# Laura Snell

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China National Hybrid Rice Research and Development Center Changsha, China

# Introduction

My name is Laura Snell and I live in Ames, Iowa. I attended the World Food Prize Youth Institute in the fall of 2003 when I was a sophomore at Ames High School under the direction of Mr. Chuck Stevens. I was interested in the internship program because it would give me a chance to narrow down my choice of majors; lead me to experience first hand food security issues; and learn about a different way of life. Scientific studies especially in biology are of great interest to me and this internship provided an opportunity to live and learn side-by-side with highly regarded international scientists.

#### The Host Center Background

I spent my internship at the China National Hybrid Rice Research and Development Center and the Changsha Hybrid Rice Research Center in Mapoling, Changsha, Hunan Province, China. The center was founded in 1984 and it is the first research institute to specialize in hybrid rice in the world. Prof. Yuan Longping is the chief director of the center and known as the "Father of Hybrid Rice." The center's 210 staff members focus on six divisions of hybrid rice production: scientific research, technology development, international cooperation and development, administration, personnel and finance. Many scientists under the scientific research division perform a wide variety of research including hybrid rice breeding, molecular breeding, agronomy, lab testing, field management, sci-tech exhibition, and observational journaling. This last year a new laboratory was built with state-of-the art technology and a large auditorium. Prof. Yuan Longping has won many awards over the years for his research including the UNESCO Science Prize, the Wolf Prize, and the World Food Prize. He continues to develop hybrid rice for the welfare of people all over the world through increasing quality and quantity of rice varieties.

#### **Internship Research**

I was assigned to work with many different programs and with many different researchers but my largest project consisted of testing SSR primers and new varieties of hybrid rice and their parent lines. As scientists create new hybrid rice varieties, the varieties must be tested against many SSR primers so they can be accurately identified and their purity rates tested. While at the center I also spent a few days with a breeder learning how to cross breed different lines of hybrid rice using many different methods. Dr. Yuan, Professor Yuan's son, taught me about molecular breeding and I performed experiments and dissected calluses in his lab. I went to Dr. Cao's lab, another lab not associated with the CNHRRDC, where I learned about more time-efficient ways to test the purity of rice seeds. His laboratory works to test all the rice varieties before they are sold to the farmers. Hybrid Rice must have a 95% purity rate or higher to be sold. In Wangcheng I went to a brand new laboratory and researched the transformation of *Arabidopsis* by vacuum infiltration and agrobacterium tumefaciences by electricity-shock. I also took classes with the hybrid rice international training course and learned much about cultivation, yields and the more technical side of hybrid rice.

#### **SSR Primer Pair Analysis**

The experiment of identification hybrid rice with SSR molecular markers is as follows. Three hybrid Rice combinations and their parental lines, totaling 8 specimens, were tested by means of SSR analysis. A total of 18 SSR primer pairs were used to try to accurately detect polymorphism in all 8 specimens.

Before hybrid rice seed is sold it must pass a purity test of 95% or above. This test is not possible without accurate SSR primer pairs that produce readable polymorphic patterns. The results of testing these SSR primer pairs include more accurate purity tests and better hybrid rice varieties. There are many new hybrid rice varieties being developed and each of these varieties has different SSR primer pairs that react well to the genetic make-up of the plant. This test of SSR primers pairs and hybrid rice combinations and their parental lines is very common and an important step to getting hybrid rice sold and grown everywhere.

In order to test the DNA it must be extracted first. This is done by means of the CTAB genomic DNA extraction method. Seedlings are first cut and placed in a mortar and pestle where they are ground finely. Liquid nitrogen is then added to the mortar and the solution is ground again into a fine powder. Add 6ul of CTAB buffer to the solution in the mortar and by using the pestle put the solution into a 1.5 ml eppendorf tube. Add 12ul more of the CTAB buffer and then close the lid. Incubate at 65 degrees Celsius for 25 minutes turning every so often. Add 500ml of 24:1 chloroform: isoamyl alcohol and then mix gently for five minutes. Centrifuge for five minutes at 13K and 4 degrees Celsius. Pipette the entire aqueous layer into a new eppendorf. Add 330ul cold isopropanol and then incubate at -20 degrees Celsius for 30-60

minutes. Centrifuge for five minutes at 13K and 4 degrees Celsius and then pour off the supernatant. Dry the left over solution under a vacuum and then add 50ul water and 10ul of RNase and incubate for 37 degrees Celsius for 1 hour. Store the ending solution at -20 degrees Celsius until ready for use.

