"Rice is Life"

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Acknowledgements

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Personal Remarks

I have lived in rural Iowa most of my life. I attended Mount Vernon High School and was a part of the biggest graduating class, only 115 students. It was during my junior year when my math teacher, Mrs. Jan Sauter announced that she had attended the annual Hoover-Wallace dinner. She found the Youth Institute and Borlaug-Ruan Summer Internship Program intriguing and decided to offer her support to any interested student. I was impressed with her description of the Youth Institute and that a research internship would be offered to a mere high school student. The opportunity she described addressed a desire I've always had, to reach outside rural Iowa, to become a part of the bigger picture and to somehow become a catalyst for positive change in our world.

As a prerequisite to the Youth Institute I completed a research paper on Haiti and improving food security through agricultural extension programs. This research opened my eyes to the need for global food security within the complex world of science, agriculture, politics and international affairs. Haiti is just one small island of people, desperately in need of advances in science, improvements in government and empowering of the local farmers. My research made me question what I had assumed about poverty and starvation in our world. With what the world has to offer, why are the people of Haiti so poor and hungry and why are there so many places like Haiti in the world? The articles and papers I read left me with the desire to get involved in the fight against global hunger, and I looked forward to the youth institute where all attending shared the same desire.

Participating in the World Food Prize Youth Institute was the most rewarding experience of my high school career. I was given the opportunity to speak with dignitaries, all of who offered their support and encouragement. Their passionate words made me feel as if I had a role to play in the fight for food security. This significant event made me realize that making a difference in the world is not only attainable, but more importantly vital; the world is desperately in need of people to stand up and affirm humankind's moral right to an adequate food supply. The event not only helped solidify my future goals, it also broadened my knowledge of our world.

After attending the Youth Institute I knew that the Borlaug-Ruan Internship would allow me to pursue my interests in science, agriculture, politics and international studies,

therefore applying became necessary. I remained hopeful through the interview process, although I felt honored just to have made it thus far. I knew that if I were chosen I would do my best to take advantage of this life-changing opportunity.

When I received notification that I had been accepted as an intern at IRRI, The International Rice Research Institute, I literally sat in silence. I was leaving the comfort of my home to gain first hand experience about the issues I had read in books and scientific articles. The experience didn't seem real until I took off my shoes and stepped into the muddy rice paddies of Los Baños, Philippines.

IRRI- International Rice Research Institute

The Ford and Rockefeller Foundations established the International Rice Research Institute (IRRI) in 1960 in cooperation with the Philippine government. Its research activities began in 1962, and it is now estimated that IRRI has touched the lives of almost half the world's population. IRRI played a significant role in the start of the green revolution when it developed a new breed of rice in the 1960s. This is estimated to have saved millions of Asians from famine, as well as initiated economic growth, which raised more people out of poverty than at any other time in history.

With its headquarters in the Philippines, IRRI is known today as the oldest and largest international agricultural research institute in Asia. It is an independent, nonprofit rice research and education organization with staff based in over fourteen nations. IRRI was established to "help poor rice farmers in developing countries grow more rice on less land using less water, less labor, and few chemicals."

IRRI's mission is "to reduce poverty and hunger, improve the health of rice farmers and consumers, and ensure environmental sustainability through collaborative research,

partnerships, and the strengthening of national agricultural research and extension systems." IRRI strives to pursue its mission with five main goals. *1) Reduce poverty through improved and diversified rice-based systems 2) Ensure that rice production is sustainable and stable, has minimal negative 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 is true to its motto, "Rice science for a better world."

IRRI is one of a group of fifteen research centers that makes up the Consultative Group on International Agricultural Research (CGIAR). CGIAR is a coordinated organization where funds for international agricultural research are administered to the fifteen different centers around the world. IRRI receives its financial support from donor governments, agencies and foundations.

Background

Almost half of the world's population, 2.7 billion people, depend on rice as an essential staple and source of income (Pisupati). Although most rice producers and consumers live in Asia, rice is also essential to many others in Africa and South America. A large percentage of these people are among the worlds poorest, making efficient rice production one of the most important agricultural economic activities. Rice fields cover about 11% of the earth's arable land area, so the development of sustainable technologies can have a large positive impact on the global environment (IRRI).

Over the past forty years an increase in rice production has resulted in a dramatic decrease in the real price of rice. This increase in productivity has provided the poor with a vital source of additional income. A demand for higher quality rice production is a result of improved economies. In the Indochina region, where countries are undergoing economic transition and rice is the staple crop, it is becoming increasingly important to ensure that rice production is high quality (Renault and Facon 2004). With the possibility of disrupting political stability, it's extremely evident why rice holds such critical importance in Asian culture (Dawe and Dedolph 1999).

Asia alone represents 90% of the world's production and consumption of rice (Pisupati). In Asian culture, the cooking and eating properties of rice are directly linked with the market value. This makes maintaining these qualities extremely important to farmers (Champagne et al. 1999). Various studies have shown that the characteristics and development of high quality rice depend upon consumer preferences, which are influenced by different cultures and groups (Juliano 2001). In rice quality evolution programs, amylose is considered the most important factor for classifying different varieties of rice. This is because rice is mostly consumed as a whole grain, and its appearance, tenderness, and cohesiveness are strongly influenced by the amount of amylose content (Mackill et al. 1996).

There has been extensive study focused on the cooking and eating properties of rice. The factors that influence these properties have been identified and genetic links have been established between the two. When rice is cooked, a series of events occurs before the raw grain is transformed into a gel. The cooking process begins as soon as water is added and as heat is applied to the grains of rice (Fitzgerald 2004). When the rice is finished, it can then be evaluated based on its texture. Amylose content affects the hardness, stickiness, and the break down (retro gradation) of cooked rice (Champagne et al. 2004). These qualities are used as a tool to classify different rice varieties (Juliano 2001).

My Work at IRRI

I spent my internship working in the Grain Quality, Nutrition and Post Harvest division. Within Grain Quality, the lab studies three main quality traits; physical properties, sensory properties and nutritional properties. My experiments dealt mainly within the sensory characteristics; amylose content, gel temperature, gel consistency and texture. Aroma is another sensory trait, although it did not pertain to my work.

Gelatinization temperature (GT) was the main focus of my work. Gelatinization temperature is the temperature that the crystalline lamellae begin to melt, in other words; it is the temperature that the rice begins to cook. The GT affects the way starch granules swell and absorb water; therefore it affects texture and cooking time. The most desirable rice variety has an intermediate gelatinization temperature that is above 70°C and less than 74°C. The reason this range is so optimal, is because rice varieties with a high GT have crystals that are harder to break down. High GT requires more fuel use, therefore a waste of energy and would be cost inefficient. Having a low GT would cause the grains to become easily damaged, which may result in an undesirable texture and consistency. To put this into context, lower GT decreases cooking time by four minutes. Saving four minutes every time rice is cooked, saves 10,056 years each day of cooking time – fuel and carbon stocks (Fitzgerald).

