Rice in the Philippines

Summer Experiences Abroad

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2008 Borlaug-Ruan World Food Prize International Internship

IRRI: International Rice Research Institute

Acknowledgements

I would like to thank Norman Borlaug, John Ruan, and Ambassador Quinn for making possible my summer experiences.

I would like to thank Lisa Fleming for ensuring that my summer was the best possible.

I would like to thank Pioneer Hi-Bred for their generous sponsorship of my internship.

I would like to thank the International Rice Research Institute for allowing me to make a contribution to food security.

I am grateful to Dr. Mackill, Dr. Septiningisih, Ms. Darlene Sanchez, and Ms. Josefina Mendoza for allowing me to work alongside all of them and teaching me about agricultural research and food security.

I would like to thank Jason and Sarah Beebout for all their help.

I would like to thank all the people I met in IRRI and in Los Baños for their friendliness and generosity.

I would like to thank my family for their continuous love and support.

Introduction

As I walked to the podium, my sweaty hands wavered as they clutched a sheet of messy notes. All I could do to give an illusion of confidence was my sure-footed walk. With every step, I could feel their eyes boring into me. The eyes of those sharp suited, well-spoken dignitaries that sat in an intimidating row of their own.

This memory from the World Food Prize Youth Institute 2007 remained with me well after that day in late October. The opportunity to discuss solutions to issues in front of people who had dedicated their lives to them was not one I had expected when I approached my AP Biology teacher to ask if I could represent Central Academy. She seemed delighted and soon I delved into a heap of articles discussing food security and biofuels; I admittedly had little knowledge of either when I began. I became especially interested in India and the role of women's rights in determining food security of a region. The simplicity of my research astounded me. By guaranteeing rural women equal rights, one could significantly increase the likelihood that their families would have sufficient food. Thus if India wanted to pursue biofuels with greater zeal, it would have to address this issue.

Having been born in India, these issues struck a chord with me but also made me realize the implications that the findings had for the rest of the world. However, living in Des Moines, Iowa, it was easy to dissociate myself from those facts, as I had never experienced them; it was impossible for me to fully understand the issues relating to food security without being directly involved in the cause. Thus when I learned that there was an opportunity to spend a summer in a nation facing issues of food security while participating in research at a premier agricultural research institute, I eagerly applied. At the end of the application process I was informed that I would be going to Los Baños, Philippines in the summer to participate in research at the International Rice Research Institute.

Background of IRRI

The International Rice Research Institute (IRRI) has a rich history in agricultural breakthroughs and bolstering food security. The nonprofit organization, started in 1960 by the Ford and Rockefeller Foundations in cooperation with the government of the Philippines, is the oldest and largest international research institute in Asia (IRRI). Their mission is "to reduce poverty and hunger, improve the health of rice farmers and consumers, and ensure environmental sustainability through collaborative research, partnerships, and the strengthening of national agricultural research and

extension systems" (IRRI). To do so, IRRI has formed many partnerships, local and international, in order to further research and to inform farmers about the latest farming techniques and technologies. The facilities at IRRI include research laboratories, training centers, and the 250-hectare experimental rice farm (IRRI).

IRRI is carrying out its mission by pursuing its goals. IRRI is reducing poverty by improving rice systems (IRRI). If famers have the necessary tools to improve the yields of their fields, they will be able to farm more effectively and have the necessary resources to provide for their families. It is also important to ensure that rice production is not negatively affected by climate change and that agriculture has minimal environmental impact. Sustainable and stable agricultural practices not only protect the environment, but also encourage farmers to embrace the beneficial practices. Another goal is to improve the nutrition and the health of the poorest rice consumers and rice farmers in Asia (IRRI). In the Philippines alone, rice accounts for 41 percent of total caloric intake and 31 percent of protein intake (FAO). Ensuring that the over one billion rice consumers are getting the necessary nutritional benefits from rice is one way to fight malnutrition, which is one of the primary results of poverty. IRRI is also focused on the distribution of information and knowledge of rice farming techniques and developing the next generation of rice scientists (IRRI). With knowledge from sources, such as the Rice Knowledge Bank and Rice Today, farmers will be more aware of factors affecting rice. With the IRRI Gene Bank, IRRI is able to provide famers and scientists with genetic materials they need to develop and enhance rice production. This five-fold method has resulted in past achievements and will bring forth future successes.

