"Keeping Rice Cool as the World Heats Up"

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Personal Remarks

Growing up in rural Iowa, surrounded by corn fields and knowing everyone in my class, has, for some reason, always left me with the desire to travel and see more of the world. Little did I know that desire would be fulfilled so early in my life. When Cindy Pottebaum, a family friend, first told me about what the World Food Prize was and what it did, I was surprised to learn that there was such a program for high school students. I was only a sophomore at the time so I waited eagerly for the next year to write my paper and attend the Global Youth Institute in the fall of 2011.

The summer before school started I began to do research for my paper on Mongolian agriculture. I didn't even know where Mongolia was located, let alone about their agriculture practices. When I started writing my paper, I began to discover the real need for environmental conservation and adaptations to better withstand climate change. While attending the Global Youth Institute, I was able to listen to the former President of Brazil and the former President of Ghana speak of their work in helping to reduce poverty in their own countries. From African women, to a reporter from Minnesota. to professors at Iowa State University, I had the chance to chat and network with some very amazing people at the conference. Although it wasn't until after hearing first-hand accounts from last year's Borlaug-Ruan interns, that I really began to have a desire to apply.

When I began the application process for the Borlaug-Ruan Internship, I could never have imagined what would be the result. After the interview process, I got the letter in the mail and

raced into town to allow my close friend to open it for me since that had been our deal from the beginning. While at work, she opened the letter as I stood at the hardware store's counter hardly able to breathe. After struggling to open the letter, in a monotone voice she finally said, "You got it." I stood in disbelief. It didn't feel real. Knees trembling, heart pounding, and hands shaking, I could hardly speak or stand for that matter. I would be leaving the comfort of my home for two months during the summer to do research half-way across the world. I've always wanted to travel, but to be traveling abroad as a 17 year-old seemed impossible. I don't even like going to the grocery store by myself, let alone flying alone. I knew this experience would force me to step out of my comfort zone and experience a world I had only seen in movies and read about in books, but I could never have foreseen how this experience would change me a person.

As the days for my departure drew closer, I got more excited and anxious. My mind was full of unanswered questions about what the culture would be like and what kind of work I would be doing. Little did I know that the second my feet hit Philippine soil, I would be welcomed with open arms by everyone I met and have the two most amazing months of my life.

IRRI - International Rice Research Institute

In 2010, the International Rice Research Institute celebrated fifty years of "Rice Science for a Better World." IRRI was founded in 1960 by the Ford and Rockefeller foundation, and has since become the oldest and largest international agricultural research foundation in Asia. The Philippines houses the institutes' headquarters where a wide variety of research is done in an effort to help farmers improve the quality and quantity of their rice in an environmentally sustainable manner. IRRI has been known for being a major contributor to the start and expansion of the Green Revolution. The work done in IRRI's laboratories, greenhouses, and experimental fields has now been estimated to have reached over half of the world's population. Since its beginning, IRRI has produced hundreds of varieties of rice with one of its first varieties being IR8, nicknamed "miracle rice," for helping to save thousands of people in South and Southeast Asia from famine. Millions of lives have already been saved because of the work done at IRRI and this work continues every day.

IRRI's mission statement 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 system."

To further devise a way to comply with their mission statement, IRRI aims to pursue the achievement of five goals. *1. Reduce poverty through improved and diversified rice-based systems. 2. Ensure that rice production is sustainable and stable, has minimal environmental impact, and can cope with climate change. 3. Improve the nutrition and health of poor rice consumers and rice farmers. 4. Provide equitable access to information and knowledge on rice and help develop the next generation of rice scientists. 5. Provide rice scientists and producers with the genetic information and material they need to develop improved technologies and enhance rice production.*

IRRI's mission and goals also aid in the efforts of the United Nation's Millennium Development Goals to eradicate extreme poverty and hunger and ensure environmental sustainability.

IRRI is a non-profit organization and is a part of Consultative Group on International Agriculture Research (CGIAR). CGIAR is a multi-donor trust fund that supplies funding for agricultural research to improve food security by uniting those who finance research and those who carry out the research, to execute projects and experiments that benefit farmers and consumers. Additional funding comes from numerous governments, universities, philanthropic foundations, the private sector, and other non-profit agencies.

IRRI is also the lead center for the Global Rice Science Partnership (GRiSP). GRiSP is a strategic plan to allow for stronger partnerships and more open communication for effective sharing and coordinating research and development. It brings together research institutes, universities, education and extension systems, international organizations, CGIAR centers, the private sector, and civil society organizations. GRiSP is the first of its kind to represent one strategy for worldwide rice research. GRiSP and CGIAR work closely together to do collaborative research in the areas of production systems, environmental issues, and many others involving rice production and food security.

Background

It is estimated that, by the year 2035, an additional 116 million tons of milled rice will be needed to meet the worldwide demand. Rice is a staple food for more than half of the world's population; furthermore, almost 3.5 billion people rely on rice for 20% of their daily caloric intake. For every 1 billion people added to the world's population, 100 million tons of rice will need to be produced to meet the demand. Rice is much more than just food for so many people, in fact, more than one fifth of the world's population relies on rice production for their livelihood, with many of those people living in developing countries (GRiSP et al., 2010).

For decades scientists have been developing and improving rice varieties by making them adapt to drought, submergence, salinity, pests, and diseases, among other things. With advances in rice technology, the global rice production has increased by 344 million tons from 1965 to 2000; however, since 2000 the rate of production has steadily decreased (FAO et al., 2012). Each day the world population increases and the amount of usable crop land decreases due to factors, such as, urbanization, desertification, and land degradation. Already droughts, floods, and other severe weather have affected rice fields all over the world. Occurrences, such as these, are only projected to increase in frequency and severity in the years to come. A major force behind this erratic weather is global warming. The Intergovernmental Panel on Climate Change (IPCC) defines climate change as any change in climate over time, whether due to natural variability or as a result of human activity. In 2007, the IPCC predicted that the worldwide temperature would increase by 2.0 to 4.5 °C by 2100 (IPCC et al., 2007). These temperature trends are particularly devastating to those who produce and consume rice, due to vital role that temperature plays in rice production. With a higher air temperature comes a variety of climate change issues including: precipitation changes leading to more flooding, melting ice caps causing higher sea levels resulting in higher saline delta regions, and an increase in canopy temperatures causing a shorter growing season, higher amounts of spikelet sterility, and greater respiration losses (Wassmann et al., 2009). These transformations in global climate makes it extremely challenging for rice farmers to meet the worldwide demand.

An increase in global air temperature, as previously mentioned, is seen as an increase in both day and night temperatures. Higher day temperature is escalating. Flowering is the most heat susceptible stage of rice production, and in order to protect the rice plant, IRRI scientists are breeding genotypes that can flower during early morning to escape early to late afternoon heat stress and with enhanced transpiration cooling to cool their microclimate. However, these adaptations are not enough to prevent yield losses. Evidence of these losses were seen in 2007, when a heat wave in Pakistan corresponded with the vulnerable flowering stage of the rice variety IR6, resulting in a 30% decrease in yields. Occurrences similar to this are projected to continue due to the flowering stage coinciding with high temperature conditions in many countries including Bangladesh, east India, northern Thailand, and southern Myanmar. In an effort to reduce water scarcity, i.e. newer techniques are tested for irrigating rice namely dry-seeded rice and aerobic rice. While these methods reduce water consumption, it does not cool rice as much as constant wet irrigation. Although day temperatures can damage rice plants, high night temperatures (HNT) have perhaps an even more profound effect. Night temperatures have been increasing faster than day temperatures and with limited stomata activity at night rice is very vulnerable (Wassmann et al., 2010). When the night temperature is higher, rice plants must respire more in order to maintain themselves. This occurrence results in the plant using more energy, thereby utilizing a larger proportion of the stored photo-assimilates, resulting in lower yields. A study done by researchers from the United States, the Philippines, and the Food and Agriculture Organization found that a 1 °C night temperature increase in China, India, Indonesia, the Philippines, Thailand, and Vietnam resulted in up to 10% decline in rice output (Welch et al., 2010). Besides causing a decrease in quantity, HNT causes a decline in quality. In rice, the appearance of "chalky grains" is when any part of the grain is opaque or white compared to the normal translucent appearance. The occurrence of chalky grains, a highly undesirable trait in almost every market, can be detrimental to the farmers' profits and reduces consumer preference. In short, both HNT and high day temperatures in general can have very destructive effects on rice plants by reducing overall growth, pollination, number of pollen germinated on the stigma, and increasing spikelet sterility and inducing yield losses.