Much research has been done on genetic explanations of gelatinization temperature. There are three recognized gelatinization temperatures: low GT, intermediate GT and high GT. Amylopectin is made up of many short and long chains of glucose units. Low gelatinization temperature is characteristic of more short chains than long chains, thus making the amylopectin weaker. More long chains than short chains are indicative of a higher GT and a stronger amylopectin structure. The gene found to be responsible for extending the short chains is Starch Synthase IIa (SSIIa). SNP or Single nucleotide polymorphisms (a polymorphism is a DNA sequence variation) in SSIIa define four alleles (An allele is an alternative form of a gene that is located at a specific position on a specific chromosome). It is believed that SSIIa activity alters the chain length distribution of single cluster chains.

There are four haplotypes (contraction of the term 'haploid genotype' or the DNA gene sequence), two haplotypes that represent low gelatinization temperature A/G/TT and A/A/GC. As explained above, these both lead to more short chains in a cluster. There are

also two haplotypes for high gelatinization temperature, G/G/GC and A/G/GC and these both lead to more long chains in an amylopectin cluster.

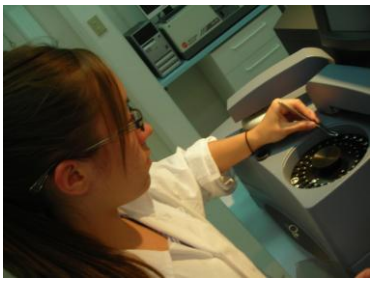
My research used the improved rice varieties, also known as the demo varieties. These are a specific group of rice varieties chosen by IRRI breeders for having intermediate gelatinization temperature. I performed three specific tests using the demo varieties; the results from my experiments will be interpreted and used by breeders, scientists and eventually national rice programs, farmers and consumers.

The following three portions explain the procedure and results of my experiments.

Functional Portion

Differential Scanning Calorimetry (DSC)

Introduction:



Differential Scanning Calorimetry (DSC) is one method used to monitor changes in heat flow. A sample is compared to an empty reference pan while both are subjected to the same heat temperature (Hohne et al. 2003). This method measures the specific heat of the sample, which changes both with temperature and any physical change. An example of physical change is a loss of crystalline.

In DSC, the reference and the sample pans are placed on a platform. As phase transitions take place, the sample would need additional heat or less heat to keep the temperature of the sample the same as the temperature of the empty reference pan. The same temperature change rate is maintained, and this allows a closer observation of more subtle phase transitions. You can then determine and observe the enthalpies (the amount of energy needed to melt a sample) by incorporating the endothermic or exothermic peak. (Hohne et al. 2003)

Materials and Methodology:

Gelatinization temperature can be measured using different methods and for my experiment, I used a Differential Scanning Calorimeter because it is the method that can determine an exact gelatinization temperature.

Four milligrams of flour was placed in an aluminum hermetic pan with 8 μm of water. The pan is sealed with a hermetic crimp and the sample was heated from ambient temperature to 120 degrees Celsius with a ramp rate of 10 degrees per minute in a differential scanning calorimeter.

An empty pan was used as a reference. When heat goes through the empty pan it has a certain heat flow, when heat goes through

a pan with a sample the scanner shows the difference in heat flows between the empty pan and the sample pan. Gelatinization temperature is the peak of the endotherm (negative peak).

Results:

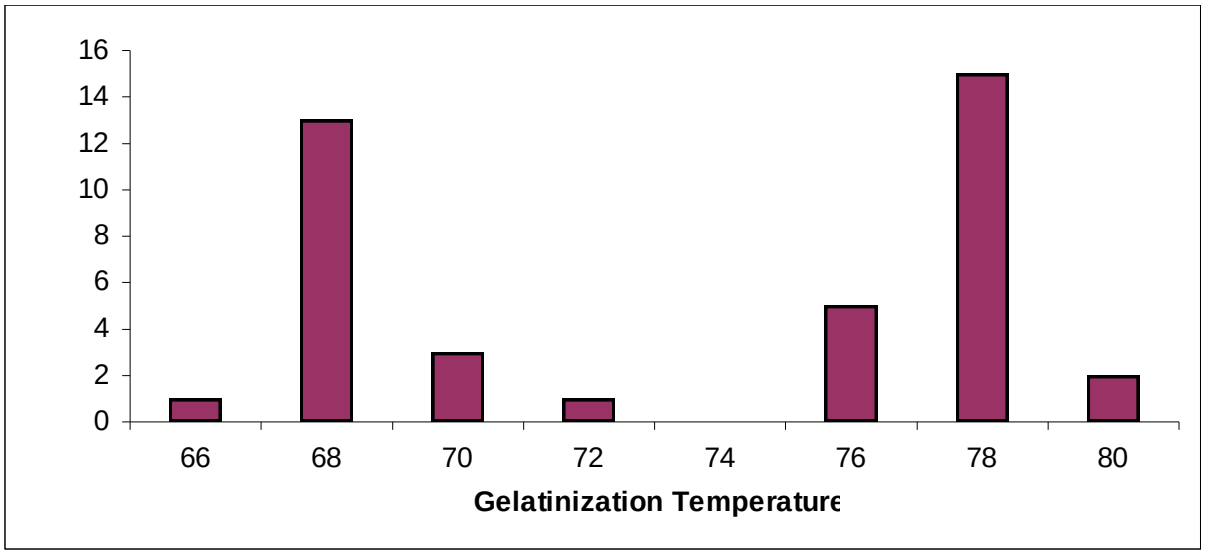


Figure 1. The distribution of gelatinization temperatures by DSC in 40 improved varieties with intermediate gelatinization temperature based on alkalized spreading value.

The distribution shown in figure one displays two peaks. It shows that gelatinization temperature is grouped into two temperature ranges, high gelatinization temperature and low gelatinization temperature.

The range of gelatinization temperature is from 66ºC to 80ºC. The first peak ranges from 66ºC to 72ºC and peak two ranges from 76ºC to 80ºC. There is no peak in the middle, representing the intermediate class.

Discussion:

The samples I chose were based on the alkalized spreading value (ASV) test, all of which fell in 70-74º C intermediate gelatinization group. ASV is an old method used to manually determine gelatinization temperature. After obtaining the results based on the DSC, I found a full range of temperatures- high, few intermediate and low. Since there are only two clusters shown in figure 1, it is obvious that there is an absence of the intermediate group based DSC. The intermediate grains would have had a gelatinization temperature of 70-74º C but only a few of my samples fell into the intermediate class. This means that my assumption that the DSC values would match the ASV test was wrong, the intermediate gelatinization class based on alkalized spreading value is not the intermediate class based on DSC.

There are a few reasons as to why the intermediate class was different based on ASV. Since a person manually performed the ASV test, they could have added a higher

concentration and in that case the results could have been different. Also, the test is very subjective, and the person evaluating the results may have judged them differently

Structural Portion

Fluorophore-assisted capillary electrophoresis (FACE)

Introduction:

The ends of each side of the capillary are immersed in the electrolyte buffer reservoirs along with the electrodes coming from the high-voltage power supply. In the detector, the portion of the capillary that passes through it is clear. The reason it is clear is so that the detector can 'see' the passing analytes because starch isn't detectable by UV. An auto sampler transfers the capillary end from the buffer reservoir to the sample vial, to collect a predetermined volume of analyte for separation.