The next process is the PCR process which separates the DNA for analysis. This process requires a mixture of 2.5ul of 10xBuffer, .5ul of dNTP (2mm), .1ul of taq, .125ul of each primer F and R, and 18.1ul of distilled water which equals 21.35ul of solution plus the 1ul of DNA which totals 22.35ul for the PCR program. PCR is a process of steps which heats the solution up and then cools it down over and over again. In this particular program the solution is first heated to 95 degrees Celsius, cooled to 55 degrees and then heated again to 72 degrees. The process is repeated 34 times then it is cooled to 15 degrees Celsius and stored at -20 degrees until further use.

Next an agar gel is made with 1-2% agarose and then the PCR solution is dyed and pipetted into the holes in the gel. The gel is then put into an electrophoresis program at 80 volts for 30 minutes. This program produces the polymorphic picture that is the final product. After the electrophoresis, the gel is placed in EB for 20-30minutes.

This cancerous material makes the polymorphism easier to see. Once this is done the gel is looked at through a UV light photography system and a picture is taken for analysis. This picture is the final product and proves whether the primer was accurate in identifying the difference in the parent and hybrid varieties.

After testing 18 SSR primer pairs I found that the primer pairs RM252 and RM337 accurately identified the genetic polymorphism in all 8 rice varieties. The primer pairs RM219 and RM519 also showed promising results but they weren't as clear as the two above.

Everything done in this experiment including the DNA extraction, PCR program, electrophoresis, and the polymorphic picture were all new learned techniques. I learned how to be careful with liquid nitrogen and EB and how to prevent the spreading of bacteria and contaminants in a lab environment. The identification of hybrid rice with molecular markers is very important and was my main experimental task.

# **Techniques of Breeding**

There are several breeding techniques that I also learned. I was taught by the center's chief breeder, Mr. Wu, during a two day in-depth study. These specific techniques are used only in field work and other laboratory breeding techniques will be discussed when studying with Dr. Yuan (son).

There are many approaches to exploiting heterosis in rice. One way is by the three-line system. The three lines are the cytoplasm-genetic male sterile line (CMS or A line), the maintainer line (B line), and the restorer line (R line). The CMS or A line should have a stable male sterility. This should be inherited from generation to generation and not be influenced by environmental changes. The line should be easily restorable withgood floral structure and flowering habits. The B line is a pollinating line used specifically to pollinate the CMS line. Therefore the function of the B line is to reproduce the CMS line. The R line pollinates the CMS line to produce F1 hybrids that will become fertile and generate seeds by selfing. The R line should be a strong restorer, have good agronomic characteristics, well-developed anthers with a heavy pollen load, and good flowering habits. Most commercial production areas use the three-line system because the male-sterility is controlled by both the cytoplasm and the nucleus.

The two-line and photo/thermo-sensitive genetic male sterility (PTGMS) systems were developed in China and are widely used. A rice plant which became male sterile under a long day length and became fertile under a short day length was discovered. This spontaneous mutation was then called PGMS and another variety that reacted with temperature changes was called TGMS. In order to create a two-line system you can use either the PGMS or TGMS variety and another pollen parent or P line. This system simplifies seed production and decreases the cost of hybrid seeds.

In tropical and subtropical areas the TGMS lines are more useful than PGMS lines since the day length is relatively short and the temperature is rather high. PGMS lines are more preferable used in higher latitudes. Under optimum long day length or high temperature they show complete pollen fertility and under moderate temperatures and shorter day lengths they show almost normal fertility and multiply by selfing. There is a very broad range of parent lines that can be used which, increases the probability of developing superior hybrids. PGMS and TGMS genes can be easily transferred to many rice cultivars to develop new lines for various breeding purposes. These lines also have no relation with cytoplasm so the unitary cytoplasm situation of WA types can be avoided. Both of these techniques go through a breeding process to ensure correct planting and hybrid pollination occurs. The first task is to plant a source nursery of CMS or PTGMS lines, and a R line or P line. The next job is to testcross the nursery. This is done by planting a check variety after every 10-20 hybrid combinations. If the combination shows male sterility and has good characteristics then the male parent should be used to develop the hybrid line. Now the three-line system must go through several more procedures of re-test crossing the nursery and then backcrossing the nursery and finally evaluating the heterosis of the hybrid combinations. After the hybrid combination has become stable the process can be made more flexible. Some of the middle testing tasks can be ignored and then plants can be planted into rows in the field. In the two-line system the plants need to be planted under a long day length or high temperature to make full expression of its male sterility. The plants that actually show complete male sterility should be selected to obtain selfed seeds. Other procedures are similar to those for breeding in the three-line system.