In previous years, higher yields were achieved through more effective agricultural practices, such as, improved seeds, inorganic fertilizers, and improvements in the irrigation infrastructure. Now more than ever, agriculturists are faced with the complex task of producing more with less land, water, energy, and emissions. Climate change has created exceedingly difficult problems for farmers and consumers alike.

My Work

My time at the International Rice Research Institute was spent working with the Crop Physiology group in the Crop and Environmental Sciences Division (CESD). The mission of CESD is to conduct research on natural resources management to ensure that rice ecosystems are profitable, sustainable, environmentally friendly, and adapted to climate change. The Crop Physiology group's primary focus is the effects of climate change on rice. Personally, I've always had an interest with climate change. I first examined this topic more fully by choosing to make it the focus of my World Food Prize Global Youth Institute paper on Mongolian agriculture responding to climate change through adapting agricultural practices. By being a part of Crop Physiology team, I was able to further my knowledge in respect to rice and the destructive effects of climate change. My experiments were mainly focused on heat stress with a particular

exploration of high day temperatures, heat stress during different stages of rice production, and the effects of heat stress on nitrogen, carbohydrate partitioning, and spikelet fertility. By exploring these concepts in greater detail, speaking directly with local farmers, and seeing results first-hand, I realized the dire need for continued work to combat climate change. Furthermore, the study of heat stress on rice is a relatively new concept compared to that of drought, submergence, and salinity, which have been around for decades. High temperature variability more than the gradual increase in mean temperature, has become a realistic and problematic aspect of rice production, particularly during the last decade.

Standing in IRRI's experimental rice fields

Introduction to rice and its key development stages

To begin learning about how rice was produced, I first had to learn the biology of rice. Rice, an annual grass, has three main growth stages, i.e. vegetative (germination to panicle initiation), reproductive (panicle initiation to flowering), and ripening (flowering to maturity). During the vegetative stage, tillering occurs, as well as, an increase in plant height, leaf emergence and biomass accumulation. The reproductive stage is defined by culm elongation, a decrease in tiller number, booting, heading, and flowering. Flowering, one of the most critical developmental stage, is defined by “when the spikelet opens, allows pollination to occur and closes leaving the anther and a portion of the stigma outside of the spikelet." Flowering typically occurs early morning from 9 a.m. to 12 p.m. and is an extremely sensitive stag. High temperatures during this time can lead to increased sterility resulting in lower yields. The final stage, ripening, is when the grains are filled, and after the kernels have hardened, the rice is harvested. The rice life cycle requires approximately 110 to 150 days from germination to maturity, depending on the genetic background and the surrounding environment (Moldenhauer & Slaton).

Growing Rice "the Farmers' Way"

Figure 1. Rice planting processes (a) sowing seeds in seedling tray and (b) transplanting seedlings in the field. (c) land preparation using a hydrotiller in the field

In order for me to understand how rice is grown, I was first exposed to initial steps to produce rice. I assisted the greenhouse technicians with seeding, pulling, and transplanting. Seeding is simply the placing of seeds into tray to grow for a short time to germinate. Seedlings are pulled when they are 14 days old and are then methodically placed "transplanted" in straight rows with definite spacing in rice fields. Transplanting is performed with several people moving simultaneously placing the seedlings in the soil then moving backwards for every new row. Two people stand at opposite sides of the rice paddy, holding sticks with a wire connecting the sticks to guide the workers in placing the seedlings. These three parts of rice production were an immense help in understanding the basics behind growing rice "the farmers' way."

By participating in these activities (Fig.1), I was able to acquire a greater sense of appreciation for how demanding rice production is for each farmer. The amount of time and effort put into every rice plant was far greater than I ever imagined. Additionally, almost all the work was done manually starting from applying fertilizer to placing the seedlings in the soil, with few farmers having the luxury of using hydrotillers. Taking part in these steps of rice productions allowed me to experience directly how so many people in the world make their living and understand how physically demanding it is to produce each and every single rice plant.

Experiment 1 - Seedling stage heat stress response in rice

Figure 2. Rice seedlings of 13 varieties in Petri dishes on a thermogradient plate.

Objective: To determine rice varietal difference to heat stress at the seedling stage

Hypothesis: Rice seedlings have definite optimum temperatures for normal growth and development.

Materials and Methodology: In this particular experiment, 13 varieties were exposed to temperatures from 20 to 50 °C on a thermogradient plate during the seedling stage (Fig.2). LED lights produce the necessary light needed for plant growth, without changing the temperature of the plate. The 13 varieties were

placed randomly in the horizontal direction. This process was repeated in a new randomly selected order for each new row to help provide more accurate observations without biases. A minimum of 25 seeds were placed in each Petri dish with soil and were placed on the thermogradient plate for a period of 10 days under well watered conditions. Over this period of time, the number of germinated, emerged leaves, rolled leaves, and dead leaves were observed to determine plant performance. Once the experiment was terminated, the leaf length, plant height, fresh weight, and dry weight were measured.

Results and Discussion: Over the 10 days, noticeable differences occurred among the varieties. The plants in the middle of the gradient plate with a temperature of 35 °C performed much better than those at either end. The growth rate in the cold and hot temperatures was much lower compared to that of the optimum temperatures. This pattern was visible due to the stunted growth and poor plant vigor of the plants at the extreme temperatures. Plants in the optimum (30 to 37 °C) temperatures tended to produce taller plants and exhibited vigor (Fig. 3). Also burnt tips were more prevalent in the higher temperatures. Whereas, plants grown at temperatures higher than 39 °C showed poor growth and some did not survive. Plants grown at temperatures lower than 28 °C were shorter than the plants at optimum but performed better than plants at the high temperature end. More specifically, N22, a rice variety which is highly heat tolerant at the reproductive stage recorded some of the highest percentage of dead leaves compared to other more susceptible varieties (Fig. 3a).

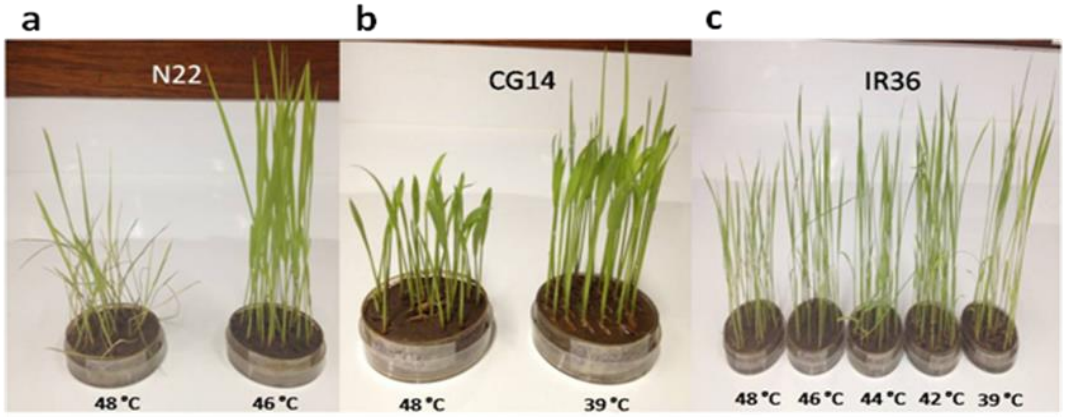


Figure 3. Comparing plant growth at different temperatures in (a) N22 (b) CG14 and (c) IR36.

