This is one set of data that shows Hypothesis 1 is correct.

Table 2-Total Nitrate in Cumulation of Water Samples

	Fast Test	Standard Method
N1P1 Leached	616.03 mg	1017.50 mg
N1P1 Runoff	11.23 mg	7.05 mg

The data comparing Nitrate Concentration of the Leaching process and Runoff process verses time showed that the general trend was that as more time passed, up

until the time of the next rainfall, the concentration decreased. For water leaching this was the case besides for a few exceptions. The most dramatic being between minutes 959-999, but even the amount that it increased to was still less than half of the starting value. For the second rainfall standard test method, the trend is that it decreases, with only a few minor fluctuations. For runoff water, the data has a general decreasing trend except for the standard method's time between minutes 95 and 100. The rest of the data has only very slight upward fluctuations.

For the comparison of the fast test method to the standard method, the data was directly related. The nitrate concentrations were found to increase and decrease at the same points.

The results of this research will lead to field studies in the Tai Hu region. There will be a greater emphasis on water leaching studies, especially immediately after the rain begins to fall. The people who work in the Soil and Fertilizer Institute and their comrades will also now feel more comfortable about using the fast test method in the field.

Application of Results to the Tai Hu Region

The Tai Hu Lake Basin is located on the Yangtze Delta in southeastern China in the provinces of Jiangsu, Zheijing, Fujian, and Anhui, as well as Shanghai City. This region is home to around thirty-five million people and comprises an area of 36,500km² with 2.66 million hectacres of arable land. It is comprised of Tai Hu Lake, many other large and small lakes, and many slow flowing rivers and canals. The total watercourse length is 120,000 km, covering an area of 5551 km². The average rainfall is between 1000-1500mm per year.

The lake and its catchment area serve many purposes such as the storage of floodwater, irrigation, shipping, drinking water supply, fish farming, and tourism. It is a major drinking water source for the municipalities of Wuxi, Suzhou, and indirectly Shanghai, as well as for the many villages and towns in the regions.

The Tai Hu region produces over ten percent of China's national agricultural output value, and the lake produces 25% of China's total freshwater fish harvest. The region annually produces 11.5 millions tons of grain, 125,000 tons of cotton, 290,000 tons of rapeseed, 60,000 tons of fruit, along with producing many other crops. The land in the Tai Hu region has been used for paddy planting for over 6000 years. The cropping system usually involves two or more different grains being grown on the same land each year. The most common combination is rice-wheat. Because of this multiple cropping method, lots of fertilizers are used to help ensure maximum yield of each crop.

Rapid industrial and agricultural developments, as well as excessive population growth during the last twenty years, have resulted in a massive increase in pollutants being discharged into the lake. In the period from 1982-1990 the Nitrate content in the lakes has more than doubled, and still continues to rise. It has reached a level of .25mg/L which is 2.5 times higher than China's allowable level.

The high nutrient influx, especially of Nitrogen and Phosphorus, causes water ultrophication. Water ultrophication is usually concentrated in closed or semi-closed lakes, and slow flowing rivers (less than 1 m/ min). The resulting ultrophication is algae bloom, especially of the blue and green variety. When the algae die, the decomposition uses up much of the precious oxygen in the water, which can lead to the suffocation of aquatic life. It is very similar to eutrophication, but in the Tai Hu region it has a special name of ultrophication, and this is very damaging to the fishing industries.

Ultrophication is also dangerous to people's health. Blue-green algae produces toxins, especially the phycotoxins Microcystin and Nordularin, which have been associated with liver and other types of cancer. The risk of liver cancer associated with drinking water contaminated by algae is two times greater than drinking unpolluted water.

Thus the water pollution is severely affecting both the fishing industries and the safety of millions of people's drinking water.

The work that I did was very important because it was one of the first steps needed to be able to successful reduce the Nitrogen and Phosphorus inflow into the waters of the Tai Hu region. Not only will this make the drinking water safer for people, but it will also help keep the fish alive. By keeping the fish alive, fish farmers can stay independent and feed their families. If the fishing in the Tai Hu lake and its catchments are destroyed, then all of those fishermen will become part of the hunger-poverty cycle that the World Food Prize and many other organizations are trying to end.