Molecular breeding is essentially breeding for the twenty-first century. I studied with Dr. Yuan (son) for several days learning the complex and amazing world of molecular breeding and rice transformation. By using methods of rice transformation, new varieties of rice that have higher yields, higher grain quality, resist pests, resist stress, cost less, and don't damage the environment can be developed. In order to perform rice transformation some requirements must be present. Some of these requirements include a target cell/tissue for plant regeneration, a method to introduce the DNA into the plant cells, a procedure to select the transformed cells/tissues and a stable expression of introduced genes in expected patterns.

To create a rice tissue culture, which is the first step to rice transformation, the plant must be cut into an explant and then grown and cut into a callus. After this step the callus is then grown into a protoplast or is transferred straight from a callus to embryogenic cells. Embryonic cells lead to regeneration and new plants with transformed genes and new traits.

Four ways to transfer genes include protoplast-mediated transformation, particle gun transformation, agrobacterium-mediated transformation, and pollen-tube transformation. Protoplast-mediated transformation includes polyethylene glycol (PEG), liposome and electroporation, and it is a true single-cell system. In this system the new DNA is directly inserted into the protoplasts and regenerate into plants with new traits. The shortcomings of protoplasts include the fact that protoplasts must be present, some genotypes are unresponsive, and the process is not very efficient. Particle gun transformation is the second most used method. It uses calluses or organized tissues that are genotype-independent. There is a low efficiency and it is rather expensive but many copies of the genes can be processed and chimeric plants can be regenerated. Agrobacterium-mediated transformation is another form of rice transformation. This widely-used method is natural, clean, simple, and has a high efficiency. The downfalls of this technique are that it produces a low copy number and it is genotype-dependent.

Agrobacterium-mediated transformation is the primary technique I used while I was working with Dr. Yuan. The procedure to complete agrobacterium-mediated transformation starts with mature seeds which then grow into primary calluses. Twenty days later the growth of embryogenic units occurs, and ten days later a R2C liquid is added to the solution and 15 minutes later it becomes an agar gel. Three days later the selection of the good calluses transpires and they are transferred to a new medium. Two to three days later the calluses become mature and the regeneration process starts. Two to three months later a new rice plant with new genetic traits is mature and is cultivated for further testing and the process restarts.

The last rice transformation technique is the pollen-tube transformation technique. I performed this technique in the field as well as in the laboratory. This technique is simple, fast, cheap, efficient and genotype independent. It produces many copies but it is very instable.

Transferred genes include nutrients like Vitamin A related genes, ferritin, lysine rich protein, and starch synthesis related genes. Yield genes include C4 genes and an IPT gene. Pest resistant genes include BT genes and a GNA gene. Disease resistant genes include Xa21, chitinase and the herbicide-resistant gene includes the bar gene. There are limitations to rice transformation which include gene silencing, position effect, efficiency, multiple gene transfer, and mutation.

# **Seed Purity Testing**

In Dr. Cao's lab I was taught by a more efficient means to test seed purity and DNA extraction. He uses his own proprietary serum for extracting DNA. The serum uses two chemicals and a heat source to "boil" out the DNA from small cut-up pieces of the plant. This is very different from the complex CTAB method which requires the cell wall of the plant to be broken and twenty steps of chemical mixing and centrifuging. He also uses a shortened PCR and

electrophoresis program which saves time. The agar gel I used in this laboratory already had EB or ethyl bromine in it so I had to be very careful pouring it into the molds. (Ethyl Bromine is cancerous). The gel is also reused and then melted again to save time and money.

In Dr. Cao's lab they had the most sophisticated ultraviolet camera I had the privilege to use while I was researching. The quality of the pictures is superior and the pictures could be printed off for easy analysis. Dr. Cao's lab is very efficient and technically advanced. The research atmosphere is completely different from the CNHRRDC.

