The International Rice Research Institute:

A Growing Experience in the Philippines

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How I Became Involved

From the time I was a young child, one of my main passions in life has been learning. It didn't matter how much work was involved, as long as I was learning something. I quickly discovered that learning didn't have to be limited to books and school. In fact, I think that if I had restricted my mind to that which my teachers were drilling into my head, I would have lost my interest long ago. Instead, I have tried my best to seize every possible opportunity presented to me to learn more. It was because of this that I first became involved with the World Food Prize Youth Institute.

As I wrote my research paper for the institute, my intentions were most likely different from that of other students. Many were probably very interested in spending eight weeks in a foreign country. To me, an internship in a foreign country seemed exciting, but it was also extremely intimidating. To my parents, sending their youngest daughter overseas alone was almost inconceivable. Once we found out that a separate application would be required later in order to be chosen as an intern, I eagerly registered for the institute. I knew I would enjoy the experience, and I thought I could consider the internship later.

In October of 2002, I attended the World Food Prize Youth Institute in Des Moines. It was an incredible experience, to say the least! I sat in the front row for the World Food Prize ceremony itself, and I discussed my paper with other students and several world leaders, including Nobel and Food Prize Laureates. The whole institute was an unforgettable event that changed my life. I still was unsure about foreign travel, but the next day the previous year's interns made presentations about their internships, and that was what really convinced me. It also convinced my parents that I would be safe. I decided to apply, and was surprised and honored to be accepted as an intern at the International Rice Research Institute (IRRI) in Los Baños, the Philippines.

The Initial Shock

When I first arrived in the Philippines, I was overwhelmed. It was late at night, and I was standing by myself in the Manila airport, feeling nervous, lost, exhausted, and tall. I quickly discovered that as a nearly six foot tall white American girl, there was no chance of me "blending in" with the five foot tall Filipina women. When the man at the international organization desk asked if I was Addie Hall, I'm not sure which one of us was more surprised. Beyond that, I was never entirely sure of what he was trying to tell me, but I saw that he was pointing at a chair...so I sat down. I stayed in my chair and tried to look like I was waiting for something, even though I honestly didn't know what it was. Eventually a car pulled up outside, and the nice man pointed that I should go.

As I stepped out of the airport, the first thing that surprised me was the heat. The air was not only hot, but heavy and humid. I was used to heat during the day in hot Iowa summers, but never during the night. My first breath also reminded me of something else I'd never had to live with before: pollution. As I was standing there staring, it occurred to me that my luggage was no longer next to me – the driver had already started packing it in the trunk of his car. I didn't know what to do next. Should I help him? Should I get in the car? Should I pay him? In the end it didn't matter, since I only had two suitcases. By the time I noticed him heaving things into the trunk, he was done. He then opened the door for me, and I stepped in.

It was these first experiences that made me realize how vulnerable I was. I had no idea where I was, who I was with, where I was going, or what I would be doing once I got there. At that moment, I was fully dependent on this man and his car. After the man started driving his car, I found I was also rather dependent on my seatbelt. In the Philippines, painted lines and road signs (including speed limits) seem to be all but ignored by most drivers.

I was so fascinated by everything that was going on, and so curious about the views from my window. Instead of endless fields of corn and soybeans, there were many separate paddies of rice. The well-built homes and farmhouses I was used to at home were here simply shacks along the side of the road, with small children sleeping on the ground and maybe one animal tied up outside. Also, since we were riding with the windows down, I got some extra exposure to that fresh Philippine air I mentioned. The hot breeze was tolerable, but the pollution from the cars was a huge shock. Every time a jeepney (the Philippine's version of a taxi) passed us, I had to repress a cough. I felt like I was breathing smoke the entire ride, but I didn't want to be rude, either. As curious as I was about the Philippines, eventually I became so tired that I reluctantly allowed my eyes to shut.

About an hour and a half later we arrived at Drilon Hall, the ladies' dormitory at IRRI. I was greeted by two security guards outside the door, a slightly confused and very tired substitute matron, and a myriad of curious geckos. I was given the keys to room number 13, and was told that I could eat breakfast at 7 the next morning. I didn't know where the cafeteria was, but I was too tired to worry about it. I just wanted to find my room and go to bed. I found that my dorm room was very nice, and I enjoyed some much-needed sleep.

After breakfast and some confusion the next morning, I was directed to the top floor of N.C. Brady Laboratory to meet Dr. David McKill, the head of the Plant Breeding, Genetics, and Biochemistry division. He, in turn, introduced me to the man in charge of the Plant Molecular Biology Laboratory (PMBL), Dr. John Bennett, who was to be my supervisor for the next eight weeks. Dr. Bennett took good care of me, and I know I was very fortunate to have had him as my supervisor.

I decided that my main task the first day should be to become familiar with the area. The lab's secretary, Minnie, gave me a map to help figure out where I was, where the important buildings were, and how and when to walk between them. I was also given a desk and a tour of the labs. The thing that surprised me most about IRRI was how diversified it was! Since it is an international center, I was surrounded by people from all around the world. I still felt tall, but also very young. I was greeted with dark eyes looking up at me, and often a response of *"Only 17?!"* when they asked my age. The people closest in age to me at IRRI were the 27-year-old scholars doing PhD thesis studies. I soon became known as the "lab baby", which I found ironic since I was a foot taller than many of the people in my lab.