IRRI has a long history of success in rice research. The first major breakthrough came in the 1960s, soon after it was established, with the dwarf varieties of rice (IRRI). The characteristic higher yield and shorter stem began the Green Revolution of rice that may have saved millions in Asia from starvation by substantially increasing rice production and decreasing price (IRRI). The poor could afford

more food with their incomes and a crisis was avoided. Rice production since has grown steadily from nearly 200 million tons in 1961 to 540 million tons in 2000. As a result of the increased productivity, thousands of hectares of forests have been saved and millions of rural poor have been able to afford basic nutritional needs (IRRI).

Challenges with Rice in Rain-fed Environments

During the Rice Production Course and the Consortium for Unfavorable Rice Environments (CURE), I had the opportunity to learn about the factors that affect rice production in rain-fed environments. Dr. Stephan Haefele explained the challenges faced by unfavorable rain-fed environments. Although rainfed rice it covers 43 percent of rice producing area, it provides only 25 percent of global rice production (Haefele). In lower land there is often higher soil fertility yet also higher flooding risk; in higher land there is lower soil fertility but famers risk drought. Soil fertility, toxicity, and mineral deficiencies are other pervasive problems throughout all rain-fed environments.

One of the topics discussed at the CURE was the detrimental affect of drought. It can reduce the yield potential and can cause spikelet sterility, especially if the drought occurs during flowering (Haefele). Haefele went on to explain that since transpiration is linearly related to biomass formation, an increased rate of transpiration induced by the hotter, drier climate results in a weaker plant. Drought also reduces the indigenous nutrient supply. As the mass flow and diffusion of nutrients to the roots is reduced, the nutrients available changes which reduces the biological nitrogen from the bacteria resulting in increases of nitrogen loss. Drought can also cause management disruptions and favor weeds against the crop leading to risk-avers input management. Weeds are favored due to the environmental stresses; as the rice plants cannot keep up with the weed growth in dry soils, their competitor easily overtakes them. Each year, 19 to 23 million hectares of rice fields are affected by drought (Haefele).

Farmers can take certain steps to manage their fields despite the risky possibility of annual drought. If the drought comes early in the season, crop establishment and transplanting of the

seedlings can be delayed. If it occurs late in the season, there are fewer options for the farmers. The only thing farmers can do is cut losses; they apply less fertilizer to fields guaranteed to incur heavy crop loss. They can also apply relatively low levels of inorganic fertilizer in drought to be profitable; the amount depends on the expected yield of the rice variety and the intensity and length of the drought. Thus it is important for famers to be realistic about their predictions for their drought-prone fields, accounting also for other factors such as water availability and soil quality (Haefele).

Another factor discussed was salt affected lowlands. Salts accumulating in costal areas due to tidal seawater cause imbalances of nutrients such as phosphorous and zinc (Haefele). There is also an unsafe concentration of sodium salts in the soil, which is detrimental to the plants. Currently scientists are trying to develop salt tolerant varieties and develop new technologies for resource management. There are also prospects of alternating cropping systems that would increase farmer incomes (Haefele). Another possibility is to adjust nutrient management to bolster the seedlings ability to survive in the environment.

Use of fertilizer practices are essential to improving crop yields despite poor soil quality. It is important to realize that the plant yield will be limited by the lowest necessary mineral; even if the amount of other nutrients are increased, its overall effect on the crop will be limited (Haefele). Farmers must determine which minerals are lacking, abundant, or in excess in their fields. They can do this by physically going to the fields and investigating them. This technique is inexpensive, however, the farmer will only see the symptoms after it is affecting the crops and the diagnosis may be difficult when the problem is the cause of multiple minerals. Another choice is a plant tissue analysis, which determines the concentration of an element below the critical level. This technique allows for an accurate diagnosis of the plant, it is possible to conduct before symptoms arise, and it is relatively simple; however, it requires a lab analysis that may be expensive. A soil analysis measures soil extracted from the field against a standard to determine quality. Although this technique can be preformed before the cropping

season and allows for specific recommendations, it is not valid for large fields due to soil variance and may be expensive. To make nutrient addition/omission plots, farmers apply one specific element that could limit yield except in one small plot to check if it is needed; this technique is cheap but cumbersome (Haefele).