Conclusion: The results indicated that there was an optimum temperature (30 to 37 °C) below or above which most of the rice varieties are negatively affected and that tolerance at seedling stage (my study) may not necessarily mean tolerance at the reproductive stage (previously published literature), as seen with N22.

Experiment 2 – Molecular study of a rice Heat Shock Protein (HSP)

Objective: To detect the presence of a 22 kDa Heat Shock Protein (HSP) gene in contrasting rice varieties

Hypothesis: Presence of Heat Shock Protein gene increases tolerance to heat stress in rice

Materials and Methodology: HSPs are previously known to increase tolerance in plants and there are different HSPs which are either ubiquitous or specific to leaves or pollen. Leaves from four

different varieties N22, IR64, IR2006, and Azucena exposed to three different temperatures were collected from Experiment 1. For N22, I collected leaves from 24, 33, 44 ^{o}C. IR64 temperatures included 26, 35, 44 ^{o}C, with IR2006 leaves exposed to 29, 33, 44 ^{o}C and Azucena leaves from 26, 35, 48 ^{o}C. These temperatures were chosen to examine the DNA from low, middle, and high temperature regimes on the gradient plate. Also the availability of leaves was a factor, since some Petri dishes contained only dead leaves, and hence could not extract leaves for all four different varieties from the same temperatures.

DNA Extraction and Quality Confirmation

There are several methods of DNA extraction and purification. For this experiment, I followed the CTAB method (Fig. 4a and 4b).The purified DNA was dissolved in sterile distilled water and stored at 4 °C (Fig. 4c). In order to check the DNA concentration and purity of the samples, a NanoDrop spectrophotometer was used by taking a small sample (2µL) of DNA and placed on a spectrophotometer that is connected to the computer (Fig. 4d). The spectrophotometer then reads the amount of DNA based on the ratio of absorbencies at specific wavelengths (260/280 nm). A ratio of approximately 1.8 is generally accepted as pure DNA. If samples are not contaminated, the process can continue.

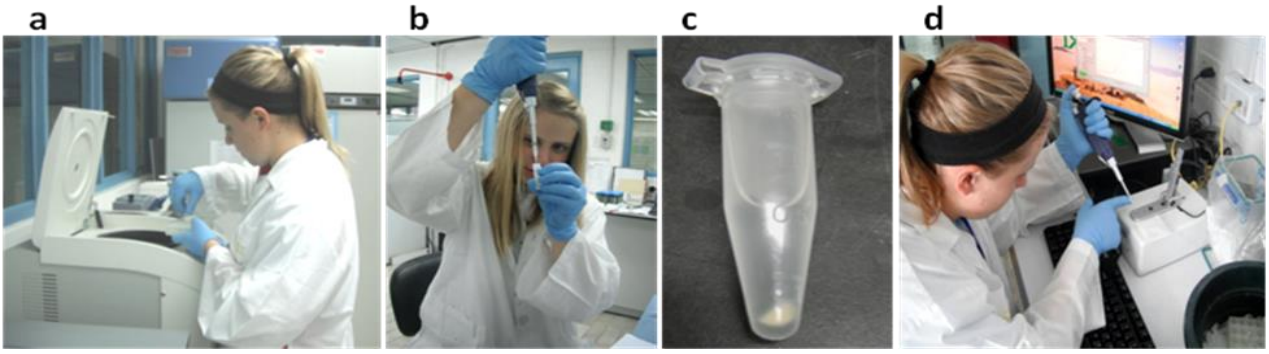


Figure 4. DNA extraction using the CTAB method (a) centrifugation of samples (b) pipetting out supernatant (c) DNA pellet suspended in 70% ethanol (d) DNA quality and quantity confirmation using the NanoDrop.

DNA Amplification through Polymerase Chain Reaction

To be able to determine the presence or absence of the desired DNA region, the amplification of the specific DNA strand must occur. To facilitate this process, the DNA must be run through polymerase chain reactions (PCR). Before amplifying the DNA in the PCR machine a mixture or cocktail must be made by calculating the correct amounts of different components and mixing them together. The cocktail contains sterile distilled water, 10x PCR buffer, 2.5 mM dNTPs, 10 mM forward primer, 10 mM reverse primer, DMSO, and *Taq* polymerase (5 U/µL). Once the cocktail is in each microfuge tube, I added the proportionate amount of DNA (50 ng/µL) from each genotype to the corresponding tube. To prevent degradation, all components are placed on ice for the duration of mixing the cocktail. Finally, I added one drop of mineral oil to each microfuge tube to help prevent evaporation that could possibly occur during PCR. I then placed each of the 12 samples on a 96-well plate and ran my PCR program, which is composed of steps with different temperatures and duration. The first stage of PCR is **denaturation**, where the hydrogen bonds between complementary bases are broken in the DNA. In order to break the

bond, the temperature in the PCR machine is raised to 94 ^{o}C for 1 minute (min). The second stage **annealing**, the temperature was lowered to 54.5 °C for 30 seconds (sec) to allow the single-stranded primers to anneal to the single-stranded DNA. The temperature is then raised to 72 °C to begin the third stage which is **elongation**. This is within the optimum temperature range for *Taq* (*Thermus aquaticus*) polymerase to attach to the end of each primer and synthesize new DNA that is complementary to the starting template with the use of the available dNTPs. The three primary stages (denaturation, annealing, and elongation) were repeated 35 times to make several million copies of the desired DNA fragment and to be able to visualize it in an agarose gel. A final elongation is done at 74 °C for 10 min to ensure that all DNA strands are fully extended. A cooling or final hold step is done at 4 °C for an indefinite period for short term storage of the reaction. The PCR machine lid was heated to 110 °C during the whole process to prevent sample evaporation (Fig. 5a).

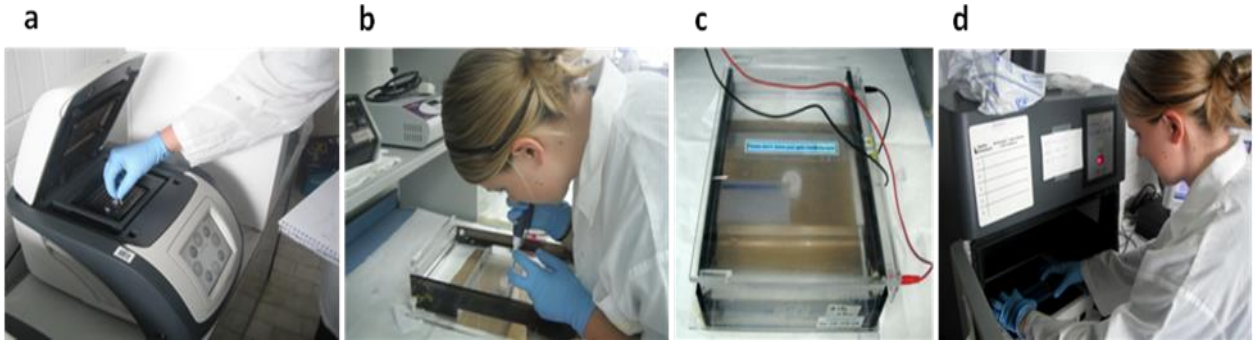


Figure 5. DNA amplification and visualization (a) PCR running, (b) gel loading, (c) gel running, and (d) gel viewing.