The next morning, I had a discussion with Dr. Bennett. He asked if I had a particular project in mind, which I did not. I knew nothing about rice, so I was open to suggestions. I did, however, tell him one thing, and that was that I didn't want to just learn what other people already knew, I wanted to DO something. I wanted my work to be something that would actually make a difference, especially after seeing a brief glimpse of the way some live in the Philippines as I left Manila. It didn't matter how much work was involved, I was ready for a challenge. I knew it would be rather difficult for me, since I was not only young and inexperienced, but I also knew nothing about the plant material or the lab equipment. Dr. Bennett realized this too, and he said those were some of the problems that new international scholars often faced. However, one problem that I did *not* have to face with him was a language barrier. At IRRI, everyone needed to know or learn English, and he noted that I could already speak, read, and write that language particularly well.

When I found out I was going to the Philippines, I was fully expecting to learn some Tagalog, the local language. From what I have heard since, most foreigners expect the same thing. However, the Philippines has been so influenced by the U.S. that almost everyone speaks English. Even all of the international staff and scholars speak English, some better than I do. At first I was a little disappointed that I wouldn't have a uniquely Filipino experience, but I realized that the diversity was even better. I was surrounded by people from around the world, so I was exposed to many different

cultures all at once. I did learn a few words in Tagalog, as well as some in countless other languages. It certainly made the world seem smaller!

Rice Problems and the IRRI Solutions

"Rice is life." I had heard the phrase before, but never really understood it until this past summer. I was obviously not used to eating rice for every meal, so people started to ask me what *my* staple food was, if it was not rice. I had no answer. Iowans grow a lot of corn and soybeans, but I didn't consider them staple foods. I thought of potatoes, since they can be mashed, baked, and even made into things like fries, chips, and hash browns. The closest food I could come up with was bread, but I told them that it still was not nearly the same. We did not have a staple food that we depended upon. There was no particular thing that I ate at every single meal, and living without any one food (even corn or potatoes or bread) would be practically unnoticeable to me. In the Philippines, rice was definitely the staple food. Without rice, people simply would not survive. Even the workers at IRRI were paid partially with bags of rice, and it was unheard of to eat a meal without rice of some kind.

Rice farmers around the world face many of the same problems, such as pests, drought, and low-yielding plants. Encouraged by Dr. Norman Borlaug's success with Mexico's wheat crop, the Rockefeller and Ford foundations combined their resources in 1958 to create the International Rice Research Institute. This was the first international center devoted to increasing rice production through science, research, and training. Rice may not be a staple in the U.S., but it is in many other places. For example, *eighty percent* of Asia's total calories consumed come from rice. That's four-fifths of an Asian's diet. To them, rice most certainly is life. So why the concern for more rice? At the time, the usual increases in rice production were dwindling, while the population continued to rise drastically. Fierce competition for land and water drove people to urban areas to find alternative work, fueling the cycle. In order to save the rice-consumers of the world, something needed to be done. That's when IRRI was born.

IRRI's motto is "Rice Science For a Better World." This rice science encompasses many things, from their international gene bank of rice to their efforts to develop higher-yielding rice varieties. In the1960s, IRRI researchers began cross-breeding varieties already improved in other countries, such as Japan, China, India, and Taiwan. This led to the development of IR8, a new rice variety with a higher yield, shorter maturation time, and greater resistance to blast (a fungal disease). These favorable traits, among others, caused IR8 to be called a "miracle rice." This development helped spark the Green Revolution of the 1960's and 1970's. It also stimulated further rice research to find rice plants tolerant to anything from drought to submergence and areas from upland to lowland.

Of course, not only is the plant itself a concern in new rice varieties. The grain quality is an important factor as well, and the intended location is something that has to be considered. Different regions prize different types of grains. In some areas, long well-defined thin grains are best. Some others prefer short sticky round grains. These qualities must be taken into consideration in order for a new rice variety to be a success. Since IR8, IRRI has developed many more successful rice varieties.

IRRI is divided into several different divisions. My division, PBGB, has had a huge impact on rice production in the past. Gurdev Khush was head of this division and the principle plant breeder for 30 years. During that time, over 300 new lines of rice were developed, and now over sixty percent of the world's rice field area is planted with IRRI varieties or their offspring. In the future, IRRI researchers hope to produce a low-tillering rice plant (See Note 1), as well as develop rice varieties resistant to numerous stresses. I was chosen to be an intern at this particular institute because of my interest in the field of genetics. While I was there, I worked with drought tolerance and screening methods to find tolerant plants.

My Project

For me, the most important part of my internship was my project. For eight weeks, my entire schedule (including eating and sleeping) was manipulated around the growth, sampling, and testing of rice plants. The following is a scientific paper I wrote during my internship after some initial research and experimentation. It describes the start of my work at IRRI with some basic principles, experimental methods, and discoveries.

Determination of Protocol for Testing Starch Presence in Rice Leaves

Summary

Methods exist to determine the presence of starch in the pollen of rice plants. Methods also exist to determine the presence of starch in *Arabidopsis* leaves. Here, work was done to adapt the starch test for the leaves in *Arabidopsis* to use in rice plants. As the method was being established, various experiments to test the method's efficiency and reliability were planned and carried out. The results of the experiments used to test the method are described, as well as general descriptions of experimental development. Several correlations found between the plant leaf starch content and other factors are also included.