There are a multitude of factors that affect rain-fed rice environments and the ways in which to deal with them. Thus at IRRI they have various divisions that deal with these issues in different ways, from the social sciences to the plant breeding. When I arrived, I was already assigned to a project concerning rice submergence with Dr. Mackill, a plant breeder. Working in the Plant Breeding, Genetics, and Biotechnology (PBGB) division of IRRI, my work took place in the laboratory.

The research goals of PBGB are to develop better germplasms for the various rice ecosystems, to explore the use of new breeding, and to discover the genes that determine the major traits by conducting genetics, molecular biology, and plant pathology research (IRRI). My project would be an extension of the exploring new breeding techniques; I would use molecular markers to find the presence of a gene.

During my internship, I had the opportunity to work with a number of talented people. The supervisor of the project is Dr. David J. Mackill, a Senior Scientist at IRRI. He is the leader of the Plant Breeding and Program 1, concerned with raising productivity of in rainfed environments. Dr. Endang M. Septiningisih, a Postdoctoral Fellow at PBGB, was the leader of my project. In the lab I worked with and was assisted by Ms. Darlene L. Sanchez, an Assistant Scientist at PBGB. Ms. Josefina G. Mendoza, a Technician at PBGB, trained and assisted me in the lab.

Submergence Overview

Over the thousands of years of rice cultivation, submergence remains a problem affecting millions of poor farmers of rain-fed rice around the world. Although rice grows in standing water,

periodic flooding in rain-fed lowland areas of Southeast Asia can cause crops to be completely submerged. Submergence occurs when the water level stays at or above the canopy; if this condition persists for more than a few days, it can result in significant yield loss or plant death. Submergence affects between 15 to 22 million ha of rice in Southeast Asia ranging from Bangladesh to the Philippines (Ismail). As the rainfed areas where there is the greatest risk of submergence are also often the poorest, the billion dollars of annual crop loss has a dire impact on the economics of food security (Ismail).

Flooding can occur during different parts of crop growth and each stage has different mechanisms to tolerate the stress. If submergence occurs at crop establishment, the farmer may delay transplanting or rely on the natural tolerance of the plant at germination and early growth (Ismail). If submergence is due to a flash flood of duration of less than two weeks after crop establishment, the Sub1 region primarily determines submergence tolerance. If submergence is due to a flash flood of duration of more than two weeks, then the *Sub1*Plus region, the regeneration ability of the crop, and water stagnation, determines tolerance. The crop can tolerate deep water flooding by facultative elongation (Ismail).

In the future, problems with submergence will only become a more prominent. As the effects of global warming grow, farmers will have to contend with more frequent extreme weather such as unexpected heavy rains and more frequent typhoons. Furthermore, the rise of the sea level will also mean greater incidence of submergence in costal areas of Southeast Asia.

Rice varieties exist that can survive flash floods with duration of two weeks. After the water recedes from the flooding, these varieties recover quickly and there is far less negative effect on the crop yields. However, rice varieties with natural submergence tolerance are often not popular as they often have low yield (Mackill). Creating new varieties by transferring traits from the known submergence tolerant varieties to popular varieties is a way of dealing with the frequent flash floods.

New varieties with submergence tolerance characteristics will increase food security for more than 70 million people around the world (IRRI).

In the early 1990s Dr. Mackill and Kenong Xu discovered the *Sub1* region on chromosome 9 of the rice genome that is responsible for submergence tolerance (Mackill). My case study is a result of the work that Dr. Mackill and his team's work in 2006 when they successfully introduced the *Sub1* trait into five mega varieties (Septiningisih).