Gel Electrophoresis

The process of gel electrophoresis allows the DNA to travel though a gel suspended in running buffer from the negative to positive charge in the electrophoresis tank. DNA is negatively charged so when placed in an electric field, it will migrate towards the positive pole. As the DNA travels through the gel, DNA molecules are separated according to size. The smaller molecules travel faster and therefore can be found in the lower portion of the gel while the bigger DNA molecules are found in the upper part of the gel. A DNA molecular weight ladder consisting of different molecule sizes was run side by side the samples to indicate the size of the accompanying samples. To form the gel, I made a 1.2% agarose solution created by mixing 50 mL 0.5x TAE buffer and 0.6 g of agarose powder. I then melted the powder into the buffer by heating the solution for approximately 2 min, or until the powder was no longer visible. After allowing the mixture to cool with constant stirring, I added 4 µL of SYBR Safe, mixed the solution then poured it into a tray with a comb to cast the gel. When the gel is formed, the comb is carefully removed from the tray and the gel is placed in a gel electrophoresis tank with 0.5x TAE buffer. Before putting the PCR product into the gel, it must be combined with 2 µL 5x loading dye on Parafilm sheet, mixed through pipetting, and put the mixture in the well of the gel (Fig. 5b). After all PCR products and DNA ladder are put in the gel, the electrophoresis tank is closed and the voltage regulator is turned on to start the electric field inside the tank. The charge was set at 160 volts for approximately 50 min. The loading dye should be approximately three fourths of the way down on the gel in order to allow proper migration of samples and DNA ladder (Fig. 5c). After the DNA has ran down, the gel can then be viewed under UV exposure (Fig. 5d).

PCR Optimization

For the primer pair used for HSP 22 kDa, the optimal annealing temperature was unknown. A PCR cycle with a gradient temperature from 50 to 58 °C for the annealing step was performed. After viewing the gel, I found that temperatures between 54°C to 57 °C were the temperatures amplifying the target molecule size. I chose 54.5 °C to use for all the samples in PCR due to the fact that this temperature had the the clearest band and the least amount of unspecific products

Results:

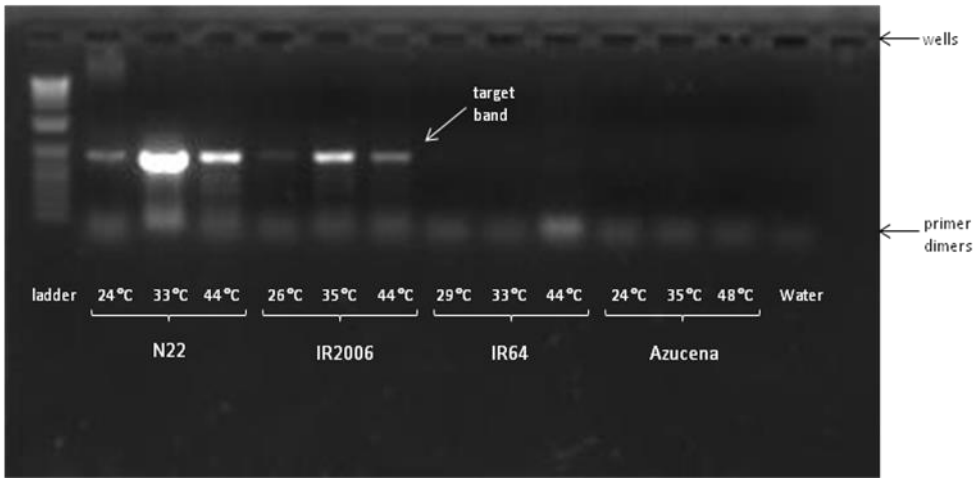


Figure 6. Gel image of PCR products of different varieties having HSP 22 kDa.

Analysis: Based on Fig. 6, N22 and IR2006 showed clear bands with a size of ~800 base pairs (bp) on the gel for all temperatures where leaves were sampled. IR64 on the other hand has a faint band on 44 °C and Azucena did not have any. No band was seen on the water control, which indicates that there is no contamination during the PCR and running the gel. The bands at the bottom are primer dimers, which are by-products of PCR where primers attach to each other due to some complementary bases rather than attaching to the DNA. However, it should be noted that PCR was repeated multiple times and all varieties produced bands. Unfortunately, I was not able to produce a gel having all bands present. Based on these results, it show that HSP 22 kDa is present in all varieties at different temperatures on the temperature gradient plate. This demonstrates that even if the plant carries the gene, it does not guarantee tolerance to high temperatures as seen with the poor growth of some of the plants on the thermogradient plate.

Conclusion: PCR only measures the presence and absence of a gene and does not measure the magnitude to which the HSP is expressed, so we cannot know from these results how much the gene influences heat tolerance. Further tests can be done to see how much 22 kDa HSP is expressed by using RNA as the starting material and analyzed through RT-PCR or quantitative RT-PCR, which will measure the expression level and produce more in depth results but have much more complicated process.

Overall, this experiment exposed me to the various laboratory instruments, and allowed me to become acquainted to scientific equipment in order to use DNA for practical application. By learning these valuable skills, I was better able to grasp complex concepts, and gain a much better understanding of molecular biology.

Experiment 3 – Reproductive biology of heat-stressed spikelets of rice genotype N22

Objective: To observe and compare pollen germination and pollen tube growth between control and heat-stressed rice spikelets

Hypothesis: If the female reproductive organ of rice is exposed to heat stress during the reproductive stage, then fertilization is still possible.

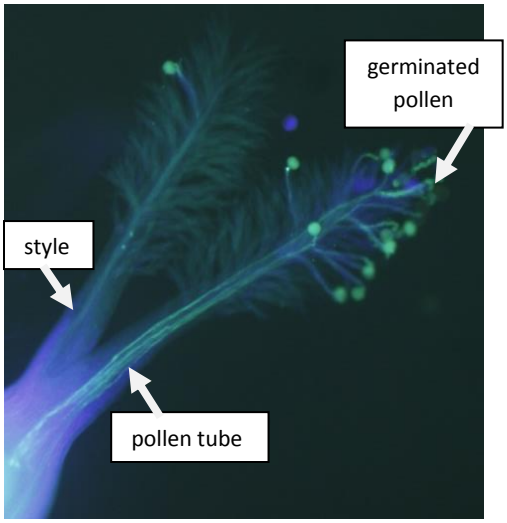
Background: A rice spikelet contains the male reproductive organ, i.e. the stamens, and female reproductive organ, i.e. the pistil, and it's within the rice spikelet that fertilization occurs. In order for rice spikelets to be fertilized, the pollen must be viable and have the ability to germinate and fertilize to set-seed. The rice plants had been exposed to 38 °C at microsporogenesis stage, 10 days before flowering. Later the plants were grown till heading without any interference. The spikelets I used in my experiment were from a different experiment; therefore, I had to take into account the process of artificial pollen dusting and treatment. This process of pollination is done by taking the just flowered panicle from an unstressed plant and dusting the pollen on to the stressed pistil, to specifically look at the effects of heat stress on the female reproductive organ. After the artificial pollination, spikelets were collected from the stressed and unstressed plants, on which I was able to work. Spikelets were from N22, a heat-tolerant rice genotype.