Introduction

Rice plants, like other plants, use sunlight as a source of energy. During light times of the day, they undergo photosynthesis to produce energy. However, during extended periods of sunlight, the plants produce more energy than they need to use right away. This extra energy is stored as starch and accumulated in the leaves. In extended periods of darkness, such as at night or on cloudy days, the plants degrade the starch. Some mutant plants exist that do not degrade starch normally. The mutants, known as *sex* (starch in excess) mutants, exist in various forms. Screening and studies have already been done in *Arabidopsis*, where the plants became saturated with starch in the light and then degraded starch in the dark. After being in the dark, plants with the sex1 mutation still showed very high amounts of starch as compared to those without the mutation. Work to isolate these mutants in rice would allow for further research to be done on the processes and regulation of starch degradation (Yu et al., 2001), particularly in the area of genetics.

Once the starch in excess mutants are isolated, the plants can be studied to identify the particular gene(s) causing the mutation(s). This would allow sex mutants to be purposely produced for further study in the controlling agents of starch content in the pollen. It is predicted that that the excess starch in the leaves is also indicative of a higher accumulation of starch in the pollen. Research has already shown that stress during flowering has a major impact on the rice plant's yield. If the pollen does not have enough starch, it becomes sterile. However, increased levels of starch in the pollen have already been linked to drought tolerance in the rice plants. It is possible that some mutant rice

plants with increased starch levels might have higher yield. It is also possible that the inability to degrade starch becomes detrimental or even lethal to the plants. None of this is known yet. Once the mutants are isolated, they could be further researched to determine what effect their mutations may have on future rice production.

An effective method for determining the presence or absence of starch in rice leaves could prove to be a valuable tool. "The diurnal cycle of starch synthesis and degradation in leaves allows starch degradation to be studied alongside starch synthesis in a short space of time" (Smith et al., 2003). Therefore, using the leaves to test for starch provides a more efficient screening method when doing research on large numbers of plants, particularly mutant populations. When testing leaves there is no need to wait until the rice plant flowers to test for starch, so testing can be done on leaves much earlier than on pollen. Here, the method and the experimental steps used to test the method on rice plant leaves are described and illustrated.

Results

Although various methods of maceration of the leaf samples and direct staining were attempted (See Note 2), it was found that an extraction of the chlorophyll was first necessary to remove the pigment. The staining was much more visible on the leaf samples without chlorophyll. The method used in testing *Arabidopsis* (Yu et al., 2001) was found to be effective in rice samples as well. The leaf segments did not turn completely white after chlorophyll extraction, but were pale enough to see the staining (See Figures 1-3).

After the method was determined, the experiments helped to uncover many other factors affecting the leaves' starch staining. When samples were collected in the morning, the samples showed little, if any, stain (Figure 1). It was determined that the leaves had not yet accumulated much starch, since much more starch was present in the rice plants in the afternoon once they had been exposed to many hours of sunlight (Figure 2). Another important factor was the intensity of the sunlight. More starch accumulated in bright light than in dim or shaded sunlight. These generalizations only held true, however, for normal, healthy rice plants; stressed plants did not seem to give such clear results, as our technician Nards had predicted (Figure 3).

It was found that the starch content was even different within the same environment. When leaves on the top blocked sunlight from the rest of the plant (Figure 4), the leaf samples showed a wide range of starch accumulation possibly due to this shading (Figure 5). The results could even be different within one plant, since the areas of each leaf are different ages, and give varied results. The leaf tips are the oldest, the bases the youngest. The old and sometimes deadened areas of the tips were determined to be unreliable places to sample because of their dissimilarity. However, the young bases seemed to stain consistently less than any other region. Because of their immaturity, leaf bases don't contain very high amounts of chlorophyll and therefore probably do not undergo as much photosynthesis. For these reasons, it was decided that the best place to sample would be in the middle section of the leaf.

More uniformity in these environmental factors can be achieved by using a controlled environment like the phytotron (See Note 3). However, it should be kept in mind that in any experiment, no environment will be perfect, and there will always be uncontrollable variations. Furthermore, it was found that if the light source is too intense,

it could kill the plants and also rapidly dry them out (Figure 6). In tests, a greenhouse seemed to provide a more natural and less stressful environment for the plants, but nature was often too unpredictable for experimental use.

Discussion

In designing an experiment with rice plants, several things should be kept in mind. First of all, the experiment should be planned in detail. The entire process needs to be thought through to make sure the steps are clear and correct. Another thing to consider in planning is the timeframe. If only two weeks are allotted for an experiment, three-week-old plants cannot be used unless planning, planting, and preparation were done well in advance.

While performing an experiment, all factors should be kept the same to make sure that only one variable is tested at a time. Samples should be taken from the same age of leaves on each plant, and from the same place on each leaf. Also, factors like water, temperature, plant type/age, and available light should be kept as uniform as possible when they are not being purposely manipulated.

One very important step of any experiment is to record and label all results for future use or study. Data should be well kept and organized, and presentable at all times so that results can be shared with others. Also, comparing data from different experiments is important. One test's results have no meaning unless there is something being compared. In most experiments, it is necessary to manipulate the variables for the sake of comparison, such as testing at different times, testing different plants or plant ages, or testing plants in different locations. However, only one variable can be changed at a time. If a sample from a seedling in the greenhouse taken in the morning is compared with an afternoon sample from a mature plant in the phytotron, no good assumptions about any particular variable can be made. There are too many differences to determine which caused the change in the results.