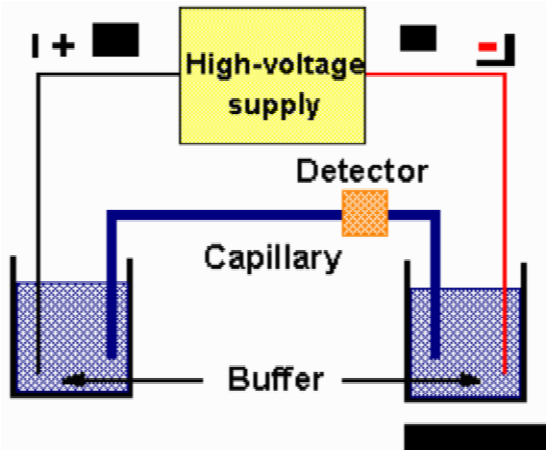


Figure 2: Illustration of CE set-up

Separation during electrophoresis is attained from the migration of charged species. This charged species contains an electrolyte and as an electric current is passed, separation occurs. Regardless of charge, all ions flow through the capillary in the same direction due to electroosmotic flow (EOF). Once the energy is applied to the capillary, the new layer of charges migrates towards the cathode together with water. The samples travel based on their electrophoretic mobility, and they are analyzed based on a function of time (Oda and Landers 1996).

During capillary electrophoresis separation, the hydroxyl groups are exposed to the high pH of the buffer solution, and these will be ionized, forming a negatively charged wall. If the pH is low, the capillary walls are not ionized, and remain neutral in charge. It is desirable to have an ionized wall since oppositely charged ions form another layer on top of the ionized wall, thus neutralizing the charge. Neutral analytes would flow in the same direction as the EOF, but charged analytes may or may not flow in the same direction.

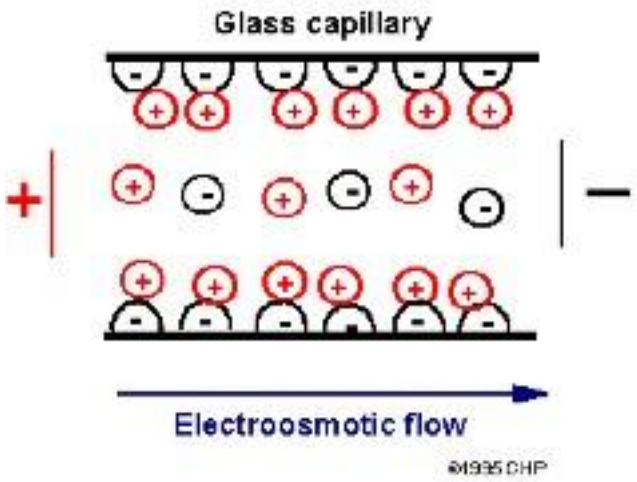


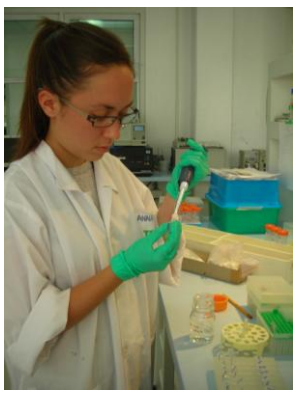
Figure 3: Illustration of the electroosmotic flow during CE

Since the EOF is generated across the length of the capillary, there is constant flow throughout. Therefore, there is no analyte mixing or spreading; the peaks are revealed as sharp and discrete. On the other hand, if the flow were laminar, molecules in the middle of the flow would move faster than those at the sides because friction lowers the pressure at the capillary walls (Frazier et all. 2000). As a result molecules of different sizes would be detected at approximately the same time; the peaks in the resulting electropherogram are broader than peaks resulting from the EOF.

The neutral molecules that travel with the EOF are then separated based on the ratio of mass to charge. Smaller molecules and those with more charge would travel faster through the capillary than molecules with less charge or molecules that are bigger. This movement of molecules is called as electrophretic mobility.

The method of determining the chain length distribution of starch by FACE has been developed by Morell and his co-workers (O-Shea et all. 1998). In this technique, the starch is debranched by enzymatic reaction. The molecule is labeled on the single reducing end on each molecule.

Materials and Methodology:



I selected improved rice varieties with intermediate gelatinization temperature based on a test called alkali spreading. I determined their DSC values and now I'm looking at the structure of the debranched amylopectin chains.

Amylopectin is highly branched, by using an enzyme called isoamylase I cut the branches to get linear chains. The first step I took was to gelatinize 10 mg of flour with 40 μm of (1 milliliter = 1000 microliters) ethanol (95%) 200 μm of sodium hydroxide (.25 M) and 600 μm of water in a boiling water bath. The sodium hydroxide destroys the chrystalin structure of the amylopectin.

After the boiling water bath, sodium acetate with glacial acetic acid was added. Glacial acetic acid reacts with the sodium hydroxide to neutralize the suspension (A solution with an undissolvable solid in it). Sodium acetate acts as a buffer (preventing a sudden change in pH), it is very important to keep the pH constant for the enzyme to work. That is when the isoamylase enzyme is added. In the two hours that isoamylase is reacting with the starch at 50°C it cuts the branches of the amylopectin chains. The samples were then boiled to kill the enzyme and a portion of the samples were transferred into empty tubes and dried. After drying I added maltose (two glucose units stuck together) as a marker. The maltose marker is used as a reference to measure the chain length of each peak. After adding maltose the sample is dried again and the APTS (the dye) and sodium cyanoborohydride are added. APTS is 8-aminopyrene-1,3,6-trisulfonic acid and cyanoborohydride helps facilitate the process.

The following day, after 16 hours of reaction at 50 °C, urea and water is added. These samples are filtered in the spin columns (test tube with a filter) and then the labeled samples are separated by capillary electrophoresis.

Results:
(x-axis is labeled chain length (N), and the y-axis is labeled mol%)