Materials and Methodology: Firstly, five pollinated spikelets are transferred into a vial containing distilled water, then under a stereo microscope the spikelet is dissected by first removing the rice hull. Once the pistil has been removed from the hull, a blade is used to cut away the anther filaments (Fig. 7). Using a small brush, the pistil is placed in a vial containing 8N sodium hydroxide (NaOH). The sample is then left in the solution for 24 hours to clear the tissue for viewing. Many procedures recommend leaving the pistil in NaOH for only four to five hours, but since the ovary is thicker than the stigma a longer clearing time is required in order to view a clear pollen tube growth near the ovary. In preparation for viewing, many steps must be followed. First, dissected and cleared spikelets are placed on a slide, and excess NaOH was removed. A few drops of 0.2% aniline blue is then placed on the slide enough to fully cover and stain all of the pistils. The slide is then placed in a dark area for 10-20 min (as aniline blue is photo sensitive) to stain the pistil. After this time, the aniline blue is removed with a pipette and replaced with water for washing excess stain. A toothpick is used to apply silicon glue to four corners of a cover slip, serving as a spacer between the slide and cover slip to prevent the ovaries from being crushed. The cover slip is then placed on the slide and water is added to any unfilled space. The ovary can then be observed under a fluorescent microscope.

Figure 7. Spikelet dissection under the stereo microscope

Results:

N22 Control Stigma



N22 Heat Stigma 38 °C

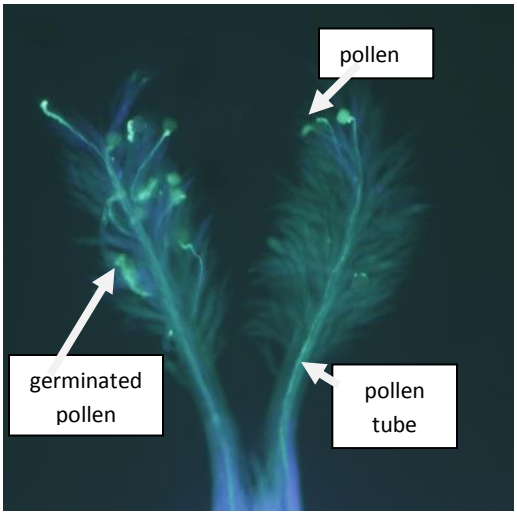
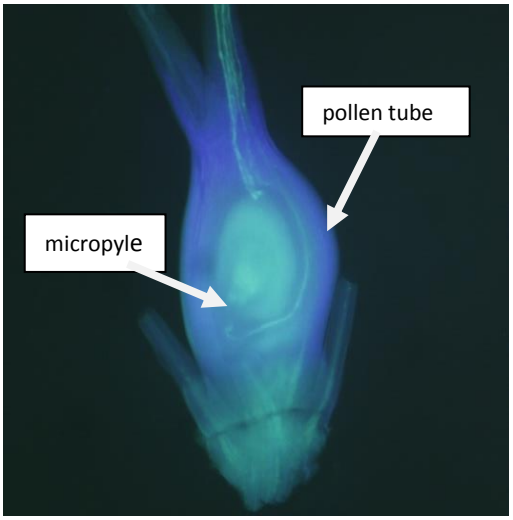


Figure 8. Stigma of N22 with germinating pollen from control and heat treatments.

As previously mentioned, N22 is known to be a heat tolerant genotype. As seen in Fig. 8, the control has approximately 14 pollen on the stigma with most of the pollen being germinated. The germinated pollen can be seen by looking at tubes coming from the various pollen and traveling down the style. In the heat stressed spikelets, there were approximately 20 pollen grains and most of them germinated.

N22 Control Ovary



N22 Heat 38°C Ovary

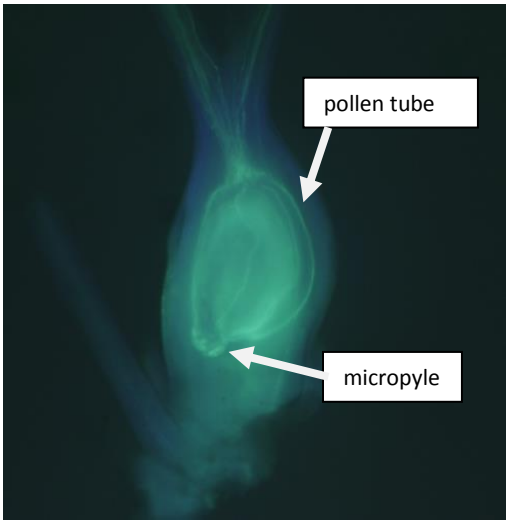


Figure 9. Ovary of N22 with pollen tube from control and heat treatments.

As the style then leads into the ovary, the pollen tube reached the top of the ovary, then travels around it, and into the micropyle. In the control only one pollen tube reached the micropyle. One pollen reaching the micropyle is sufficient for fertilization. In the heat-stressed ovary, the process of fertilization was still occurring, which otherwise would have lead to sterility and no seed-set.

If the above pattern was repeatedly seen among many spikelet samples then it indicates that heat stress does not have an effect on the female reproductive organ and fertilization proceeds as normal in N22, even though it was exposed to heat stress.

Experiment 4 – Vegetative and reproductive stages heat stress response in rice

Objective: To determine the effect of heat exposure at vegetative stage on the nitrogen, carbohydrate partitioning and spikelet sterility.

Hypothesis: (i) If rice plants are exposed to high temperatures at vegetative stage, the nitrogen and non structural carbohydrate production and partitioning are altered; and (ii) Heat stress at vegetative stage enhances tolerance at reproductive stage.

Figure 10. Rice plants in pots during the vegetative stage in the greenhouse

*Materials and Methods: A*fter working in the lab and the field, I was able to further extend my experience to the greenhouse, where I had 60 potted rice plants with six different varieties. Variety 1 - IR40, Variety 2 - IR54, Variety 3 - NSICRc158, Variety 4 - NSICRc222, Variety 5 - HHZ, and Variety 6 - Mestizo3 (a hybrid). Each variety had 10 pots with two plants each. Five of those 10 pots were maintained in the greenhouse for the duration of their growth to serve as control in the experiment; the other five were placed in a growth chamber and exposed to high temperatures. Since these were my own plants I was able to perform each step of the experiment first hand. After learning the math behind fertilizer calculation I prepared, Nitrogen, Phosphorous, and Potassium (NPK) fertilizer in 2g:1g:1g proportions and mixed into 6 kg of soil to give the plants proper nutrition. I then transplanted the fourteen-day-old seedlings into the pots. After transplanting, I waited 15 days to put the plants in the temperature-controlled chamber. By waiting, this allowed the plants to recover from transplanting shock. Thirty plants were exposed to 40 °C in the chamber for 12 days during the vegetative stage. The exposure to heat mimics the effects of heat waves and their effects on rice yields and on plants nutrient distribution. After the treatment, the plants were transferred back to the greenhouse. I then took one pot (2 plants) from each variety to test the content of nitrogen and nonstructural carbohydrates (NSC) in the leaves and stems. The remaining pots, to be used for spikelet fertility analysis, were monitored for the heading stage, and the main tiller of each plant was tagged. At the first day of flowering, two pots (4 plants) from each variety that were heat-stressed at the vegetative stage were placed back inside the chamber set at 38 °C to impose heat treatment for seven days. Plants were then maintained in the greenhouse until maturity. Data on growth analysis and spikelet fertility were then collected (The spikelet fertility and maturity data was kindly collected and shared by my IRRI friends as rice crop takes nearly 3 months to complete its life cycle).

Nitrogen Testing

Objectives: To determine the nitrogen level of different varieties in both control and heat treatments

Hypothesis: "Heat stress does not affect the N accumulation in rice plants."

Background: Expected results of the nitrogen would be a decrease in nitrogen under higher temperatures; however, some genotypes may be able to better withstand heat, meaning their production will be more similar to the control plants. Varieties exposed to 40 °C could result in decreased nitrogen level which would reduce the overall photosynthesis rate, leading to decrease in yields.