Finally, all experiments should be repeatable experimentally, biologically, and in an assay. This is not to say that repetition will produce the same values each time. If the same experiment is performed many times, the results probably will not be the same because of uncontrollable environmental factors. The results should, however, be located around a central mean value. If two similar rice plants are used, the results should also be close to that mean value. In an assay, the rice plants should give consistent results over time. Other people should be able to perform the same experiment and obtain relatively similar results.

Materials and Methods

The general procedure used to grow IR64 (wild type) rice was as follows: I germinated the seeds by placing them on a wet paper towel in a sealed petri dish and incubating it. In order to germinate, rice seeds must be moistened and warmed slightly (Hoshikawa, 1989). After the seeds had germinated, about 60 of them were planted about 1.5 cm apart in rows 3 cm apart in a plastic tray of soil. The seeds were lightly covered with soil and sprinkled with water. Then the whole tray was put in the dark to prevent further stress on the young plants as they grew and adjusted to the soil. Only about 50 plants were needed, but twice as many were germinated in case the seeds didn't

develop properly. Then 60 were planted in case any of the plants died. The rice plants used for these experiments were IR64, but from previous plantings so they were more mature.

Several plants, both old and young, were placed in phytotron chambers. One chamber was light (Figure 6) and one was dark (Figure 7). By having one dark cabinet and one light cabinet, I could control how much light and dark my plants were exposed to simply by moving them back and forth between the two cabinets. The temperature remained a constant 29 degrees Celsius, as it is a temperature conducive to rice growth. Another cluster of plants was located in the greenhouse (Figure 4). Leaf samples were collected from different plants at different times and put on ice to carry to the lab to keep the enzymes from being active. For my preliminary experiments, the ice and foam bucket were sufficient. Later I used tubes both filled with and surrounded by liquid nitrogen, storing the samples in the -80 degrees C freezer. After the leaf samples were collected (from the middle part of the leaf), they were heated in ethanol to extract the chlorophyll (Figure 8). After most of the color had left the leaves, the samples were removed from the alcohol and put into separate wells. When the IKI stain was added (Figure 9), areas of starch appeared a dark black color. If the leaf was saturated with starch, it turned completely black. If the leaf had no starch, it had no color change. Varying degrees of starch content in between produced lighter or more localized areas of starch staining as opposed to the entire leaf turning dark black or staying light. After staining, the samples were rinsed in water to remove any excess IKI.

Project: Establishing Protocol

I had already learned enough about the materials and the lab that I could work fairly independently, with the supervision and excellent advice of Dr. Bennett. At this point, I could apply what I learned and use the new information to plan further tests. I discovered many things in my first experiments, but I still had a lot more to learn. After I had determined this basic protocol, I had to face the challenge of adapting my assay to be one that was not only reliable, but that could be used on many young rice plants in a short period of time. The first challenge was to find out how old the rice plants needed to be before they began to accumulate enough starch to test positively. As in my preliminary experiments, I began by germinating and planting seeds. I had three main reasons for the continued use of seeds:

1. I could test the plants at any stage of their early development to find the youngest reliable age for testing starch accumulation.
2. The plants would not need to be transplanted, and therefore would not be under any additional stress.
3. I could control the spacing. The seeds could be planted far enough apart that shading would not be as much of a problem.

After testing the plants and performing various experiments, I concluded that the rice plants needed to be two weeks old before they could be accurately tested for the presence of starch. Two-week old plants tested well; they stained dark when kept under light, and showed no stain when left in darkness. The plants were also just small enough that they did not shade each other with their leaves, so there was no variation among plants in the same tray.

I planned my next set of experiments to determine the light-dark schedule. First, I had to determine how long the plants needed to be in the light before they were saturated with starch. Then I

had to find out how long the plants should be kept in the darkness. Determining the amount of time in the light was not too difficult, since once the plants were saturated it didn't matter if they tried to accumulate more. However, once the plants started degrading starch I couldn't let them continue indefinitely. If the plants remained in the dark for too long, even the sex mutants (especially those only partially mutated) might degrade their starch, giving me no positive results. If I didn't leave the plants in the dark long enough, none would have degraded starch and I might think they were ALL sex mutants.

Using two-week old IR64 rice plants, I began this experiment with the simpler question of light. First I had to make sure the plants were starting with no starch. I kept the plants in the dark for almost 3 days (about 65 hours), then moved them into the light to start the experiment. I tested several plants after 12 hours in the light, and still saw some slight variation. I tested again at 20 hours, and all appeared to be saturated. The stain turned all of the samples completely black, so I decided that I should use a minimum of 20 hours light in my assay.

I knew I'd have a more difficult time determining the length of darkness, since I was searching for a smaller time span. After an initial sampling at 0 hours, I placed the plants in the dark. I knew they would not show much of any accumulation until approximately 12 hours later, so I decided I would sample four random plants after 12 hours, then every 6 hours until I saw no stain. I ended up testing at 0, 12, 18, 24, and 30 hours dark, which created an interesting sleeping schedule for me. During this experiment I stayed up late to sample and test, then slept with a container of liquid nitrogen and sampled plants at 4:00 AM! I knew that if I obtained good results it would be well worth the extra effort and lack of sleep.