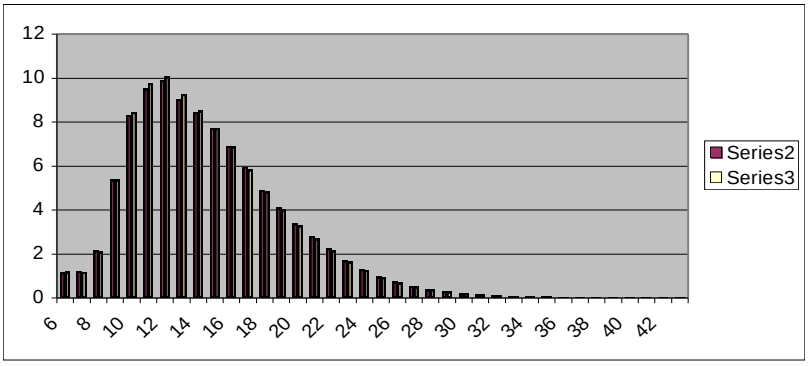


Figure 4: Chain length distribution for debranched amylopectin in PSBRC52 flour

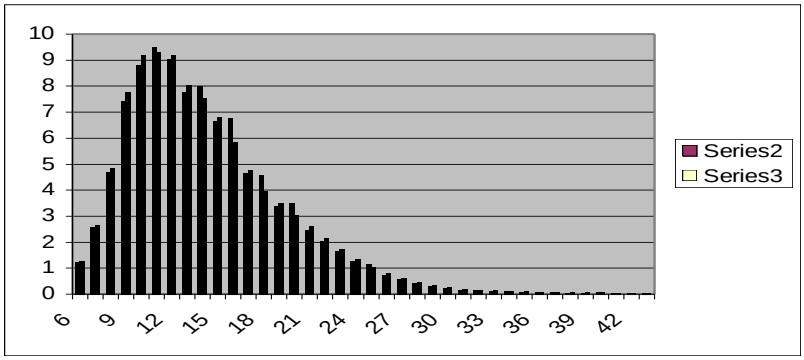


Figure 5: Chain length distribution for debranched amylopectin in PSBRC48 flour

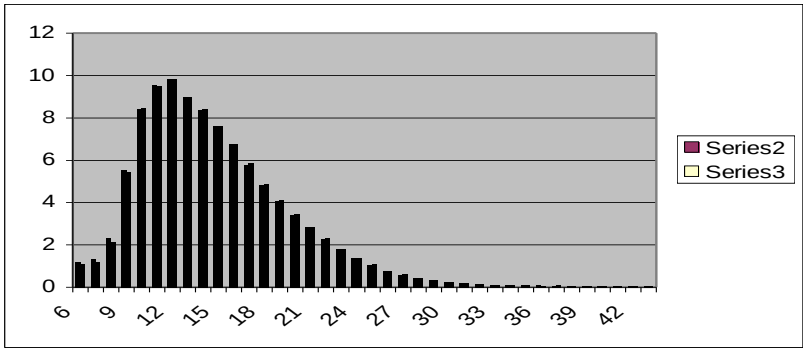


Figure 6: Chain length distribution for debranched amylopectin in PSBRC18 flour

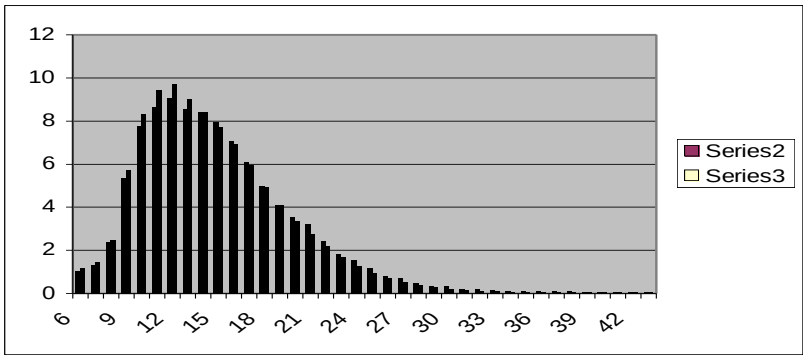


Figure 7: Chain length distribution for debranched amylopectin in PSBRC1 flour

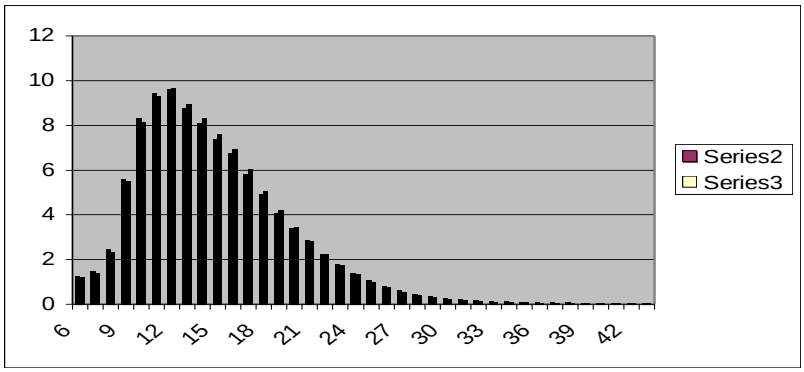


Figure 8: Chain length distribution for debranched amylopectin in NSIC112 flour

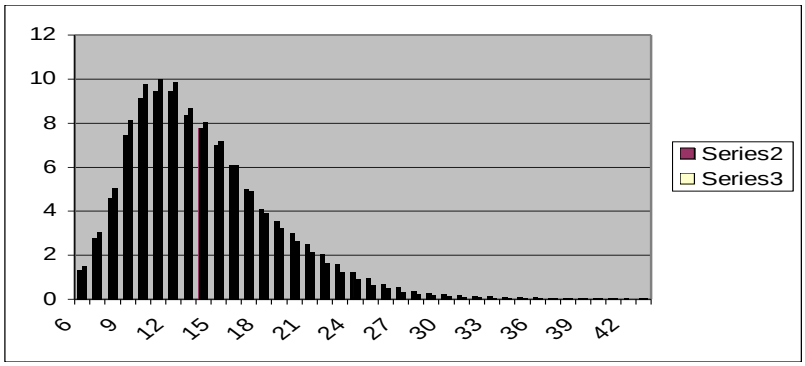


Figure 9: Chain length distribution for debranched amylopectin in IR68 flour

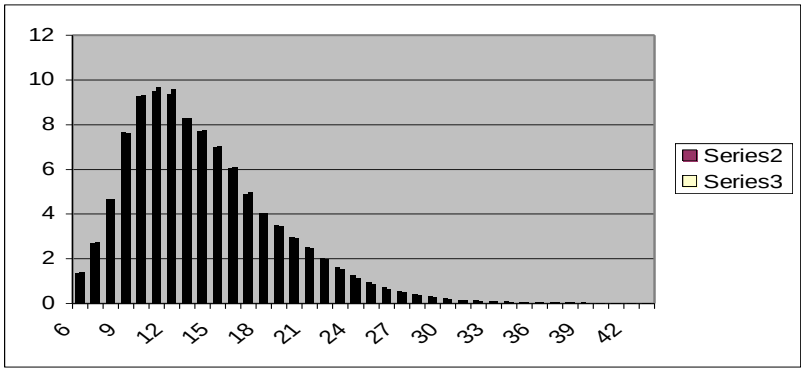


Figure 10: Chain length distribution for debranched amylopectin in IR60 flour

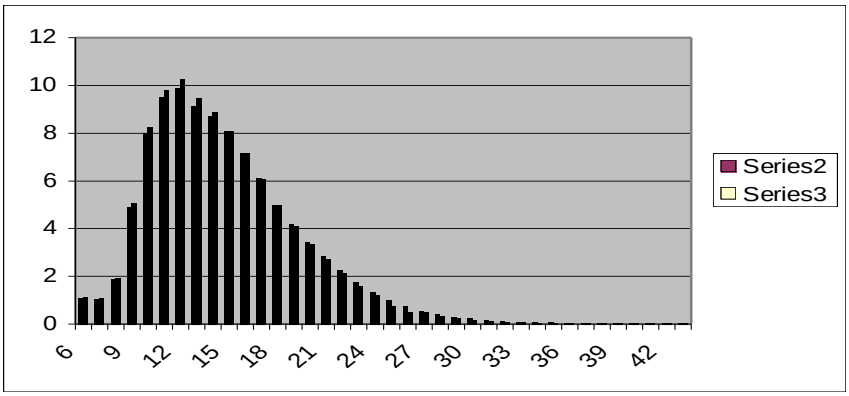


Figure 11: Chain length distribution for debranched amylopectin in IR48 flour

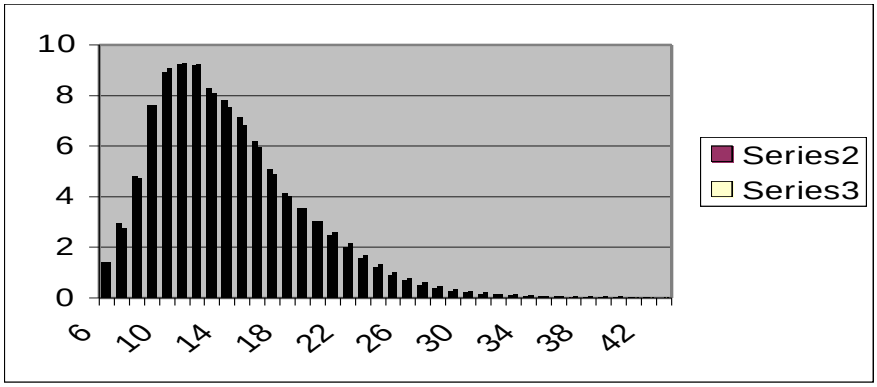


Figure 12: Chain length distribution for debranched amylopectin in IR45 flour

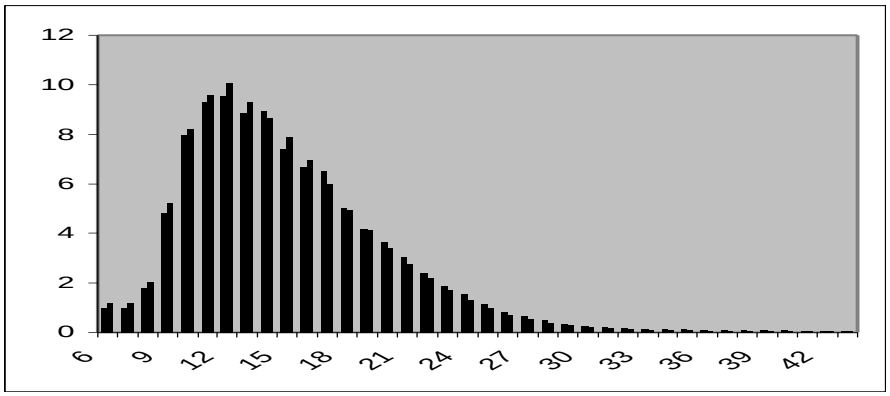


Figure 13: Chain length distribution for debranched amylopectin in IR30 flour

Discussion:
Not all forty samples were tested by FACE during the time I spent in the lab. I created the graphs above for the ten samples that showed the most accurate results based on DSC. The chain length distribution results by fluorine assisted capillary electrophoresis will be used in further studies.

Genetics Portion

Introduction:
When I began polymerase chain reaction (PCR) my samples were just long strands of DNA. The activity of the PCR is to mimic the conditions of what goes on inside of a cell, thus you can amplify the certain strand that you want. There are four ways to break the hydrogen bond, the first is by using protein, the second is by using high temperatures, the third is by using a high salt concentration and the fourth is by using a low pH concentration. For PCR, I used high temperature to break the hydrogen bonds in the DNA.

The first phase in PCR is called “denaturation.” During this phase, the temperature is raised to 95ºC in order to break the hydrogen bonds. I then have to identify the segment, which we want to amplify. By using primers/DNA markers (small segments of DNA, 18-25 base pairs) we can target the SSIIa gene. This second phase is when the primers target specific portions of DNA, it is called the “annealing” phase. When you receive the primer from the manufacturer it’s a double stranded helix, so in order to break the hydrogen bonds of the primer, you have to set the machine to a certain temperature (this temperature depends on your specific samples) before the primer will anneal to the specific sample. The third phase is elongation, after the DNA marker/primer anneals to the sample; Thermus Aquaticus Polymerase extends the segment and provides the complimentary bases.