A Change of Tasks

Halfway through my internship, CAAS and the WFPF co-hosted a conference honoring Dr. Yuan Longping, the 2004 World Food Prize Laureate. At this conference, I was able to hear inspiring talks from world experts about how far the world's and China's food security has come, but was also reminded how far there is still to go. Hearing the Millennium Development Goals for the world, and then learning about how well China is doing in meeting these goals was somewhat comforting. However, I also realized how far there still is to go to reach global food security.

At the time of the conference, we had finished the lab work part of the non-point source pollution experiement, and I was doing data analysis on the computer all day. I wanted to do something else meaningful my last half of my visit here. With the help of Ambassador Quinn and Dr. Borlaug , I was introduced to Dr.Fan, the head of the Agricultural Biotechnology Institute. She warmly invited me to work with her, and after a few days, I was transferred to one of her labs.

I was placed in Dr. Zhou's lab. His lab focused on plant resistance to abiotic stresses ie: salinity, drought, hot temperatures, and cold temperatures. The current focus of the lab is on the ABp9 gene.

ABp9 is a gene from maize, which acts as transcription factor for anti-oxidant genes in plants. While it is from maize, they are analyzing it for the use in other crops too, such as rice. It was initially identified through the following process: When plants are subject to abiotic stresses they produce Reactive Oxygen Species such as Hydrogen Peroxide, Superoxide, Hydroxyl Radical, and Single Oxygen. It was found that the gene Catalase 1 (Cat 1) can digest H2O2, one of things that is necessary for a plant to resist abiotic stresses, because if the amount of H2O2 in the plant becomes too high, it will kill the plant. Cat 1's CIS element, a fragment of DNA which regulates promoter activity in result from environmental cues, was found to be ABRE. Using ABRE in the yeast one-hybrid system, it was found that ABp9 is the transcription factor for Cat 1. During previous research, my lab found that ABp9 is not only the transcription factor for Cat 1, but also for many other anti-oxidant genes.

Gene expression is influenced by environmental or developmental cues. The mechanics of how abiotic-stess-resistor gene expression is regulated must be dissected in order to modify the plants in a way that they will have improved tolerance to abiotic stresses. In order to genetically engineer the plant, the critical genes which enhance the plants response to stresses are needed. To get these critical genes, the whole signaling pathway must be dissected.

When the plant is under stress, the stress signal is received by a receptor in the cell membrane. It then transmits the signal through a chain of proteins until it reaches the transcription factor protein, which then activates the CIS element, which then activates the gene needed to combat the stress. If the promoter is ABp9, then one of the genes activated will be Cat 1, which will produce a protein that digests H2O2.

This is where the project I worked on comes in. I worked with a PhD Student named Wang Hanqian on his doctoral project. His project is focused on using the yeast two-hybrid system with ABp9 as bait in order to indentify the proteins involved in the signaling pathway. This project had no hypothesis because there has been no previous research with this field, so there was no evidence on which to base a

hypothesis.

The yeast two-hybrid system is a molecular genetic tool which facilitates the study of protein-protein interaction. The protein that is already had is called the bait, and is fused to a CIS-element. A protein with a matching activation domain will come to interact with the protein. If the proteins interact, then a reporter gene such as His 3 is transcriptionally activated. This process was then used to identify proteins (prey) which interact with the ABp9 gene (bait).

This project was started in March of 2004, so Wang Hanqian had already done much work before I joined him. He had already made a reporter construct (bait construct) through the fusion of proteins, and then transformed it into yeast (Reporter Yeast). He used the reporter yeast to screen a cDNA library and get a positive yeast clone. He extracted the DNA from the positive yeast clone and inserted the DNA into E. Coli bacteria in order to get a transformed E. Coli positive clone.

While I was working with Wang Hanqian, what we were doing was extracting the DNA from E. Coli and doing single or double digestion using restriction enzymes. After digestion we tried to classify the positive clones into groups based on digestion patterns. A pattern is based on the number of DNA fragments that are produced and the fragment size.

My role was to extract the DNA in the form of plasmid from the E. Coli, and then prepare the plasmid DNA by Alkaline Lysis with SDS for Digestion. The protocol for the plasmid preparation is as follows:

Preparation of Cells

1. Inoculate 2 ml of a rich medium (LB) containing appropriate antibiotic with a single colony of transformed bacteria. Incubate overnight at 37 degrees Celsius with vigorous shaking.
2. Pour 1.5 ml of culture into a microfuge tube. Centrifuge at 10,000 rpm for 1 minute.