The samples after 24 hours dark showed almost no staining, and there was little or no difference between 24 and 30 hours. Therefore, I knew that 24 hours was the right amount of time for the plants to remain in the dark before they were sampled and tested. Now I knew all the details of my screening method. *I* could test the plants and tell the difference between samples showing starch accumulation and those without. The next step was to find out if *anyone* could determine this same thing. To test my method, I used a blind assay. It was only a single blind experiment, since I would still be sampling and testing, but someone else would be evaluating the results. When asking for evaluations, I used a random numbering system and asked people to group the samples according to color. Everyone grouped the four samples from the light plants as the darkest, which was what I had hoped for. My protocol was a success! The following is a complete description of my blind assay procedure, as well as a picture of my results.

Blind Assay Procedure

I germinated 100 seeds by placing them in petri dishes with a moistened paper towel and incubating them for three days. After three days, most of the seeds showed growth, so I planted the seeds in a tray of soil. After planting, the tray was covered for the remainder of the day to prevent further stress on the plants. Then the tray was uncovered, and I allowed the plants to grow in the greenhouse under normal conditions for 7 days. Then they were taken to the phytotron and placed under 12 hours light, 12 hours dark for 2 days, after which they were moved to another cabinet for 16 hours of light. Late afternoon the next day, the tray in the phytotron was covered. The tray remained covered for 55 hours, at which point I collected 4 leaf samples and the lights in the phytotron cabinet were turned on. The lights remained on for 20½ hours, at which point I collected

another 4 leaf samples and the lights were turned off. I left the tray in the dark for 24 hours before collecting another sample. These were my results:

After 55 hours dark.

After 55 hours dark, 20½ hours light.

After 55 hours dark, 20½ hours light, 24 hours dark.

When selecting a leaf to sample, I looked for leaves that were very green, as well as wide and healthy. I did not use small skinny leaves, or leaves that showed yellow, white, or brown coloring, since they had not been staining as well in my other trials.

Project: Mutant Screening

After the blind assay, my protocol was established! I was extremely proud, and I was also anxious to screen some mutant rice plants using my new method. At this point, I only had about three weeks left in my internship. Since the plants needed to be two weeks old before screening, I had to start planning my experiment and planting seeds right away. I met with Dr. Bennett and Dr. Wu to discuss what type of mutant seeds I should use. Since *sex* mutants would probably be slow growing, I wanted to use mutants showing this particular phenotype. I hoped that the mutants would not be so slow growing that they would not be large enough for an effective assay.

We decided to use mutants from the EMS mutant collection, meaning that they were point mutated using Ethyl Methanesulphonate. Each plant contains about 200 point mutations, giving me a better chance to find some mutants. I used the M2 line (the second generation) so that any homozygous recessive traits would have a better chance of existing and surviving. Using M4 is usually even better for finding mutations, except that in my case, this particular mutation might be sub-lethal or even lethal. By M4, the healthiest plants would have been selected, so the lethal traits would already be removed. DEB mutations were also available, but this type of mutation involves complete deletion of genes. Therefore I could have had the same problem: the mutants I wanted to find might not grow at all.

Before I could start planting the seeds, I had to plan how I was going to do the assay. Because of my limited time frame, we decided I should screen only one of the four boxes of mutant seeds. Even that would still be a challenge, since that one box contained 470 small packets of seeds. Because of time constraints, I decided against pre-germinating the seeds. To decrease the risk of seeds that did not grow or naturally-occurring albinos, Dr. Wu suggested that I plant 3 seeds from each packet of mutant seeds.

Next I had to spend a few days determining how I would plant that many seeds. Just planting the seeds would have been relatively easy, but I needed to keep track of each plant so that I could determine exactly which mutants showed the excess starch characteristics. I decided to plant the seeds

in rows of 10, to make the numbering system easier. I planted 6 rows per tray, with 3 from the same packet on each side. I called these sides A and B, and labeled both sides of each tray with tape (1A, 1B, 2A...24A). I also put the same number/letter on two wooden pot markers per tray, as well as the mutant numbers for that particular section. For this planting scheme I needed 23½ trays, all filled with soil, each tray containing 60 seeds and representing 20 different packets. I also needed a lot of space in the greenhouse, someone to water my plants daily, and both of my phytotron cabinets available when I needed them. I learned a lot of organizational skills and preparation tactics during this stage of the experiment!

With the help of two of my fellow lab workers, we spent an entire afternoon planting 1,337 seeds. With so many seed packets, I should have had 1,410 seeds to plant. However, some mutant plants had produced more seeds than others, so there had been a few nearly-empty packets originally. Also, since I was not the only researcher who had used the seeds, some packets had been used up completely or only contained one or two seeds. This made my planting method a little more challenging, but I instructed my planter helpers to maintain the rows. If a packet did not contain three seeds, we would simply leave a space wherever those seeds should have been. I also wrote this information down, and kept a detailed planting chart to show exactly what seeds were planted where. That way I could look back later and see which seeds did not grow, and which specific plants we had planted and sampled.

After all of my seeds were planted and organized and growing happily in the greenhouse, I had two weeks to wait. I used this time to prepare a presentation for our weekly lab meeting. Using PowerPoint slides, I explained where I was from, the World Food Prize program and Borlaug~Ruan Internship, and the work I had done so far in the Philippines. I also used this time to plan my sampling procedures. The ideal sampling method would have been to use one labeled test tube per seed packet and keep all of the test tubes in liquid nitrogen. Unfortunately, that would have required 470 test tubes, about 70 sampling containers, and many gallons of liquid nitrogen. We did not have that much equipment or any convenient way to transport it, so I had to be creative. Another potential problem involved opening and closing that many test tubes and containers while I was sampling; the plants that I sampled last would have been exposed to different amounts and intensities of light than those that I sampled first.