# **DNA Transformation**

At the newly built Wangcheng laboratory I studied and researched the basis of molecular biology and DNA transformation. *Arabidopsis* is a small flowering plant that is widely used as a model organism in plant biology. The plant is not of major agronomic significance, but it offers important advantages for basic research in genetics and molecular biology. The reason this plant is used is because of its small genome (that was sequenced in 2000), there are extensive genetic and physical maps of all 5 of its chromosomes, it has a rapid life cycle (about 6 weeks), and an abundant seed production with easy cultivation in a restricted space. Arabidopsis has proven to be efficient at using transformation methods utilizing Agro bacterium tumefaciens, and contains a large number of mutant lines and genomic resources. It reacts well in many circumstances to many environments and climates.

The first set of experiments I did with the Arabidopsis was DNA transformation by vacuum infiltration. This experiment suspends plants in an infiltration medium consisting of salt, B5 vitamins, sucrose, benzyl amino purine, and silwet L-77. Then the plant is put into a vacuum infiltrator and the rapid increase in pressure after the vacuum pump is turned off forces the bacterial cells into the plant tissue.

The second experiment I performed on *Arabidopsis* was the transformation of foreign DNA by electricity-shock. In this experiment the DNA sample had to be electro orated using a machine called the Gene Pulsar Xcell. The machine sends a shock of 2.4kv into the DNA cells and the foreign agro bacterium is pushed into the DNA.

The last experiment I performed with *Arabidopsis* was the one I learned the most about. I learned many new techniques including how to use a fume hood, how to streak agro bacterium, how to properly vortex a solution, and I learned many new vocabulary words along the way. In this experiment I transformed DNA by isolating the plasmid part of the DNA. This process was very interesting because the solution took on many different forms and the chemicals used to extract the plasma were very toxic.

#### **International Hybrid Rice Training Course**

I took classes with a group of 30 people from all over Asia through the International Hybrid Rice Training Course. These classes proved to be very useful in learning about the technical cultivation and planting aspects of hybrid rice production and also gave me the chance to work out in the field. The classes are all taught in English and the professors came from the local Hunan Agricultural University and from the Longping High-tech International Exchange Center. The exchange center is a branch of the Yuan Longping High-tech Agriculture Co., Ltd., and was established for dealing with the businesses of international scientific and technological, economic and trade cooperation. The center focuses on hybrid rice extension all over the world, and started the Hybrid Rice Training Course to provide an effective way to educate people about the importance of hybrid rice. The week of lectures focused mainly on hybrid rice cultivation techniques.

The first lecture covered the topic of photosynthesis and dry matter production. I learned about the "harvest index" of hybrid rice and why dry matter production is a fundamental factor for productivity of hybrid rice plants. I learned that dry matter is almost all a product of photosynthesis and that Changsha is in the inland-monsoon humid climate region of the subtropics. The maximum rate of photosynthesis happens at around 9 am and hybrid rice has a higher assimilation rate than conventional rice. In simple terms, more sunlight equals more photosynthesis, which equals more yield.

Factors that effect photosynthesis are compiled into two categories, internal factors and external factors. That means genetic factors and environmental factors. Some internal factors include leaf area, unity assimilation rate, and light transmission coefficient. External factors include light intensity, temperature, and rainfall. The balance between photosynthesis and respiration is favorably maintained at the early growth stage of plants and then declines towards the later stages. The same happens when dealing with optimum leaf area. Dry matter production reaches a maximum value at a certain leaf area and then declines; the optimum area is called the

optimum leaf area index. Temperature has a major effect on hybrid rice. An example: as temperature goes up, the net assimilation rate goes up but the leaf area index goes down.

The second section the lecture covered was the growth and development characteristics of hybrid rice leaves. The flag leaf is an abnormally large leaf that is not usually the first leaf to appear on the stock. Primary leaves are grown to protect the panicle at the time of development. The duration of these upper leaves on early hybrid rice is 30-35 days where as that of late hybrid rice is 40-45 days. The longer these leaves maintain their health the more rice the plants will produce. Leaf growth is controlled by increases and decreases in temperature. Leaves stop growing at temperatures around 9 degrees Celsius and the optimum temperature is about 28 degrees Celsius. Leaves have many nutrients in them. Nitrogen tends to be concentrated at the top of the leaf and Calcium tends to be concentrated towards the bottom. The leaf area index or LAI is the magnitude of leaf area relative to ground area. The number of leaves for early hybrid rice is about 12 and the number for late hybrid rice is about 14. Without leaves there wouldn't be any rice.