With the SSIIa gene, this process is cycled 40-45x to ensure that I can detect the DNA once it is in the gel.

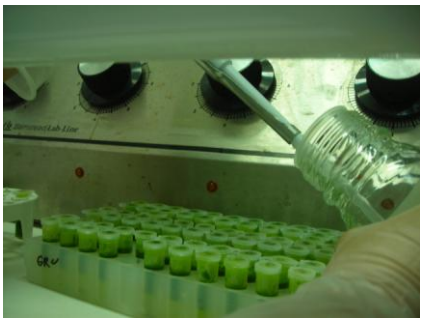
DNA Micro Extraction

Materials and Methodology:

After harvest and freeze-drying the rice leaf samples overnight, I weighed 50 mg of the

freeze-dried leaves, and placed them into 2 mL micro centrifuge tubes. I added one metal grinding ball to each tube containing the leaf tissue, and then placed the samples in a paint shaker. The tissue samples were then ground for twenty minutes. Immediately after the tissue grinding, I mixed 30 mL of extraction buffer (5 mM EDTA (Ethylenediamine tetraacetic acid), .1 M Tris, .35 Sorbitol, 3 mL HCl, and 20 mM sodium metabisulfite) along with 30 mL of nuclei lysis buffer (200 mM Tris pH 8, 50 mM EDTA, 2 M NaCl, 2% CTAB) and 12 mL of 5% sarkosyl into a beaker and stirred it until the sodium bisulfite dissolved. The 800 μm of the mixture was then added to each centrifuge tube.

I vortexed each sample for two to three seconds until all the leaf powder was mixed with the extraction buffer mixture. The leaf samples were then placed in a water bath at 65ºC for an hour and a half. During this time the samples needed intermittent shaking every fifteen to twenty minutes. The mixing is to facilitate the extraction buffer; during this time the cell membrane is lysed because of the detergent (CTAB).



After removing the centrifuge tubes from the water bath, I added 700 μm chloroform: isoamyl aclcohol (24:1). I shook the tubes by inversion for at least 10 minutes. After I shook the samples, I put them in a centrifuge for eight minutes at 13,000 rpm.

The centrifuging process separated the DNA from the other compounds in the leaf tissue. The other

compounds settled at the bottom of the tubes as a dark green mass and the supernatant was the resulting solution in the tube containing the DNA. Before I transferred the supernatant material, I added 70 μm of sodium acetate to each individual centrifuge tube. Then, I was careful in transferring the supernatants by pipette into a new 1.5 mL centrifuge tube by avoiding the area close to the green mass that lies at the bottom of the tube. This mass contains chlorophyll, cell debris and the other heavier organelles. Including some of this compound would have reduced the purity of the supernatant and may cause contamination.

After transferring the supernatants, I added 700 μm of cold isopropanol and mixed each tube gently by hand to avoid breaking the DNA. After a few motions, I could already begin to see the DNA as a milky white cloud in the solution. I then allowed the samples to cool in the -20ºC freezer overnight.