Project Description

My project was based on the *Sub1* locus positioned on chromosome 9 on the rice genome and its contribution in developing rice varieties that are tolerant to complete submergence for up to a two-week period. The Japan Ministry of Foreign Affairs funded the project. One of the objectives was to develop new converted lines that were submergence tolerant using some popular varieties in Southeast Asia countries. For my case study, the rice variety PSB Rc 18, a popular rice variety in the Philippines, was chosen. To develop its submergence tolerant version, a marker-assisted backcrossing (MAB) strategy was used; the tolerant gene was transferred from the donor IR64-Sub1 to the popular rice variety or the parent, PSB Rc 18, by backcrossing the F1 plants to the recurrent parent, PSB Rc 18. This strategy had been successfully used in converting five intolerant mega varieties. After the backcrossing the progenies were selected based on the presence of the *Sub1* locus and genetic background most similar to the popular rice variety using the DNA markers SC3 and ART5 with the use of marker assisted selection (MAS). In this genotyping exercise, various techniques were used including polymerase chain reaction (PCR) and poly-acrylamide gel electrophoresis (PAGE).

In my case study, it was predicted that one backcross would be sufficient because the parent (PSB Rc 18) and the donor (IR64) have similar backgrounds. From this experiment, the progenies with the *Sub1* locus and the most similar background to the parent were selected. To determine which ones had similar backgrounds, 53 background markers were used, each corresponding to a different region of

the rice genome. The hypothesis would have been disproved if the progenies selected with the *Sub1* locus and the PSB Rc 18 background had failed to tolerate submergence to a significantly greater level than the recurrent parent check. At the end of the experiment it was expected that there would be several progenies that had both the *Sub1* locus and background that was the same or similar as PSB Rc 18. The number of seeds will be multiplied from the best plant of the chosen progenies. A submergence test then will be conducted using a relevant statistical design, such as Random Complete Block Design (RCBD) to test the viability of the product. In addition, other tests such as yield, grain quality, and other agronomic traits will be conducted.

Techniques and Procedures

For my case study, laboratory techniques were used to determine DNA concentration, amplify DNA, and analyze DNA composition. DNA amplification and analysis of DNA composition were executed in succession and repeated for each PCR assay.

The first technique that I learned was the analysis of DNA concentration using the nanodrop. After DNA is extracted from the plant tissue, it must be diluted in solution with water. The concentrations of the different tubes are analyzed using a machine called the nanodrop. One microliter is placed on the lens and the machine determines its DNA concentration against a control of distilled water. The concentrations of ten random samples from each tray are found and analyzed to ensure an acceptable variation of concentration.

Polymerase chain reaction is a technique used to amplify the amount of DNA fragments. DNA can be amplified through a process of alternating heating and cooling. Before preparing the PCR assay, the DNA must be diluted again with distilled water in a ratio of 4:46 of DNA solution to water. Then the PCR cocktail must be prepared in a 1.5 mL tube in a bucket of ice to maintain a low temperature. The

cocktail consists of sterile water, TBE buffer (Tris base, boric acid, EDTA), dNTP (nucleotides), the forward and reverse primer for the marker, and Taq polymerase. The cocktail and DNA template are pipetted into the 96 wells of the PCR plate in a concentration of 1:4 of DNA solution to PCR cocktail. A drop of oil is added to each well of the PCR plate before it is placed in the PCR machine.

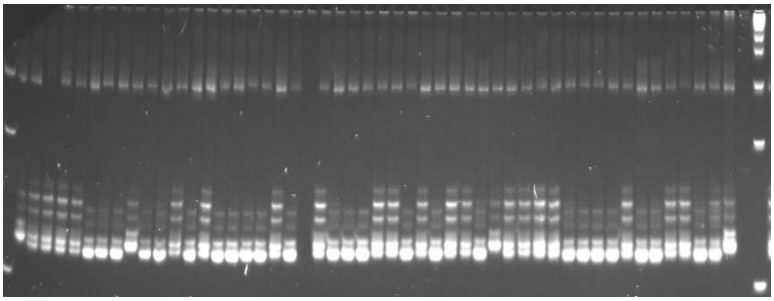

Figure 1: PAGE Image

PCR is a three step process starting with denaturation of the DNA at a temperature of 95°C for 25 seconds. In this step, the DNA template is dismantled; hydrogen bonds that hold the opposing bases in the double helix arrangement are broken. The result is two separate strands of DNA, which serve as the template for the amplification of the DNA. The next step in the process is annealing of the DNA when the temperature is lowered to 55°C for 45 seconds for a three digit primer and 60°C for 30 seconds for five digit primers. The forward and reverse primers bind to the single strands of DNA. The hydrogen bonds that held together the complimentary base pairs now hold the primer to the DNA template as the Taq polymerase begins DNA synthesis by binding to the primer-DNA complex. It synthesizes DNA by assembling the dNTP (nucleotides) into the pattern of the complimentary base pairs of the DNA fragment. At the optimum temperature, each extension step will double the amount of DNA as all the target DNA fragments are split in two and reformed each cycle. The cycle of denaturation, annealing, and elongation is repeated 35 times and then held at a stable 10°C.