Lysis of Cells

3. When centrifuging is complete, pour out medium and invert tubes on paper towel to get the bacterial pelet as dry as possible.
4. Resuspend the bacterial pellet in 100µl of Alkaline Lysis Solution I by vigorous vortexing for 30 seconds.
5. Add 200µl of freshly prepared Alkaline Lysis Solution II to each bacterial suspension. Close the tube, and mix the contents by slowly inverting six times. Store tubes on ice for 3-5 minutes.
6. Add 150 µl of ice cold Alkaline Lysis Solution III. Close the tube and disperse solution III through the viscous bacterial lysate by slowly inverting 6 times. Store on ice until centrifugation.

7. Centrifuge at 10,000 rpm4 D C for 10 minutes.

Recovery of Plasmid DNA

8. Pour supernatant into fresh tube.
9. Add 880 µl of Ethanol A. R. 95 %. Invert tube several times.
10. Centrifuge for 5 minutes 10,000 rpm 4 D C. This collects the precipitated nucleic acids.
11. Pour out supernatant and invert tube on a paper towel until all the fluid has drained away.
12. Add 500 µl 70% ethanol to the pellet and invert the closed tube several times. Recover the DNA by centrifuging the tubes for 5 minutes at 10,000 rpm 4 D C.
13. Repeat step 11 except allow tubes to sit until all the ethanol has evaporated.
14. Add 30 µl of Doubly Distilled H20.

Use electrophorisis to check that the samples have the Plasmid DNA or the desired construct.

ABp9 is a self-activating gene. This means that it contains both a DNA Binding Domain (DB) and a Transcription Activation Domain (AD). To ensure that when we were using the yeast two-hybrid system that it wasn't just the ABp9 activating the reporter gene, the ABp9 gene was split up into ten different constructs. By doing this, it is possible to determine where in the gene the DB and AD are located. Wang Hanqian found that the DB is located at the C-terminal and the AD is located at the N-terminal.

Wang Hanqian made an initial batch of five constructs which were focused on the DB end. While I was there, we started working on making the cloning fragments to make the bait constructs to test the second five constructs. These were all from the N-Terminal because we were trying to determine exactly where in the gene the AD is located. Also, because it is the whole ABp9 gene that interacts with the proteins, it wouldn't give all the proteins that interact with it if only the proteins that interact with the DB end were found. While I was in the lab, we were working on cloning the constructs of ABp9-5, ABp9-8, and ABp9-9.

The general order for cloning a fragment is as follows: A vector is digested with a restriction enzyme and a PCR fragment of DNA, which has also been digested with that same or another compatible restriction enzyme. After determining which of the fragments is the right one, that fragment is ligated into the vector. The vector will have some kind of select marker gene, usually one that carries the resistance to some antibody is then transformed into E. Coli Bacteria. The transformed E. Coli is then plated on a plate which contains either Ampicillin, Kanamycin, or both. Only the bacteria which grows on the plates is the properly transformed E.coli. That E. Coli

to clone itself many times. To check that it is the right fragment, a DNA extraction is done, and that DNA is then digested with the original restriction enzyme. An Agarose gel electrophorisis is then done. If a fragment turns up the correct size, DNA sequencing is done it to check if it is the right fragment. If the DNA sequencing shows that it is in fact the correct fragment, these clones will then be used to construct the reporter bait genes needed for the yeast two-hybrid system.

Wang Hanqian was incredibly trusting and patient with me, and allowed me to do almost everything that he did on his experiment. I had had almost no prior lab experience, and all the equipment and methods were new to me. He would teach me how to do something and as soon as I could do it reasonably well he would allow me to do it by myself, and when I did something wrong, he would just tell me that it was just practice, and didn't matter. After a week of lab work, I was allowed to prepare the plasmid alone.

Improving plants' resistance to abiotic stresses is extremely important. If drought tolerant plants can be grown in twenty percent of the world's lands which are normally too dry for crop production, there will be a gain of 1040 Mha. Similarly, if Cold tolerant plants are grown in twenty percent of the land which is normally two cold, there will be a gain of 740 Mha. If salt tolerant plants are grown in forty percent of the high salinity lands, then there will be a gain of 2,380 Mha.

As the world's population continues to grow, it will no longer be possible to continue to feed all the world's people on the current agricultural land. By increasing plants resistance to abiotic stresses, it will be possible to increase the yields on lands that previously had low yields due to the effects of abiotic stresses.