In the morning, I centrifuged the samples for five minutes at 13,000 rpm and washed the DNA pellet by pouring off the supernatant and added 500 μm 70% ethanol to each individual centrifuge tube. I then centrifuged the samples a second time for three minutes at 13,000 rpm and then removed the ethanol by pouring the supernatant without disturbing the DNA pellet. The mouth of each tube was dabbed on a kimwipe to remove excess ethanol. The process of washing with ethanol is to ensure the purity of the DNA pellets obtained.

I allowed the DNA pellets to dry for an hour and then suspended each sample in 100 μm TE pH7.8 (Tris EDTA). The samples were then incubated at 65ºC for ten minutes in a water bath. After ten minutes, the DNA was not completely dissolved in the TE, so I waited another half hour for the DNA pellet to dissolve completely. After forty-five minutes, I mixed 7.5 mL TE and 75 mL RNAse. Then 100 μm of the mixture was added to each sample. The samples were then placed in a 37ºC water bath for one hour.

The samples were removed from the water bath, and 20 μm 3M NaAOc (Sodium Acetate) and 440 μm absolute ethanol were added to each centrifuge tube. I then gently inverted each sample individually until the DNA started to precipitate. The DNA 'pillow' slowly started to form. I then placed the samples in a freezer at -20ºC and left them overnight.

In the morning, I put the samples in the centrifuge at 13,000 rpm for eight minutes. After the eight minutes, I continued to purify the DNA by adding 500 μm 70% ethanol to each individual centrifuge tube. I then spun the samples again in the centrifuge at 13,000 rpm for three minutes. I let the samples air dry under the chemical hood for an hour. After one hour, I dissolved the DNA in 100 μm TE. I then placed all the samples in a water bath at 50ºC for twenty minutes to dissolve.

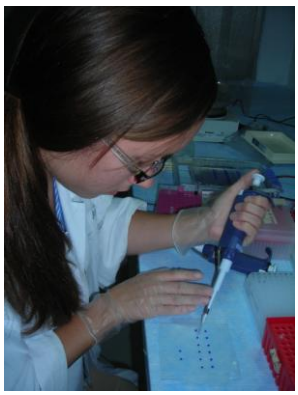
I then added 200 μm of water to each centrifuge tube to dilute the DNA. I put the samples in the centrifuge for one minute at 10,000 rpm. After being centrifuged, I transferred 10 μm into the 96-well plate and made sure to wash the tip in the water to ensure that the DNA is completely out of the pipette tip.

During the process to remove the supernatants from the dark green mass of plant material, I accidentally added two samples to the wrong tube. Thus, I had to eliminate four samples due to contamination. I then had to repeat this process for the four samples I had contaminated.

DNA Quantification Analysis

Materials and Methodology:

I used an agarose gel electrophoresis system for the DNA quantification analysis. The DNA quantification process was necessary because varying amounts of DNA were extracted from the different plant samples. This manual method is not the only way to achieve DNA quantification, but it is the most accurate.



I first distributed 2μ of loading dye onto a sheet of parafilm and then added 4μ of Millipore water to each drop of loading dye. I then used a 5μ pipette to dispense 2μ of each DNA sample to its own individual bubble of loading dye and Millipore water. Each sample was then loaded in a well of the agarose gel with a standard DNA whose quantification is known (usually 50 nano gm per microliter). After the gel had been run, I then compared the band intensity of my DNA with the standard in order to give it scores.

Polymerase Chain Reaction: PCR Amplification

Materials and Methodology:

The next stage in the process was amplification of the DNA samples using polymerase chain reaction (PCR). I first prepared the DNA cocktail containing proportionate amounts of Sd water (sterile distilled water), 10x PCR buffer, 50% glycerol, 25mM MgCl2, 2.5 mM dNTP (deoxyribonucleotide), 10 μm Fprimer, 10 μm Rprimer, 10 μm ASP (allele specific primer) and Taq Polymerase.

When preparing the cocktail, it is important to keep all components on ice so that degradation does not occur; the DNA and reagents are sensitive to high temperatures. I obtained all the primers from the -20ºC freezer and put them in the centrifuge for thirty seconds at 6000 rpm in case of contaminants, they would settle at the bottom of the centrifuge tube. I then transferred 8 μm of the cocktail into the 96-well plate. I converted two μm of

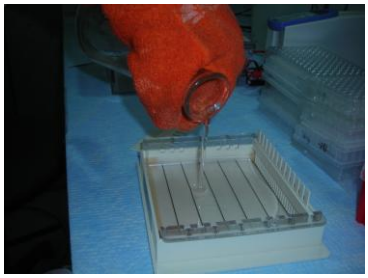
each DNA samples to the 96-well PCR plate and secured the wells tightly with a silicon mat to ensure no evaporation. I put the samples in the centrifuge for one minute at 3000 rpm so the DNA and PCR cocktail would mix.

I put the 96-well plate into the PCR. I screened a total of seventy-four samples for my experiment using the SSIIa profile in the PCR machine. Each run of the PCR machine took approximately an hour and a half and during this time I prepared the agarose gels I would use to run the PCR products.

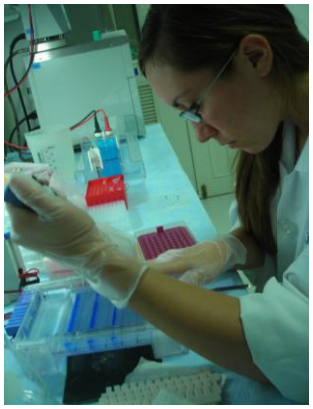
Gel Electrophoresis

Materials and Methodology:

The optimum volume of the gel caster is 80 mL, so I prepared a 2% gel. I weighed 1.6 g of agarose into a flask and then added 80 mL of 1X TBE buffer. I then placed the agarose, diluted with the buffer in the microwave to melt the agarose. After one minute, I removed the flask and swirled the contents gently to completely dissolve the agarose granules. I then put the flask back into the microwave for thirty seconds. I then took the flask out of the microwave and swirled its contents for a second time, to ensure that the agarose had dissolved.



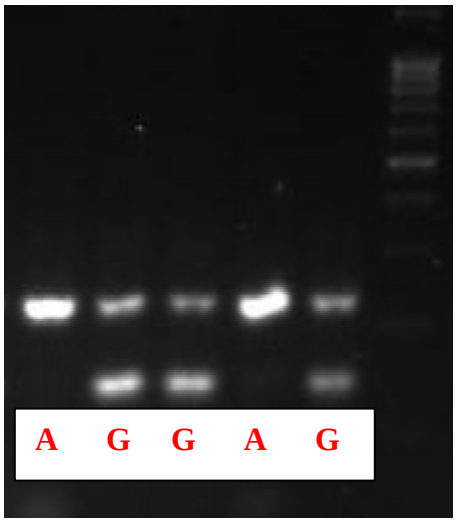
I waited a few minutes to cool the molten agarose and then added 2.5 μm SYBR safe DNA Gel Stain into the mixture. I then waited another minute for the gel to cool and then poured the solution into the gel caster. At this time, I removed the bubbles from the gel with a metal spatula to ensure that they did not interfere with the DNA results. Gently, I inserted the comb into the comb slots in the gel caster. The number and orientation of the combs to add depends on the number of samples to analyze, and for my specific use I used between two and four 26-well combs. I then allowed the gel to solidify for approximately forty minutes, but to facilitate faster gelling; I often did the gel casting inside the cold room. This allowed the gel to solidify in about fifteen minutes.