The two lectures above were two of many but they were the most interesting and thought-provoking. My favorite part of the training course was the opportunity to work in the rice paddies. I learned how to plant rice in the two-line, and three-line systems. I also tried a new planting technique called parachuting. In this technique, instead of trans-planting each plant one by one into the mud, the seedlings are grown in a plastic sheet that has hundreds of little "bowls" for the seedling's roots. When the seedlings are grown and it is time to trans-plant you take the seedling out and there is a clump of mud on the bottom of the plant that acts as a weight. The seedling is then thrown up in to the air and hopefully towards the field and it looks like a parachute as it descends to the ground. The field looks haphazard but the seedlings end up growing straight up and the outcome of yield is still very good. On my last day with the training course I went out to the fields and watched people harvest the early rice. It was very interesting looking at the different varieties and comparing the hybrid rice to conventional rice.

I was assigned to work with all of these groups so that I would learn about a broader aspect of hybrid rice instead of just the laboratory work that occurs in Professor Yuan's laboratory. All of these programs strive to increase the production of hybrid rice in China and around the world. Whether the lab is testing purity for rice being sold to farmers, testing new primers for purity tests, conducting experiments on gene transformation, or educating people so that they can go home and perform their own experiments, these programs are important to the future of hybrid rice. I enjoyed working with all aspects of hybrid rice production and I think it made my experience much more worthwhile.

Professor Yuan's (father) mission is developing hybrid rice for the welfare of the people all over the world. Rice is a major food crop in Asia. It feeds more then half of the world population. It is estimated that with population growth the world will need to produce 60% more rice by 2030 than what was produced in 1995. Therefore, to increase production of rice plays a very important role in food security. Hybrid rice has more than a 20% yield advantage over improved inbred varieties, and hybrid rice has been proven to be a very effective approach to greatly increasing yield not only in China but also around the world. Through educational programs, international testing and scientific gene transformation, hybrid rice is becoming better than ever.

The international hybrid rice training course strives to teach some of the most prominent agricultural officials from countries all over Asia and Africa about hybrid rice. The course lasts four months and goes through every process of hybrid rice production. The trainees plant crops in their own test plots, listen to lectures, and take field trips to different seed companies in China. The trainees can then take the knowledge that they learn back to their home countries and teach others what they have learned. This process leads to higher yields, better experimental processes and more food security in Asia. The training course also has a team of people that travel to many countries and set up experimental plots to test hybrid rice varieties in different climates and find out which varieties are best for each country and climate zone.

#### **CNHRRDC** Missions

The next mission the CNHRRDC has is to make rice healthier and more nutritious to help solve malnutrition in families, especially for children. By methods of gene transformation and nutrient research, scientists at the center are working to put vitamin A, protein, starch and other nutrients into hybrid rice. This process is very lengthy and difficult. Scientists are working every day to find new gene combinations that show nutrient significance. These nutrients can reduce sight problems, anemia, and weakness in people all over the world.

The CNHRRDC also has a mission to help the environment by studying chemical pesticides and herbicides, research irrigation, and water saving techniques. In China, on average,

a farmer uses 60% more chemical on their rice paddy than needed. Rice already has a built-in pesticide in that it is grown in water. The water keeps many bugs away from the stalk of the plant. This over-usage of chemical runs into the ground water systems and threatens the clean water supply. The center also studies irrigation and water saving techniques. These techniques make sure that people at the end of the water source still have water to hydrate their plants.

One of CNHRRDC's biggest goals is the development of phase three super hybrid rice and ever increasing yields of hybrid rice in general. This accomplishment would not only help China but the entire world to strengthen their food security. Super hybrid rice looks like a golden waterfall before harvest. The plant appears as though it is going to tip over because of the heavy weight on the panicle. Super hybrid rice can produce yields over 12 tons per hectare. This variety of hybrid rice must have a good plant type, a large sink and source, be highly resistant to lodging, and have high productivity and harvest index. The plants must also be highly resistant to major diseases and pests, have good grain quality, and have no senescence.

#### **Scientific Mentors**

I worked with many people at the CNHRRDC. My host mother, Mrs. Yeyun Xin is Professor Yuan's personal secretary and laboratory assistant. She is married and has one son. Mrs. Xin studied in Hong Kong and the United States as well as with Professor Yuan at his laboratory. Her specific study was on the identification and purity test of super hybrid rice with SSR molecular markers. Her expertise led me to perform my experiment on the SSR molecular markers also.