At first I was going to assay all of the plants twice: after the light period to make sure they had accumulated starch, and then after the final dark period for the actual screen. Since the sample after light would have just been a check, we decided I should just sample one plant at random from each tray. After all, my assay had been tested and I trusted the methods I established. I also knew that I really didn't need to sample EVERY plant in the final assay. Since I had planted three seeds from each packet, I only had to use one plant out of those three. I came up with a plan that would use 47 test tubes, and I was told that if I could use one of the large sampling containers that I could fit up to 50 test tubes in it at one time! Using just one container would make my sampling a lot easier, but my creative method was slightly complex. Ten leaves would be placed in the same test tube, and we had to be able to tell them apart back at the lab so I could match them with the packet number. For this, I used different types of cuts (different angle and length) to differentiate between plants.

My initial check after the light period was a success, and my sampling went well since I had good helpers. I stored these samples in liquid nitrogen in the -80 degree Celsius freezer until I was ready to assay them that night. By this time, my internship was very close to being over. In fact, the night that I was testing all of my samples was the night before I left at 4 AM for Manila to catch my plane! I hadn't even started packing yet when I began my tests. I knew I'd be short on time, but I didn't realize that my testing would take as long as it did. Thankfully, two more wonderful people

volunteered to help me, so I was able to get all of my plants screened and my things packed before I left for Manila. I hadn't slept at all, which made for an interesting trip home, but I did make it. The most exciting thing was that I found some potential sex mutants! They are currently being analyzed in the lab. The tissue cultures of the seeds are being stained, and I am continually updated as to what is discovered. I do not know much more right now, but I will hear more in the future as my project is continued.

Mid-American Energy in the Philippines (CalEnergy), PhilRice, and the Philippine Carabao Center

During my stay in the Philippines, I was given the chance to visit Nueva Ecija, a province in central Luzon. While there, I visited the Casecnan Project, PhilRice, and the Philippine Carabao Center. Mr. Robert Eugenio, Vice President of CalEnergy, invited me and a chaperone to tour the Casecnan Project. The project uses large pipelines to divert water from the Casecnan and Taal Rivers. This water is used to produce hydroelectric power and irrigate rice fields in Central Luzon. This recent Casecnan Project began operation in 2001, with plans to power 200,000 homes and irrigate 137,000 hectares of land. This extra water would produce an additional 465,000 tons of rice each year.

Zahed, a scholar in my lab, agreed to be my chaperone on the trip. CalEnergy provided us with a driver, who braved the nine hour journey to Pantabagan Lake. While there, we toured the site, the powerhouse, the intake sites, and the actual tunnel containing the pipeline. It was a very interesting and educational visit. We stayed the night there at the project, with extremely nice accommodations. The people at the Casecnan project were wonderful, and the experience was absolutely amazing. I even got to take my first ride in a helicopter on our way to the intake!

In Nueva Ecija, we also went to the Philippine Rice Research Institute (PhilRice). We learned about their facilities, services, and research as we toured the various buildings and laboratories. It was interesting to compare the work at PhilRice to that of IRRI. Both institutes had some of the same goals and were researching some of the same things, but they utilized different methods and equipment to do so. Like IRRI, PhilRice also had a collection of different types of rice seeds, although it was not nearly as extensive as the gene bank at IRRI. PhilRice also develops various foods from rice, including rice flour and rice wine.

The Philippine Carabao Center (PCC) was the final place we visited on our trip. I had never seen a carabao (water buffalo) before, but now I have seen several! Carabaos are very important to people in the Philippines. Not only are they used as horsepower in the rice paddies, but they are also a source of milk, meat, and hide. As we toured the labs, we learned that researchers at the PCC are working to preserve the natural biodiversity of the buffaloes by establishing gene pools. They also crossbreed the Swamp and Riverine Buffaloes to try to create better genetic combinations. Using modern biotechniques such as embryo transfer and *in vitro* fertilization, the center is heading towards genetic improvement of the carabaos.

The Philippine Carabao Center is also striving to develop new carabao-derived products that utilize the animal. I got to taste carabao milk back at IRRI, and I thought it tasted wonderful! Along with the new products, the center also focuses on marketing of such products and tries to create a demand for them. This would provide farmers with an additional source of income. Like many other centers around the world, the work of the PCC is ultimately intended to help achieve food security and alleviate poverty. Such a task can be daunting, but I think the PCC is definitely on the right track.

Although this trip was only two days, I saw and learned a lot while in Nueva Ecija. I also had a great time. It was one of the most exciting trips I took while in the Philippines, and I'm very grateful that I got the chance to go. Seeing different parts of the Philippines was an eye-opener as well, and I was still continuously surprised at how nice everyone was. It was a pleasant experience to learn so much and have fun at the same time.

Other Activities and Friends

Of course, my internship was not ALL work. I also took several trips in the evenings and on weekends. In fact, on one of my first days at the institute I was approached by a scholar from Iowa named Pamela who invited me on a weekend trip to White Beach! We went with another new scholar from France, and we had a wonderful time. I was very surprised that another Iowan was at IRRI, and I was still not used to the diversity. White Beach even had a wide variety of nationalities: Korean, French, American, Filipino, Chinese, Japanese, and many others. White Beach also had a wide variety of things for sale. Everywhere you turned, someone wanted to sell you something. The people on the beach made their money from tourism, and we were the tourists. Some offered goods, some offered services, and some young children simply asked for money. It took some getting used to, but I eventually adjusted to the atmosphere. I also found some children playing who were eager to get their pictures taken. I couldn't understand how everyone could be so poor, but yet so happy.