Materials and Methods: To properly collect and test for distribution of nitrogen, many steps must be followed. First, the plants were pulled out of the pot and placed in a plastic bag with water in it to help prevent shock. The roots and stems were washed and separated by hand. After the leaves and roots were separated they are put in a paper bag and heated in a microwave for a minute as quickly as possible to keep the enzymatic reactions in check. The samples were placed in the oven to dry for three days. After the samples were dried, I ground the samples using a mortar and pestle and liquid nitrogen (Fig. 11a). The liquid nitrogen at -196 °C works to freeze the samples while I ground them into a fine powder. I then measured the dry weight of each sample. In order to be able to measure the amount of nitrogen the samples must go through three major processes of digestion, distillation, and titration. Next, the reagents concentrated sulfuric acid (H_2SO_4) and salt mixture are made. The salt mixture is 250 g of K_2SO_4 with 50 g of $CuSO_4$ and 5 g metallic selenium. Then 200 mg of the dried sample are placed in a 100 mL Kjeldahl flask. The flask was then placed in an empty tin can and heated over a flame in order to digest the sample. After the sample was clear, 10 mL of distilled water was added to the cooled solution and mixed thoroughly.

Next, a number of reagents were prepared including boric acid, a mix indicator, NaOH (40%), sodium carbonate, methyl orange indicator, and standard hydrochloric acid. After the reagents were made, I put the digested sample from the Kjeldahl flask into the distillation apparatus. Once empty, the flask is rinsed three times with distilled water. The water is emptied into the apparatus. Then by using a quick delivery pipette, I added 10 mL of the NaOH to the distillation apparatus.

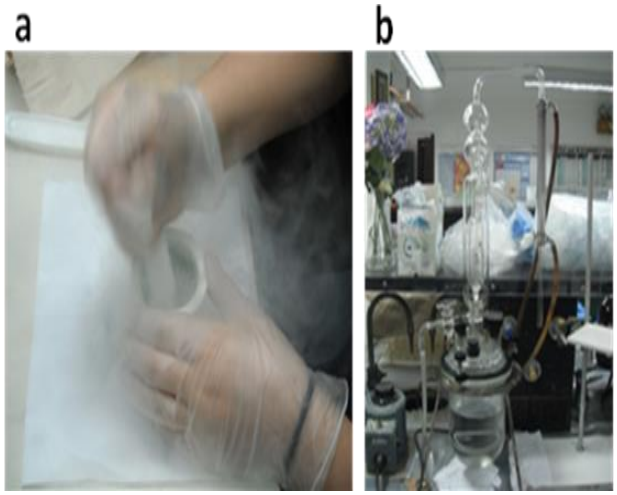


Figure 11. Steps in nitrogen testing (a) grinding samples, and (b) distillation.

I then prepared a 125 mL Erlenmeyer flask with 10 mL of boric acid reagent and three drops of the mix indicator. The flask was placed under the condenser of the apparatus. Then the steam from the boiler passed through the sample to distill off the ammonia into the flask with the boric acid. The sample was distilled for 7 min then the solution from the condenser dripped into the flask for 1 min (Fig. 11b).

The next step is titration, which is the process of making a substance with an unknown concentration react with a solution of a known concentration to find out more information about the unknown solution. I titrated the solution of boric acid and mix indicator with the hydrochloric acid. From there I then used the information I had collected to calculate the nitrogen percentage by using a formula.

$$\% \text{ nitrogen in sample} = \frac{(\text{sample titer-blank titer}) \times \text{normality of HCI} \times 14 \times 100)}{\text{sample weight (g)} \times 100}$$

Results:

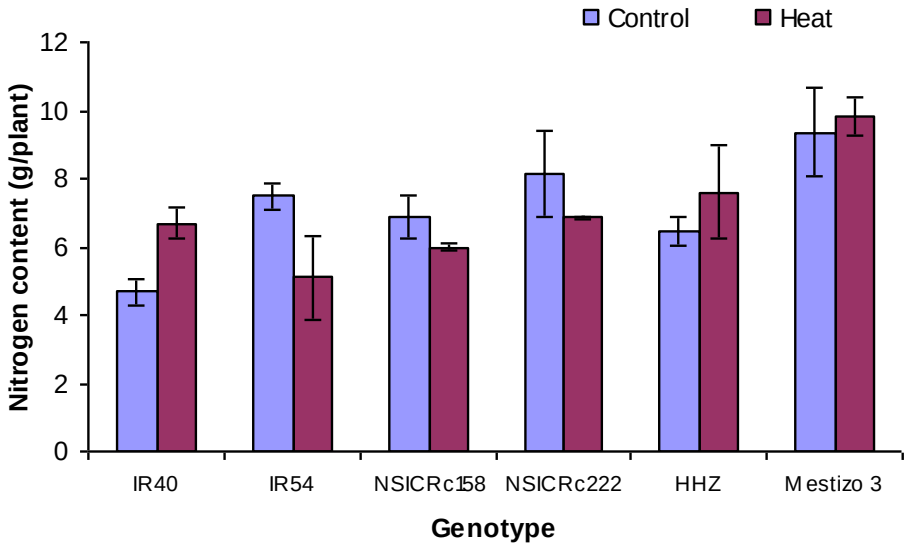


Figure 12. Nitrogen content of control and heat-treated varieties.

Conclusions: Based on the results, the nitrogen content of whole plant (leaf + stem) behaved differently between genotypes under control and heat conditions. Nitrogen content significantly decreased in IR54, NSICRc158 and NSICRc222 when exposed to high temperature stress. However, there were minor differences in HHZ and Mestizo3. Interestingly, in IR40, the nitrogen was more under heat stress than control, which indicated a very dynamic behavior of N uptake patterns across diverse rice genotypes.

Nonstructural Carbohydrate (NSC) Estimation

Background: Nonstructural carbohydrates are the combined starch and sugar content in the rice plant tissue. The carbohydrates in a plant play an important role in overall growth and development. In this experiment, the expected results would be the NSC levels being lower in the plants exposed to heat because when rice is exposed to heat there must be an increased level of respiration and reduced photosynthesis, which in combination will reduce growth significantly.

Materials and Methodology: The samples collected for nitrogen test were also used for NSC estimation. First preparations of the solutions were made which included 80% ethanol (EtOH) and an anthrone reagent.

Standards were then made for the standard curve. The standards are glucose-ethanol solutions with specific/known levels of concentration. The standard curve is used as reference to calculate the concentration of glucose in the samples.

In the extraction, 100 mg of the dried sample were placed into a 12 mL test tube and 7 mL of 80 % EtOH. The tubes were then placed in a water bath at 81°C for about 10 min. Marbles were placed on top of the tubes to prevent any spillage. The samples were then centrifuged at 3000 rpm for 10 minutes then the supernatant or i.e. top part of the solution is transferred into a 25 mL test tube, and another 7 mL of EtOH is added to the remaining residue and then placed back in the water bath to follow the same steps again. The supernatant was then combined with the first extract and for a third time 5 mL of EtOH was added and put in the water bath and centrifuged for one final time.

After these steps, a colorimetric determination was used. First, 5 mL of sugar extract and 5 ml of anthrone reagent were added to tube and mixed using a vortex, then boiled for 10 min and cooled. The mixture was then vortexed again and colorimetrically determined using a spectrophotometer. The machine sends a beam of UV light through the sample which is in a clear glass tube, and is connected to the computer that then reads the amount light being absorbed. From there, the NSC can be calculated using the glucose calibration cure and the weight of the sample assessed. *Results and conclusion:* As Fig. 13 shows, the NSC content (leaf +stem) were markedly reduced in IR54, NSICRc158 and NSICRc222 under heat condition compared to control. In contrast, NSC content of IR40, HHZ and Mestizo3 under heat were as good as control or decreased slightly. These results indicated that sensitive three may have had reduced photosynthesis or increased respiration rates while the tolerant three were relatively less affected.