Professor Yuan (father) is a very important person in China. The three-line hybrid rice system was invented in 1973 and then production of hybrid rice began in 1974. He founded the Hunan Hybrid Rice Research Center (HHRRC) in 1984 at Mapoling, Furong District of the city of Changsha. He has led the HHRRC to 83 scientific prizes, 12 of which are state-level prizes and more than 40 are provincial or ministerial prizes. Hybrid rice has become the fifth invention after the "four great inventions" in China and is a monument in the history of science around the world. Professor Yuan's expertise in rice production has not only saved China from starvation but has the ability to save the world from starvation also. His family consists of his wife, a son, and a daughter. He also is blessed with beautiful grandchildren. His English is very good and he has the only oven I could find in the town. Everyone else only cooks on the gas grill.

Dr. Yuan is Professor Yuan's son. He majored in molecular biology and mainly works in that field. He is married and has a son. His English is also very good and his knowledge in molecular biology and gene transformation is amazing. He is currently working on several large projects to make rice healthier and more filling.

Mr. Lui Bing is head of the educational part of the International Hybrid Rice Training course and taught me a lot as well as traveled with me on some occasions. He is a teacher as well as a scientist. When I was confused about something he could usually explain it to me better. His knowledge of hybrid rice is also very good and he is very cultured. His sister lives in Canada and he is married.

#### **Experimental Responsibilities and Contributions**

I had many different responsibilities and contributions to the data that I obtained and analyzed. Each laboratory I was in gave me diverse types of assignments. These ranged from dissecting rice calluses to extraction DNA and doing PCR research.

The first project I did was my own experiment on the SSR molecular markers and identifying genes in a variety of hybrid rice strains. My responsibilities started with extracting DNA from eight specimens. This was a technical process by the CTAB method. Next I had to choose two primers I wanted to test that day. The first day I tested primers RM104 and RM 472 and then I progressed through 18 pairs. In order to prepare the DNA and primer pair for the PCR program, I added four liquid solutions to the vials and then shook them. These vials would then go into the PCR program, which takes several hours. I would do this in the morning session of laboratory work along with setting up an agar mold for that afternoon's electrophoresis. After lunch I went back to the laboratory and took the DNA out of the PCR, dyed it purple and then pipetted it into the agar gel holes. Then I would let the electrophoresis react for about an hour then move the gel into the EB solution and let it sit for another half an hour and then set up the ultraviolet camera and computer for analysis. The gel goes on the UV light source and the camera fits over top and takes a picture. I would study the picture and determine if the results were good enough or if I needed to do the experiment over again. When I first started doing the experiment I would forget something or not keep the solution cold enough and the results would be faulty. I would repeat the experiment until it was right.

Mr. Wu let me work along side him in the rice paddies. My contributions included performing rice stalk gene transformation or pollen-tube transformation on a group of plants, inserting genes into the seed pods of many rice seeds and transplanting rice plants. Mr. Wu also gave many lectures on hybrid rice and its importance and about super hybrid rice and its great potential. I took lots of notes and listened intently.

In Dr. Yuan's lab I essentially went through the steps to create a rice tissue culture. On day one, I learned how to pour agar Petri dish molds in the morning of all the different types of gel that are needed for gene transformation. In the afternoon I performed a PCR analysis for the genes that we were going to put into the plants. Day two, I cut rice seeds into explants which are the growing part of the seed, not the nutrient. This process is very important and I learned about the structure of a rice plant. That afternoon I dissected calluses and placed then into a different type of agar. These responsibilities are the same responsibilities that everyone had in the molecular biology unit.

In the Wangcheng laboratory I was usually given a sheet of paper and instructed to perform the experiment that was listed. One scientist took me through the DNA transformation by vacuum infiltration experiment and then she let me finish the remaining plants. The task was a bit repetitive but I contributed in a meaningful way to the overall success of that project.

In the international hybrid rice training course I was a student of hybrid rice. My contributions to the class included a presentation I did on my home country and a small speech on why we grow corn and soybeans in Iowa. Many people were very surprised to hear that we feed most of the corn to the cows and pigs. I sat in on many lectures and took notes on hybrid rice, which in turn helped me to understand and perform experiments much better.