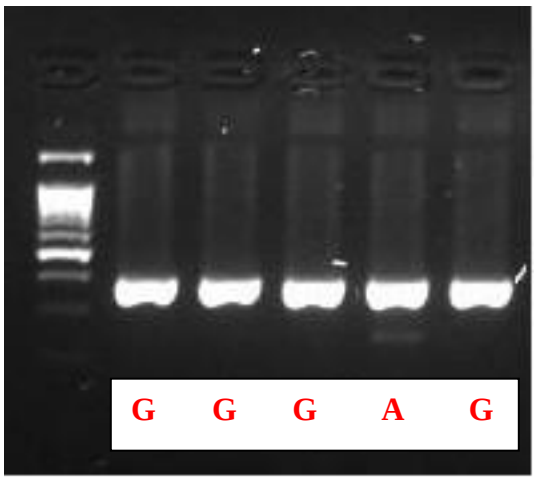
After the gel finished solidifying, I poured approximately 450 mL 1X buffer into the running trough. I then removed the solidified gel with the tray from the caster and placed the entire setup in the running trough. I then added 1.5 μm loading buffer (10x buffer) to the 10 μm PCR cocktail and then loaded the wells individually with the PCR products, using SL20 manual single channel pipettor. I loaded 7 μm of PCR product/dye into each well and ran the machine for 40 minutes at 135 V.

After the electrophoresis machine was done, I removed the agarose gel and photographed it under a UV light.

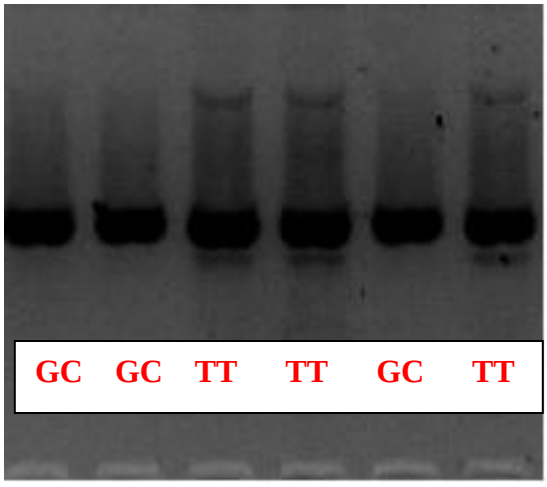
Results:



SNP 2



SNP 3



SNP 4

Discussion:
The genotypes found generally corresponded to the known gel temp haplotypes (see appendix). There were two nonconformers IR58 and PSBRC 30, in this case they will be reported, and it's assumed that environment effects or epistasis with other genes might have caused the increase in gel temp. IRRI will continue to study the effects of other genes on gel temp. The primers I used: SNP2, SNP 3 and SNP 4 are located on the SSIIa gene at chromosome 6, SSiib and SSIIIa are enzymes that can be further studied for their involvement in starch synthesis.

The Big Picture and Coming Full Circle
A Personal Reflection

My eight-week experience as a Borlaug-Ruan intern at the International Rice Research Institute exposed me to the world of agricultural research while working with dedicated and accomplished scientists from around the world. I arrived in the Philippines barely knowing how to use a pipette and left with knowledge that I could never have gained anywhere else.

Everyday in the lab was different, new students, scholars and staff where constantly in and out working on various experiments. It wasn't unusual to walk through the lab and hear conversations going on in over three different languages, nor was it unusual to be invited to a dinner party where much of the world was represented. I thought going to the Philippines would mean getting to know the Filipino people and their culture, but I also met and learned of the cultures from those in India, China South Korea, Myanmar, Ghana, Australia, Greece, Zimbabwe, Bangladesh, just to mention a few.

Every conversation was new and exciting: fighting world hunger, politics in the US and how it affects the world, discussion of different cultures and traditions, as well as the differences in the educational systems in various countries. Everyone I met spoke with optimism and a passion. That is what truly inspired me; I am fortunate to have met so many wonderful people.

The beauty of the Filipino people matches the splendor of their environment. The islands are scattered with tropical plants, outside my window I could see the IRRI rice fields and a wall of great mountains and dormant volcanoes. I was able to travel into Manila to try exotic fruits, observe traditional Filipino attire, eat the famous buko (coconut) pie and visit Manila's Ocean Park where I had my feet cleaned by fish at a 'fish spa'! I was able to visit the beautiful ocean and the great Taal Lake. I took a boat from Tagatay city (which is on a volcano) across Lake Taal. Within this lake was another volcano and inside this volcano was another lake (Volcano, lake, volcano, lake)! It was fascinating and the view was breathtaking.

I truly enjoyed my side trips as a tourist with my new friends at IRRI, but after I had spent three weeks in the lab, I felt like I was missing something. My lab experience had immersed me in the precision and detailed work of a research scientist, but I felt

disconnected from the local people. I was curious about the Philippines and the people outside the IRRI gates, outside the international research bubble, I wanted a deeper emersion. I wanted the 'big picture'. I asked my supervisor and my mentors in the lab about how I could get involved in community service. I wanted to know how I could give back to a community that had already done so much for me. A few days later I received a call from two different groups, The Society of IRRI Nonresearch Professionals (SINoP), and the Rotary Club of West Bay (RCWB).

I was warmly invited to the Rotary Club's meeting where I was kindly introduced to each member and taken in as family by President Sonny, Johnny Goloyugo and others. I started attending the club's meetings and began assisting in the implementation of a breakfast feeding program at Tranca Elementary School. After meeting with two of the schoolteachers, we found out that the school of 69 children had a monthly budget of 275 pesos or five dollars and eighty-five cents. We were also informed that the 69 children at Tranca Elementary School suffered from malnourishment and about half the children have stunted growth (see appendix).

To help these children, the Rotary Club of West Bay provided Vita Meal, a high protein and vitamin supplement, with a monetary value of 3120 pesos (sixty-sixty dollars and thirty eight cents) three days a week for eight straight months. Before we were able to give the Vita Meal, we had to teach the parents how to add it as a supplement in the children's food. That night the children had their initial weights recorded and each student was given a tablet in order to 'de-worm' each child, in case the children had parasites that would hamper weight gain.

On Monday, Wednesday and Friday mornings before my lab work began at 8 am, I went with a member of the RCWB to Tranca Elementary School to assist in the food preparation but also to interact with the children and their parents. The 'canteen' or cafeteria is a small room with two tables, enough for each grade to rotate in and out. The hot room was filled with children, and I stood over the steaming pot of food dishing out helpings to each child barely tall enough to see over the top of the table. I couldn't help but think back to the lab at IRRI, knowing that the 'Arroz Caldo' or rice porridge that they were eating was provided by the efforts of so many.