Everyone was remarkably nice to me, even though there were tensions between America and many other nations. I couldn't help but think about times I had traveled in the U.S. when people had not been nearly so kind to me as the people at IRRI. The people of the Philippines were not only friendly, but they were almost always happy. They were smiling or laughing, and oftentimes I would hear a security guard or traffic officer singing. Even though many people are poor and the economy is not great, most Filipinos didn't seem to be worried. It was a less stressful and less hurried place. Every time I walked into or out of a building past a security guard or worker I was greeted with a big smile and "Morning, ma'am!"

In the beginning I was quite shy. Among all the diversity, I soon learned that when I did talk, it was way too fast. Most people spoke English, but they were not nearly as fluent as I was and I had to keep reminding myself of that. Otherwise I got a lot of blank stares when I tried to speak to someone. The first source of evening entertainment I had at IRRI was the piano in the dorm lounge. I played American music, and sometimes I even attempted the Philippine's national anthem, although I don't think it was recognizable. One day I went in to play and someone was watching the television. I hated to play while she was trying to listen, so I sat down with her instead. That someone turned out to be Rachel, and we became friends. She familiarized me with the area and the local shopping centers, and even took me on a trip to Pagsanjan Falls, where we canoed down a river looking at beautiful waterfalls. She also introduced me to soccer.

Soccer is a popular sport in the Philippines. Soccer also led me to discover the Association For Scholars, Trainees, and Researchers of IRRI (AFSTRI). This group scheduled weekly movie nights, and even occasional shopping trips to Manila. I was able to go on one of the shopping trips and attend several of the movies where I met even more people. However, I think that the most exciting AFSTRI activities were their sporting events. AFSTRI had their own sports team, and they played games against other teams (teams of IRRI security guards, particular labs within IRRI, even the university's international students), depending on what sports season it was. When I arrived, soccer was in full swing! I couldn't play in actual games without a uniform, but I settled for being the team's photographer. I did get to play one game just for fun. I was a little scared and I had no idea how to play soccer, but I sure had fun trying!

I made a lot of new friends through soccer and AFSTRI. At the end of the soccer season we had a soccer party, and I volunteered to make pies. After all, people do refer to apple pie as "American". I had wanted to bake pies before, but I could never find an oven! Apparently people in the Philippines don't bake as often as in the U.S. I could find available microwaves and sometimes stoves, but the only oven I had located at that point was at Paul's house, which was, luckily, where we had agreed to have the party. I made five pies that night, and quickly discovered why people don't bake much in hot climates! The lab enjoyed the leftovers, and when I heard about another oven in IRRI's fire brigade I made five more pies a few weeks later to share with them. The Filipino firemen seemed very curious about what I was making, so I left them a pie, too.

The people at the lab were like family members to me. Among my lab family were several scholars doing PhD thesis studies. Students from all over the world come to be scholars at IRRI; in my lab alone we had Japan, China, and Bangladesh all represented! Sometimes we would get together for supper, and we'd each make food from our own country to share with everyone. Those were the best kinds of parties, since I got the chance to be around people and food from around the world. The get-togethers were a break from my daily cafeteria meals of rice, although Riceland (the name of the cafeteria) did have interesting food as well. During my stay, I tasted two types of squid, beef tripe, lots of fish, and many other unusual things. I was not picky, and strived to try at least one new food or drink at every meal.

One kind lady in particular, whom everyone called Beng, became my mother while I was in the Philippines. She and her husband, Glenn, took me to church, took me shopping and out to eat, and even let me stay the night at their house on weekends. Church in the Philippines was a very unique experience, and many Filipinos are quite dedicated and passionate about it. Beng and Glenn also took me on a trip to the Taal volcano. It was very beautiful, but the volcano was not all I saw, of course. My eyes were again opened to the poverty in many areas. There were women in high heels or sandals leading horses up and down the rocky slope for a small fee, and people at the top selling drinks and buko (young coconut).

I remember one particular trip to town where I rode on an old railroad track. A small young boy wearing thin sandals lifted a big cart onto the track, and proceeded to run behind and push three adults for what seemed like more than a mile. When another cart came towards us on the track, the boy with the least number of passengers on his cart would stop, wait for his riders to dismount, and then lift the cart off the track so the others could pass. I felt so bad, I wanted to help him. The cart was about twice his size, as was I. I still don't know how he managed to do it, but I think it must have just been out of necessity. When you have nothing, you do whatever you can to earn a small amount of money to buy a meal.

People with poor standards of living, families and individuals surviving against odds in poverty-stricken areas, and children starving or people going hungry are EVERYWHERE. This is not an isolated problem. It is world-wide, and one that must be improved by a conscious combined effort. A few people cannot fix the world on their own, but they can help. After all, the world certainly will not fix itself. Looking back, I still agree with what I wrote in my research paper for the 2002 youth institute:

> *It may be idealistic to believe that all of the world's problems can be solved. It may also be extremely optimistic to think that the problems will be given the attention and priority that they deserve. However, if one attempts to save billions of people and does not succeed, there are still thousands upon thousands that WERE saved in the effort. Therefore, their lives make the battle a worthy one to fight.*

One of the most interesting aspects of my internship was the ability to keep in touch with other interns around the world. We shared our experiences and stories, our laughter, and our trials and tribulations. Our observations of people were remarkably similar. Would people like these around the world benefit from improved rice varieties? I am confident that rice science will, eventually, create a better world. But I also keep wondering what would be the best thing to help, both immediately and in the future. I've found that I can't answer that. I may not have any solutions yet, but at least I am aware of the problems. I also plan to help. My goal is to attend Iowa State University and major in genetics, and to continue in genetics research.