My main project of testing the identification and purity of eight different hybrid rice lines and their parents by SSR molecular markers has much value in the long run to improve food security. If hybrid rice lines and their parents cannot be identified, then the rice can not be tested for purity and therefore cannot be sold. The hybrid rice then can not be tested for certain gene combinations and regular molecular patterns. New rice varieties are being created all the time, and with out a means to test their reliability, they are not suitable for human consumption or growth. Dr. Cao's lab is one of many labs that specialize in the purity testing of hybrid rice. Every bushel of seed must be tested before it can be sold. If they are not able to receive accurate information about definite molecular markers identifying certain rice varieties, they can not perform their job. Without tests on molecular markers, research on gene transformation and how to improve yield and develop super hybrid rice is impossible. Molecular marker tests are some of the first tests that must be performed for hybrid rice to bring improved food security to the world.

Advances in molecular biology and gene transformation have the potential to significantly improve food security. The body requires approximately 20 amino acids for the synthesis of proteins. Protein is required for growth and development. Foods such as grains, fruits and vegetables are low in proteins. With the development of vitamin A related genes, ferritin, lysine rich protein, and starch synthesis related genes will become more nutritious and improve global nutrition. Vitamin A rich hybrid rice would help children to see more clearly and help their bodies to resist disease and infections. Diseases like measles and dysentery can kill undernourished children. Malnutrition resulting in disease contributes to 10 million deaths of children under the age of five each year. Ferritin is a protein containing iron that is synthesized in the liver for use in the body. Around 25 percent of men suffer from anemia caused by iron deficiency and 45 percent of women also suffer from that same anemia. Ferritin can also help to improve nutrition and food security. Lysine is one of the basic amino acids. Lysine enriched proteins that are transferred into hybrid rice can help reduce protein-energy malnutrition, the most lethal form of malnutrition. Without protein, many children do not have the motivation or energy to think, move around or play. Starch synthesis related genes make small amounts of rice more filling and help families in poverty-stricken countries become more nourished and satisfied with the small amount of food that they have. Gene transformation also has the potential to increase yields by inserting C4 genes and an IPT gene. C4 plants such as corn have more photosynthesis ability and therefore have the possibility to create more products. The IPT gene also works with the hybrid rice plant to create higher yields and happier farmers. Many genes concerning the usage of pesticides, herbicides, and the problems of rice diseases are also being added to hybrid rice. These genes help to increase yield and to lessen the amount of harmful chemicals that are put into a population's drinking water. Molecular biology and gene transformation will improve food security by tremendous amounts.

In Wangcheng experiments on new technological ways to transform genes and studies going back to the basis of molecular biology is used to help improve food security. Since *Arabidopsis* was discovered in 1873, it has been a plant of curiosity. F. Laibach first summarized the potential of *Arabidopsis* as a model organism for genetics in 1943. This was a long time after he published its chromosome number in 1907. Scientists still research its small genome and its effect with new types of gene transformation. *Arabidopsis* may hold the key to solving the gene silencing problem in gene transformation and consequently help solve the food security problem we have today. One new technique of DNA transformation that is costly, yet efficient is the electricity-shock transformation. This technique requires an expensive machine called a Gene Pulsar Xcell. New techniques like the electricity-shock transformation are studied to help improve the quality and quantity of food all over the world.

Education is the key to success in every field. Through the International Hybrid Rice Training Center people from all over the world can learn about more efficient ways of producing hybrid rice. Hybrid rice has more than a 20 percent yield advantage over improved inbred varieties. With more yield there is more food for some of the most impoverished regions of the world. The training center teaches all aspects of hybrid rice. They start out with basic hybrid rice cultivation and teach the technicalities of the two and three-line systems. Students are taken out to test plots to plant hybrid rice. The center teaches new techniques for planting, breeding, and how to develop super hybrid rice. With education food security can be improved and there will be enough food for everyone in the world.

# Changes

I changed a lot while I was in China. My views on communism, living styles, and quality of life had definitely been reviewed. In every history class I can remember, communism has been made out to look like the black plague of government. Communism is bad and makes bad people. I was never taught why it was bad; just that is was bad for you, for me, and for everyone. Terms like the "red scare" and countries like Vietnam, Russia, and China come to mind. Wars that cost thousands of lives pop into my head and make me wonder what it was all for. In central China, communism is looked at like a savior for the farmers, industrialists, and families. Mao Zedong is praised for his uprisings, speeches and knowledge. His childhood home is toured by thousands of people each day just wanting a peek at where this great man started his life. Mao Zedong's statues are everywhere in order to "protect" the people of China. You find his head plastered on everything from medals, buttons, busts, and in cars, restaurants and on street corners. The communist party is still very strong in Hunan Province.