After performing the PCR, the assays are prepared for the polyacrylamide gel electrophoresis. First a blue dye is added to the PCR assays. The gel equipment is prepared to form the glass slot that will hold the gel. The gel is formed by first preparing an 8% gel premix of 345mL of sterilized water, 50mL of 10X TBE, and 100mL poly-acrylamide. Then 630uL of APS and 60uL of TEMED is added for every

50mL of the premix. The resulting solution is poured into the constructed gel apparatus and allowed to polymerize for 15 minutes. An 8% gel forms a relatively loose polymer matrix, which will allow even relatively large particles to move through the gel. Then the gel is loaded with the dyed PCR product. The first well is filled with KB+, a DNA ladder so relative size of molecules can be compared during analysis. The next 48 wells are filled with the PCR product in consecutive order. Then the parent (PSB Rc 18), donor (IR64), control (water), and the DNA ladder are added. The next 48 wells are filled with PCR product including the parent, donor, and control. The last well is filled with DNA ladder. This loading strategy is necessary, as it will facilitate analysis of the PCR product columns after PAGE.

The gel electrophoresis works by separating different molecules of the amplified DNA fragments by molecular weight. After the gel is loaded with the PCR product, an electrical current is induced causing the macromolecules in the gel to move toward the anode with the opposite charge. Since different molecules have different sizes, they will move at different rates through the polymer matrix creating a banding pattern. The bands closest to the bottom are composed of the lightest particles whereas the bands closest to the top are composed of the heaviest particles. The banding patterns create unique fingerprints of the DNA that can be used to analyze the DNA's composition. Subsequent comparisons of the columns indicate the variability of progenies. The variability is dependent on the molecular makers used; by using a range of molecular markers, each corresponding to a different region of the rice genome, one can analyze each locus of the genome of the progenies against that of the parent and donor.

Marker assisted selection is a process that screens progenies based on their genotype determined with the use of genetic markers. A genetic marker is a fragment of DNA that is associated with a certain region of the genome that is analyzed. With the use of genetic markers one can identify the presence of the region of DNA being analyzed because each marker is specific to a certain length of a chromosome. The marker identifies whether the progeny's genotype is derived from the parent, the

donor, or both (heterozygous). In the first round of selection, the progenies carrying the *Sub1* locus received from the donor were selected. From this pool of candidates, 53 different background markers were tested to determine which candidates had a background most similar to that of the parent. Each of the 53 background markers corresponds to a different part of the genome of the progeny. Thus we were able to determine how much the progenies differed from the parents.

Project Results

The project resulted with the conclusion that one backcross is not sufficient to yield progeny that have both the background of the parent and the *Sub1* locus. Thus another backcross must be preformed with the population that will yield a product that has both the *Sub1* locus and an acceptable amount of background matching the parent. However, the results are convincing that after another backcross, the PSB Rc18 line will be submergence tolerant. The graphical genotype of PSB Rc18 in relation to IR 64-Sub1 shows the regions of where the progeny's genome deviates from the parent's.