Learning about Life

Besides eating almost every meal with chopsticks for eight weeks, I noticed during my stay, many cultural differences between China and Iowa. In China, people will work wherever they can get jobs, even if it is many hours by train from their immediate family. Because of this distance from loved ones, they will create families out of the people they are with wherever they go. They call all their graduate level classmates brother and sister, and when they eat, it is a group experience. Everyone gets dishes for the group, and then using their chopsticks they just eat anybody's food from anybody's tray. Once it is on the table, anybody can have it, no matter who originally ordered it.

One thing that has greatly impressed me about the Chinese people is their commitment to work. People who were just one or two years older than me were happy to work seven days a week, twelve hours a day with only a ten minute break for lunch. They were just so happy to have a job. In the States people want jobs and nobody wants to be unemployed, but in China the people put forth such effort. They will split up their families. Some husbands and wives live hours apart and only see

each other a few times a year. People will do anything for a job. The Chinese people I have met are all hard-working and are very proud of any job that they possess. There is no bad job, and while some jobs may be considered more desirable than others, any job is worthy of respect.

One of the greatest changes I noticed in myself was the speed and quantity in which I progressed through my day. During my first few days there, when I would walk down the sidewalk, I would pass everyone just at my normal walking pace. Because I was so busy back home, I was moving at a pace that is hyper speed in China. Everyone there strolls after dinner, the men with their arms held behind their backs, walking the proper conservative distance from their friend or wife. I found that in China our hyper activity level is simply not possible; there was no way I could continue to move at that pace. I slowly learned how to live at a more relaxed pace, that I hope I will be able to continue once I get home.

In China, I learned that while words matter, they really don't. In the first lab in which I worked, two of the lab workers didn't speak English. But still we became close friends because we found the same instances funny or aggravating and spent hours every day together. We had friendship based on shared experiences.

One of things that took me the longest to get used to was the women and the role of women in their relationship to men. The women are very conservative. All the clothing is very modest by American standards. The women are active with their work, but most are not fond of extra exercise. In China, I noticed that most men and women are considered to be intellectual equals, but in terms of anything physical, there was no comparison between men and women. Men would carry all the bags, and do the physical labor. In China, I also noticed that men and women did not publicly show their affection for one another; everyone kept their distance.

Every evening I played basketball with a group of male students and workers. This took some of them a while to get used to because not only were they physically competing with a female, but basketball is a semi-contact sport, so they had to make contact with a girl. I think they finally decided that since I was American, it was okay. The guys who I played basketball with became some of my closest friends and made many of my favorite memories.

After my summer experience in China, I am a different person. In China, my thirst for learning and knowledge has been renewed. In my free time in China, I found myself wanting to read biochemistry textbooks and learn all about DNA. If before I left, someone would have told me I would do this, I would have laughed in disbelief. I now want to learn about everything.

For the first time, I now really realize how fortunate I am compared to so many millions of people in the world. I realize that I am wasting my opportunities if I don't

use them to my full advantage every day. One of my goals for the future now is to take full advantage of the wonderful opportunities that I encounter everyday and to learn and grow from them as much as I can.

Before I went to China I knew that I wanted to do something in my life that would improve life for my world family. In China, I was able to meet some of the people who need my help. These people became my friends and family. It felt so amazing just to know that what I was doing could to make their lives better. It was the first step towards fulfilling my dream.

I am now even more inspired every day to do what I can to make the world a better place. I believe that the difference between having an ordinary impact and an extraordinary impact on the people of the world, is putting in that little extra every day, which is something I am now committed to doing.

I want to express my deepest gratitude for the World Food Prize Foundation for sending me to China. Before I went I was tired, complacent, and scared, but my internship rejuvenated me and I am now ready to actively and excitedly embrace the future.

Special Thanks to:

The World Food Prize Foundation
The Chinese Academy of Agricultural Sciences
Lisa Fleming
Ambassador Quinn
Dr. Borlaug
The Ruan Family
Dr. Fan Yunliu
Dr. Zhang Weili
Dr. Lu Qinguang
Dr. Qinfang Wang
Zhai Li
Wang Hanqian
Dr. Zhang Renlian
Lin Qinghui
Dr. Luo
Dr. Xu
Amber
Ms. Wu Ningfeng

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Texas Natural Resource Conservation Commission: Nonpoint Source Pollution

This is a map of the Tai Hu Region that I made using geographical software in the Soil and Fertilizer Institute.