I also found that the relationships I had with the men from Vietnam were some of the best of my trip. At first I was very nervous because I expected Vietnamese people to dislike Americans but I came to realize that they are amazingly sympathetic and caring. This idea that I had about Vietnamese people was brought up because of the conflicts between the United States about communism that I had been taught through history courses. I had never been taught anything else about Vietnam other than events associated with the Vietnam War. My views on communism went from an uneducated dislike to a responsible appreciation. I now understand why countries become communist and the positive outcomes it can bring, not just the negative.

My attitude towards living styles also was tested and analyzed. With the introduction of the one-child policy and the huge population that China already houses, living styles and family life are very different. I saw three one-family houses during my stay. These families were all wealthy and very privileged. More people live in high-rise apartment buildings usually paid for by the government. For example, many farmers in Hunan are given a small apartment with a 30square-foot piece of land behind it for subsistence farming. The farmers then work together on a larger plot of land that the government has set aside. Since the government controls how much the farmers can make from their crops and controls their living environment, there is little chance for immediate changes in living conditions. This provided me with a few challenging questions about the state of the government and about their future in the farming industry. In these governmental housing districts, all the houses look the same and everyone has the same job. Some say that country folk are lucky because people who live in the country can have two children but this also supplies more people to work in the fields and the chances that their children will become anything other than farmers is very poor. From my experience in visiting urban areas of China, most families followed the "one child only" rule and the children I met were often pampered. Boys, in particular, tended to be indulged. Although in Hunan Province, girls were also highly valued. When I told people in Hunan about how I was the only girl in a family of brother, they praised me by saying I must be the apple of my family since I was the only daughter. In Northern China and Beijing, boys were generally considered to be the most esteemed gender as the heir who would continue the family name and legacy. And, too, in rural areas, boys were well thought of in their physical capacity to work as laborers on family farms. Although rare, one Chinese person I met was able to arrange to adopt a daughter from the government since there are so many girls who were abandoned or orphaned in both rural and

urban areas. Geographical locations, individual family lifestyles, cultural norms and housing accommodations taught me quite a lot about Chinese culture and society.

Another thing I found quite surprising was the circumstances in which Chinese people work and study. Generally everyone goes to school for 12 years including kindergarten. This is one year less then we go to school but their last year does not include a summer break. The last year of studying is concentrated on college entrance exams that everyone must take if they want to enter a facility of higher learning. Unlike the United Sates, there are not enough colleges or universities for everyone who wants to attend a place of higher learning. Acceptance is solely based on college entrance exams and on knowledge of foreign languages, especially English. I found it interesting that students also do not get jobs to pay for their educations and scholarships are very rare so their parents pay for their studies and other expenses. Students rely on their parents until they get their first job, which could be until the student turns 30 depending on their field of study. Jobs are also a scarcity. Even if you do attend college and graduate school there is still the big struggle to actually get a job. Students take on very difficult non-paid internships for months and sometimes years before they are actually offered a paying job. Also, once you get a job you never quit that job until you are ready to retire or until your children are ready to take care of you. It is interesting because I think about how many people we have to work in department stores and imagine four or five times that many people splitting that same pay check. That is what every store, hotel and restaurant is like. Instead of one waitress, I always had at least two if not three and instead of picking out clothes myself, I had three ladies doing it for me. Most of these jobs pay less then a dollar an hour and are the jobs that these people will keep for a very long time. The environment in which Chinese people work and study is very different from the environment in the United States.

During my Borlaug-Ruan Internship I learned a lot while studying and traveling in central China. I grew emotionally and culturally through the many experiences. Many people helped guide me through the eight-week internship. I want to thank and acknowledge Professor Yuan and everyone who made my internship a possibility. Everyone at the center was very generous with their time and their assistance. I learned not only about the complexities and history of hybrid rice but also about the hardships that Chinese people face everyday. This internship has helped me to define my future goals and has led me to devote my life even more to food security around the world.





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Crossing Hybrid Varieties