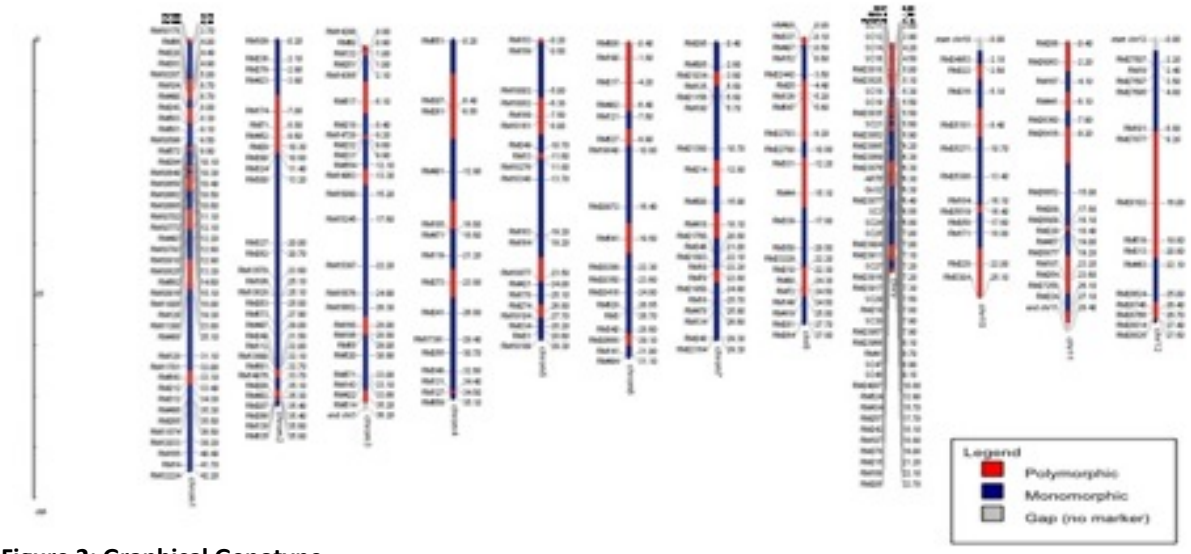

Figure 2: Graphical Genotype

Personal Reflection

My experiences in the Philippines changed my views on food security and the world and allowed me to grow as a person. The internal change was a slow process precipitated by certain experiences.

In the Rice Production Course we had the opportunity to go into the fields. I looked forward to the opportunity to experience what the farmer did each day. I was especially excited, as thus far I had only worked in a laboratory: this was my chance to discover another aspect of rice production. When we arrived at the IRRI experimental farm, I surveyed the area and decided that we must have been in the wrong fields as there was no rice to be seen. Only mud covered the fields. Soon Eugene Castro, the course instructor, walked to where we stood and instructed us to step into the field. As I leveled the mud in the field with my hands, I could not help but notice the stark contrast between the whitewashed laboratories and the sticky mud; it was hard to believe that both were used for a common purpose.

After leveling the mud with our hands, a slow and laborious process, he introduced us to the hydro paddler and the cattle. Both were alternate ways to level the field and both were much faster and more efficient. Next, were given beds of seedlings. We were instructed to place either one or two seedlings from the clump in our hands into each one of the intersections, or hills on the field. After we had planted them in a small section of the hectare lot, we were allowed to rest. Back on dry land, I looked back at the small messy grid that the ten people had spent half an hour planting; the work was labor intensive. That day I realized the amount of work that it took to produce even one grain of rice. That day I gained a deep respect for those that depended on farming for their livelihood.

During my stay at IRRI, I decided that I wanted to learn more from the farmers so I joined a visit to a local village. I noticed the number of houses dwindle until we arrived in an opening with a cluster of houses amid a magnificent sea of green rice fields. I was taken to a small hut sitting aside a gentle stream. When the farmers arrived, I was amazed at how forthcoming they were and how willing they were to discuss issues of food security. As I talked with them I came to know that most of the farmers did not own all of their land. The amount of rice that they grew was not sufficient to support a family and so they had to earn money through other means; some fished while others carved wood. Although most wanted their children to continue farming, they realized that it was best for them to receive an education and move to the cities. As we discussed the other issues facing farmers, it became clear that these problems were common throughout not only Los Baños, but also rather all of the Philippines. Corruption and greed in politics had left the famers with poor subsidies and government programs that failed to deliver. These problems could not be solved in the laboratory or in the field. To truly improve the lives of the rural poor, we cannot stop at science. That day I understood that the fight for food security must also be addressed in politics and policy.

By reaching out and searching for opportunities, I believe I was able to reach a better understanding about the factors that are critical to advancing food security. My experiences not only

shaped a fuller understanding of issues, but they also helped shape me. They have allowed me to become a more independent and cognizant individual. Through them, I have become an individual that will forever be tied to the cause of food security.

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