During my stay in the Philippines, my views of the world changed drastically. Not only did I gain invaluable experience in the Philippines, but I became interested in other cultures and tried to learn as much as I could. I think I also became more accepting of people's differences as I adapted to a new environment. It was certainly strange coming back to the homogeneity of my own small town in Iowa! One unexpected gain from my internship was self-confidence. Even when faced with obstacles and challenges, I did my best and often surprised myself with the outcome.

My internship was an amazing experience. I traveled to one country and experienced the cultures of almost twenty. I was well-supervised, yet still given the freedom to explore and learn independently. I new almost nothing about rice when I arrived, but I was still treated as a fellow researcher. Working each day in the lab gave me access to advanced technology, information, and techniques that I could have never learned here in the U.S. Still, the best part by far was all of the amazing people I met and came to know. Small towns have many advantages, but they tend to lack the opportunities and diversity that can be found elsewhere. This was the real benefit I found at IRRI. Not only was the experience challenging, but it was also very exciting to be surrounded by experienced researchers from around the world. I found new areas of interest in science and agriculture, as well as new life-long friends.

BIG Thank-You's

- Dr. Norman Borlaug and Mr. John Ruan for this wonderful opportunity
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- Mr. Robert Eugenio, the great people at CalEnergy's Casecnan Project, and Mid-American Energy for coordinating my visit to Nueva Ecija
- PhilRice and the Philippine Carabao Center
- My parents, family, and friends at home for their encouragement and support

Notes, Figures, and Photographs

Note 1: A low-tillering rice plant is one with fewer stalks at the bottom.

Note 2: The final goal of my project was actually to screen mutant plants for sex mutants, but first I had to determine the protocol. With much help from the PMBL, we tried the following methods on both regular IR64 (normal, or wild-type, rice) and a stressed plant:

- Boil in water, boil in alcohol, stain with IKI (entire leaf)
- Boil in water, boil in alcohol, rinse with water, stain with IKI (segment)
- Rinse in TritonX, macerate, stain with IKI (segment)
- Macerate, stain with IKI (segment)

Note 3: The phytotron is a building of indoor growth cabinets that allow the researcher to control how much water their plants receive, the hours of light and dark, light intensity, temperature, and humidity.

Figure 1 – Results of IKI staining on leaf samples taken from plants at 11:00 AM, showing little or no starch present.

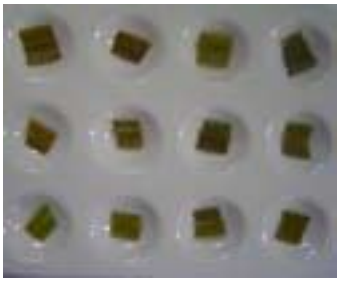


Figure 2 – Results of IKI staining on leaf samples taken from plants at 2:30 PM, showing moderate starch accumulation.

Figure 3 – Results of IKI staining on leaf samples from the phytotron. Top row: dark; middle row: light; bottom row: stressed plants in the light.

Figure 4 – Rice plants growing close together, showing the possibility of top leaves shading those underneath.

Figure 5 – Results of IKI staining on rice plants in the greenhouse that were closely spaced, taken at 4:00 PM, showing a range of starch accumulation.

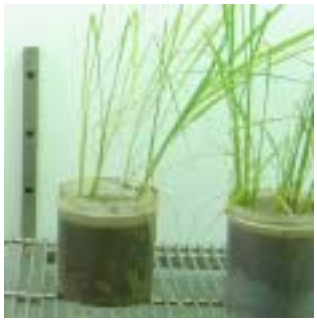


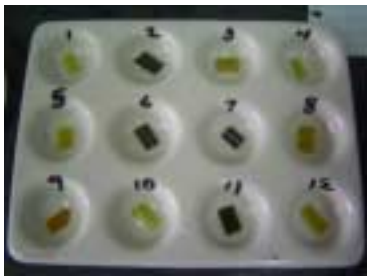
Figure 6 – Rice plants in intense light in the phytotron, starting to dry out. Leaves are bending and becoming a pale yellow-green.

Rice plants in the phytotron in the dark

Extracting chlorophyll with heated ethanol

Using IKI stain on the leaf samples

Leaf samples tested after 0, 12, 18, 24, and 30 hours dark



Randomly numbered samples for the blind assay.

The four boxes of mutant seeds; I screened the top one.

Francis and Blesilda (Beng) helping me plant seeds.

Samples from mutant plants after 20 hours light to test for starch accumulation.

One of eight large groups of mutants I screened; one good mutant is in the middle of the far right row.

Zahed and Beng helping me sample the mutant rice plants.

This is a picture of me with my supervisor, Dr. John Bennett, after he stayed up with me until 4:00 AM to hear my screening results.

The three of us at the Casecnan Project outside the tunnel

The pipeline at the Casecnan Project

This is a picture of me about to ride in the helicopter

One of the sites of the Casecnan Project

Carabao at the Philippine Carabao Center

The rice food products at PhilRice

The collection of rice seeds at PhilRice

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