Results:

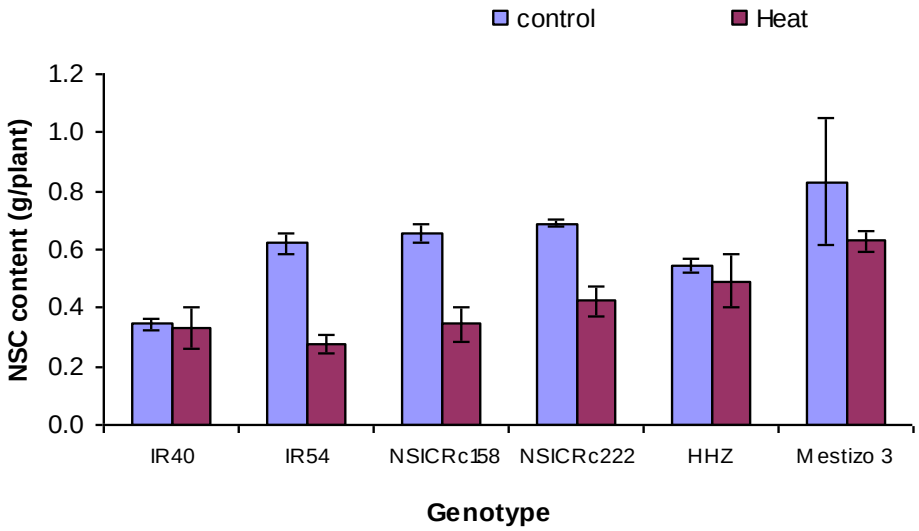


Figure 13. Nonstructural carbohydrate content of control and heat-treated varieties.

Growth Analysis and Spikelet Fertility

Methodology: For each of the eight pots (16 plants) per variety, growth analysis was performed by recording the height and number of tillers of each plant. The tagged main tillers were then harvested. By convention, harvesting is done around a month after the plant has flowered, where grains become hard and yellow. However, due to time limitations, harvest was done although not all of the six genotypes were fully mature but sufficiently filled to ensure fertility data was reliable. The harvested tillers were manually threshed, after which filled grains were separated from the empty ones. Spikelet fertility was computed as follows:

$$\text{Spikelet fertility} = \frac{\text{Number of grains formed}}{\text{Total number of spikelets}} \times 100$$

Results: Growth analysis showed that both plant height (Fig. 14) and number of tillers (Fig. 15) were not significantly affected by exposure to high temperature. On the other hand, high temperature treatment reduced spikelet fertility. Plants exposed to heat stress during the vegetative stage showed higher fertility compared to plants exposed to high temperatures at both vegetative and reproductive stages (Fig. 16). Among the tested entries only NSICRc222 recorded a reduced fertility level when exposed to high temperature at the vegetative stage. In general, these indicate that high temperature exposure during the vegetative stage does not enhance the plant's tolerance to heat stress at the reproductive stage.

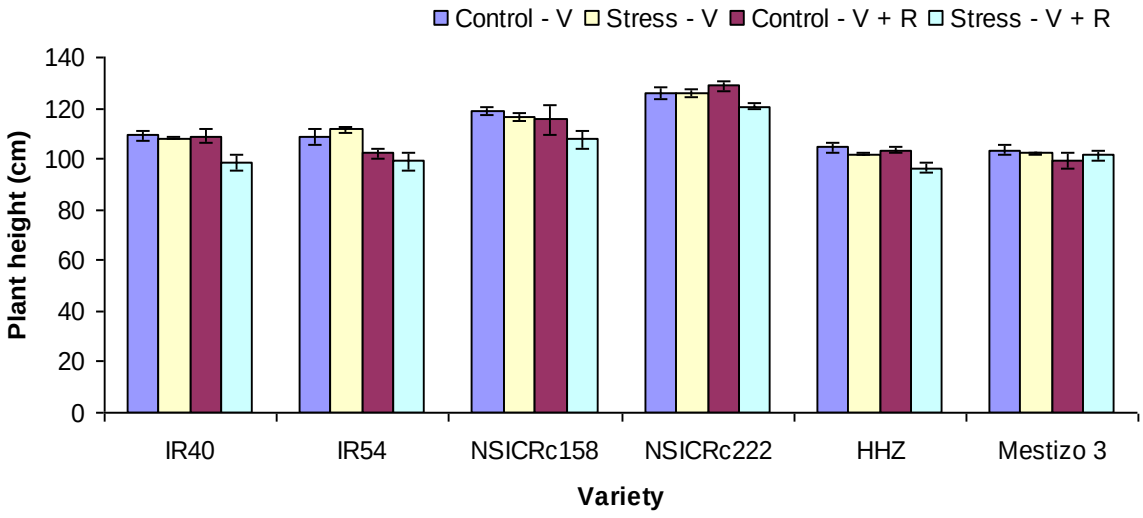


Figure 14. Effect of high temperature treatment on plant height.

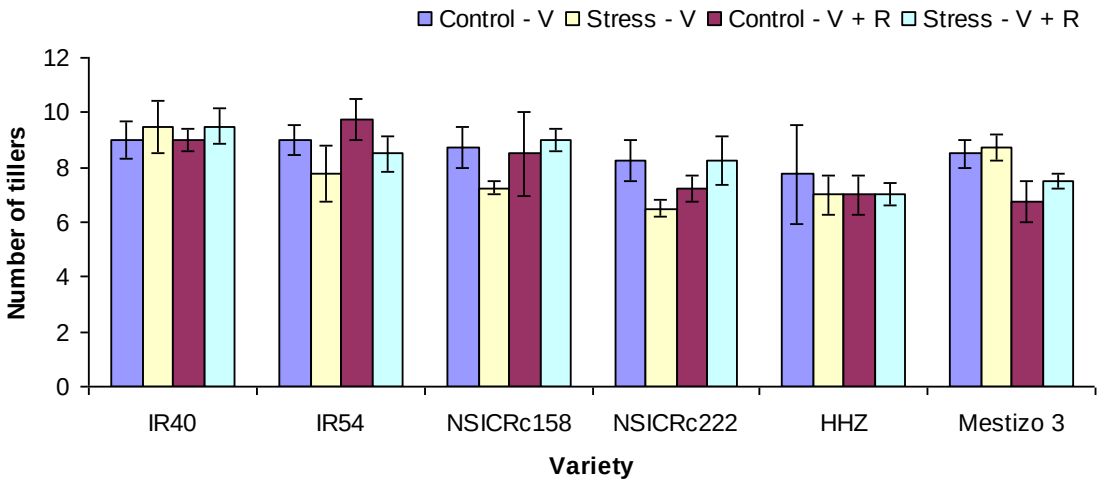


Figure 15. Effect of high temperature treatment on the number of tillers produced.

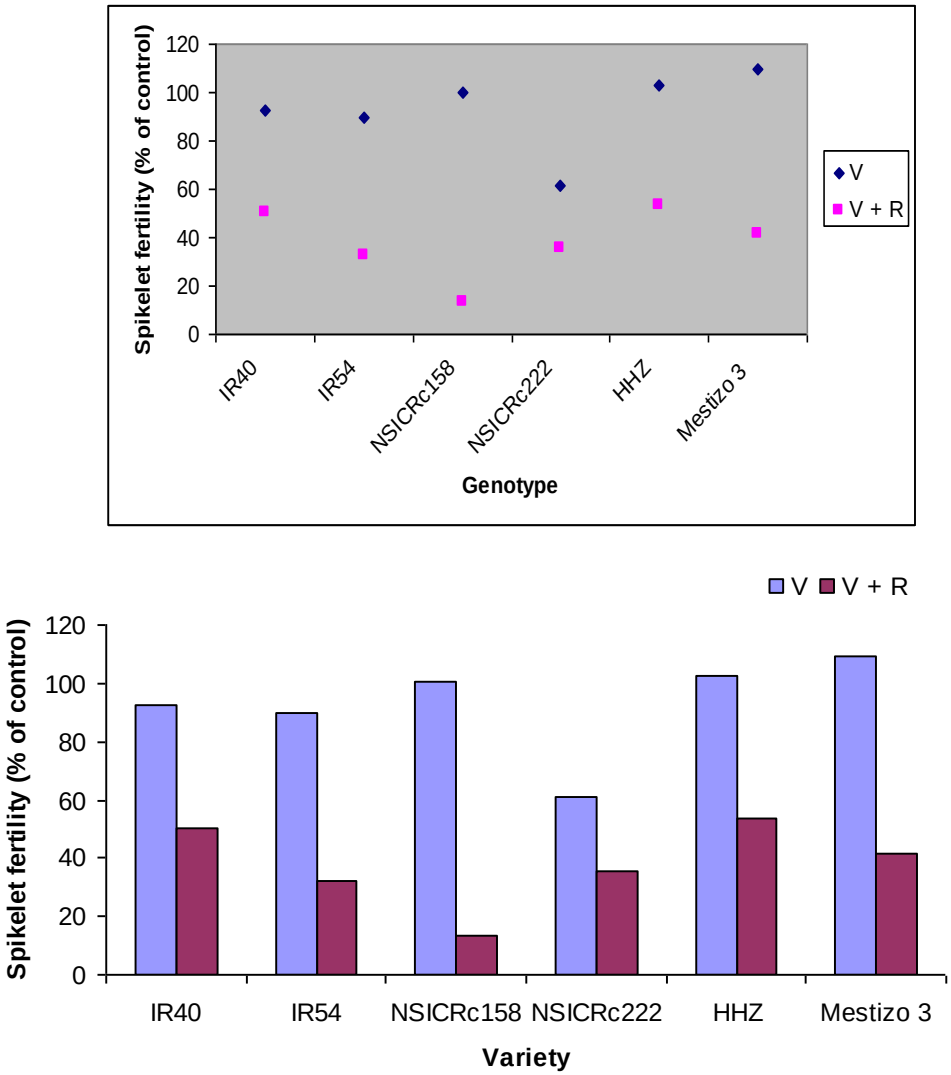


Figure 16. Effect of high temperature treatment on spikelet fertility exposed at the vegetative (V) only and vegetative and reproductive stage (V+R).

Farmer Interviews

Finally, after working in the lab, greenhouse, and field I was able to combine everything that I learned by having the opportunity to spend time visiting two different farmers and speaking with them about farming practices, economic issues, and climate change. The farmers are a part of a program at IRRI that provides farmers information on best seed sources and fertilizer management practices to promote the use of better agriculture practices to uplift local farmers out of poverty.

The first farmer, Bernardo, spoke little English, but with the help of my Filipino colleagues I was able to have an interesting and engaging talk with him. Bernardo spoke of the hardship caused by typhoons and insects which could significantly reduce rice yields; however, with the help of the IRRI program he was able to double his yields in only three growing seasons and increase his income, which was especially important because farming was his main source of income providing livelihood for his family, a wife and two kids. The increased quality in seeds and fertilizer not only bettered his farm, but illustrated to other famers in the area the importance of better technologies. While speaking with Bernardo was very rewarding, the trip to his home was equally gratifying. I was able to see a very rural area of the Philippines, and how life there was no more than simply producing enough food to live each day. The children along the road gathering sticks and the shacks people lived in did more to expose me to poverty than any geography class in school could have ever done.

Figure 17. Interview with Bernardo in his rice field in Victoria, Laguna.

The second farmer, Diosdado, was the President of the Puypuy Farmers Association, which is a voluntary program comprised of local farmers. As the president, he often tested the new seeds and other technologies then if he was successful other members would also use the new applications in their own field. He said, "To see is to believe" in reference to the other members of the association. His work helped break through the problem of farmers especially some of the older ones being somewhat resistant to adopting new practices. Even with the best technologies in rice, scientists and farmers must work together to grow better rice.


Figure 18. Interview with Diosdado in his office in Puypuy, Bay, Laguna.

When I asked the farmer his main problem, with a doubt in his voice, he replied climate change. I spoke with him in length about problems with typhoons wiping away entire crops and leaving him with nothing then at other times experiencing water shortages that cause him to use guns to protect his fields. In a discouraged tone of voice he said "You can't control climate change, you can only pray." After spending the majority of my time this

summer learning and understanding the effects of climate change on rice, the farmer's words about his struggles took the facts and charts about climate change that I had spent so much time researching and made them real to me. It's one thing to read about climate change, but another to listen to a person whose lifestyle can so drastically be changed by it. Before leaving, his last words to me were "Please help us. Farmers have a lot of problems."

The farmer interviews were a major highlight of my internship. By being able to see the work that IRRI does from the laboratories to the actual fields, and applying all of the things I had learned about rice production and getting to learn even more, I felt that this day completed the rice production experience in a way I could never have predicted.

Food Security and Conclusion

As food security continues to be an issue in the world, The International Rice Research Institute is taking scientific steps to increase the efficiency of rice farming not only in the Philippines but the entire rice growing regions of the world. As a more applied research institute, the work done is primarily used to produce rice with more desirable qualities. The Crop Physiology Group's works with high temperature and heat and drought stress interactions, can ultimately lead to breeders using the data to produce a heat or a heat and drought stress tolerant variety that will not be affected by major drivers of climate change. By studying the mechanisms of the N22, which is not widely grown, scientists will be able to introduce the high heat tolerance into widely grown farmer preferred varieties like IR64 which is grown in millions of hectares across Asia. In the future, with the use of molecular markers for specific heat shock proteins, the process of plant breeding could be sped up through using Marker Assisted Selection to five years or less. This would mean producing better rice varieties faster and more efficiently. As climate change continues to threaten sustained rice production, the research in this field becomes increasingly important to the future of global food security.

Personal Reflection

Two months may sound like a long time to some people, but I can now say without a doubt in my mind, two months is hardly anytime at all. Never would I have imagined fifty-nine days could change my life so drastically. This internship exposed me to things I had only seen and read about in textbooks. To see first-hand children and adults alike sifting through garbage for food, begging on the streets for money, and depending on rice for so much of their daily food, did more than inspire me. It fostered and further promoted a deep desire of mine to help those less fortunate than myself. I can only hope to keep this aspiration throughout my life as I enter college and go out in to the world to incorporate everything I've learned in school and through this program.

From the first day in the Philippines, the Filipino culture embraced me and even months later it is relentless in letting go. Whether it is accidently saying "Aye" or craving my daily cup of rice, the Philippines changed my perspective on the world as I knew it and taught me invaluable lessons. I was able to engage in my fair share of cultural adventures, some of which attempting to learn the Tagalog language, eating local foods like Balut, which much to my chagrin turned out to be duck fetus, and traveling in the interesting public transportation system. All of these experiences were amazing, but possible even more incredible were the people who accompanied me on these endeavors.

One of the most rewarding aspects of IRRI is the fact it is an international institute. Because of this, I was able to meet and talk with people from not only the Philippines but also India, China, Australia, the Netherlands, America, Greece, England, Germany, Japan, Thailand, Nigeria, and many more. I will always cherish the memories made with my friends and "family" in the Philippines. I will continue to be reminded of these remarkable people each time I pick up a basketball, sing Karaoke, or eat rice. I met more people and developed more relationships than I could ever have imagined. I went my entire life with even knowing so many people, but after spending two months with them I cannot picture my life without them. When I first found out I had received an internship position, I counted down the days until I would leave my home, then I counted down the days until I had to leave the Philippines, and now I'm counting down the days until I can return. I simply cannot imagine spending the summer of 2012 in a more beneficial and inspiring place.

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