I was also called by Ms. Eva Reyes of SINoP, inviting me to join their group on a trek to the foothills of Mt. Makiling to visit their adopted elementary school. Our goal was to help the children forget about the worries of everyday life, to have fun and to help lift their spirits. We played games, ate a healthy meal and distributed snacks, a personal hygiene kit, many books and school supplies for each child. The children rustled through their new belongings. One boy was so excited to open the candy we had given him, and the first thing he asked was, "Do you want some?" Even though he had so little, he was willing to give me what he had. One of the older members of SINoP was having trouble walking in her shoes on the rocky, steep terrain and a little girl gave her worn sandals to the woman. The little girl then trekked the rest of the way bare-footed on the rocks. I will never forget these children, I went that day to bring hope and happiness into their lives,

but they had given more to me than I could ever give to them. This day is a day that will stay with me forever.

I initially thought community service was a way to give back, but I feel like I gained more than I could have imagined. The children of the Philippines gave me a new perspective; I learned to respect the difficulty they face each day while I experienced their dignity first hand. I often think about the children I helped feed in the feeding program, the boy who offered me his candy and the girl that gave up her shoes.

My volunteer experiences are some of the fondest memories of my internship, but they seeded some of my most troubling thoughts. My mind circled back to what I had researched and learned about Haiti. Like Haiti, the Philippines face similar roadblocks to an adequate food supply. There is poverty with malnourishment, great disparities in wealth, a lack of government solutions, inadequate infrastructure and technology that isn't reaching into the places it's needed the most, but there is also the story of hope.

While working in the lab I experienced the optimism and enthusiasm of dedicated researchers. While volunteering with SINoP and the Rotary Club of West Bay I assisted citizens doing their part in the fight against poverty and hunger. First hand I witnessed families with a willingness to improve the condition they were born into. It is obvious that none of these groups alone can solve the problem of food security in the Philippines, much less in the world as a whole. Everyone needs to stand up and do their part, from the field, into the lab and from the average citizen to governmental agencies. It is my aspiration to stand up for food security and extend the optimistic passion I saw in the labs at the International Rice Research Institute in Los Baños, Philippines.

Appendices

Sample Name	Gel Temp	Haplotype
IR 8	68	AGTT
IR 20	76	GGGC
IR 28	68	AGTT
IR 30	78	GGGC
IR 34	70	AGTT
IR 36	80	GGGC
IR 38	76	GGGC
IR 44	68	AGTT
IR 45	68	AGTT
IR 46	76	GGGC
IR 48	78	GGGC
IR 52	68	AGTT

IR 58	76	AGTT
IR 60	70	AGTT
IR 64	78	GGGC
IR 65	72	AGTT
IR 66	78	GGGC
IR 68	68	AGTT
IR 70	78	GGGC
IR 72	76	GGGC
IR 74	68	AGTT
PSBRC 1	78	GGGC
PSBRC 5	68	AGTT
PSBRC 9	80	NO DNA
PSBRC 10	78	GGGC
PSBRC 18	78	GGGC
PSBRC 30	78	AGTT
PSBRC 46	78	NO DNA
PSBRC 48	68	GGGC
PSBRC 50	68	AGTT
PSBRC 54	78	GGGC
PSBRC 60	67	AGTT
PSBRC 68	78	GGGC
PSBRC 70	68	AGTT
PSBRC 80	78	GGGC
PSBRC 82	78	GGGC
PSBRC 86	68	AGTT
PSBRC 92	78	AGGC
PSBRC 96	68	AGTT
PSBRC 102	70	AGTT

Tranca Elementary School
Breakfast Feeding Program

*I didn't post names for privacy reasons

Name	Date of Birth	Age	Weight (kg)	Height (cm)	Height (2)	BMI	Nutritional Status Weight	Height
Pre-Elem								
1	09-30-03	70 mos	17	110			BN	S
2	08-21-04	58 mos	11	98			BN	S
3	08-16-04	46 mos	13	98			BN	S
4	09-14-04	51 mos	13	101			BN	S
5	01-13-04	53 mos	13	108			BN	S
Grade I								
1	03-14-00	9.5	20	111			BN	S
2	12-25-01	7.5	16	101			BN	S

3	09-16-02	7	16	96			BN	S
4	03-28-02	7.5	16	104			BN	S
5	02-07-02	7.5	17	104			BN	S
6	09-10-00	9	19	108			BN	S
7	09-15-00	9	19	113			BN	S
8	08-16-03	6	15	102			BN	S
9	05-20-03	6	15	96			BN	S
10	04-04-02	7	17	104			BN	S
11	12-21-01	7.5	17	99			BN	S
12	05-11-03	6	15	100			BN	S
13	07-08-01	8	17	105			BN	S
Grade II								
1	09-22-99	9.9	21	123			BN	S
2	02-11-01	8.5	18	117			BN	S
3	02-09-00	9.5	19	117			BN	S
4	01-15-01	8.6	21	120			BN	S
5	12-31-01	7.6	18	111			BN	S
6	02-06-02	7.5	20	111			BN	S
7	03-05-02	7.4	20	110			BN	S
Grade III								
1	05-16-97	12.2	28	140	1.96	14.28	BN	
2	09-02-97	12.9	29	148	2.19	13.24	BN	
3	06-23-98	10.1	22	128	1.63	13.49	BN	
4	04-13-99	10.3	21	130	1.69	12.42	BN	
5	06-04-99	10.1	23.5	134			BN	S
6	11-02-00	8.7	18	115			BN	S
7	12-11-99	9.7	19.5	123			BN	S
Grade IV								
1	12-06-94	14.6	35	156	2.43	14.40	BN	
2	01-25-98	11.6	28	139	1.93	14.51	BN	
3	02-17-99	10.5	26	135.8	1.85	14.05	BN	
4	08-02-98	10.10	26	136.2	1.85	14.05	BN	
5	11-08-08	10.7	24	131.5	1.72	13.95	BN	
6	05-05-97	12.2	30	1141.7	2.02	14.85	BN	
7	02-07-99	10.5	21	123.5	1.51	13.91	BN	
8	10-08-98	10.8	28	141.5	1.99	14.07	BN	
Grade V								
1	03-15-99	10.4	24	130	1.69	14.20	BN	
2	09-12-98	10.9	24	132	1.74	13.79	BN	
3	06-27-98	11.1	20	124	1.54	12.99	BN	
4	09-09-98	10.9	20	124	1.54	12.99	BN	
5	01-22-98	11.6	35	150	2.25	15.56	BN	
6	06-27-98	11.1	29	140	1.96	14.80	BN	
7	03-22-96	13.4	35	150	2.25	15.56	BN	
8	03-25-98	11.4	28	138	1.90	14.74	BN	

9	09-03-98	10.10	22	132	1.74	12.64	BN	
10	12-31-94	14.6	30	147	2.16	13.89	BN	
11	04-05-99	10.3	25	134	1.80	12.89	BN	
12	04-20-99	10.3	24	131	1.72	13.95	BN	
13	10-27-98	10.8	26	136	1.85	14.05	BN	
14	05-06-99	10.2	22	128	1.64	13.41	BN	
15	07-22-97	12.0	23	134	1,79	12.85	BN	
16	11-06-97	11.7	28	140	1.96	14.29	BN	
17	07-20-98	11.0	24	130	1.69	14.20	BN	
18	05-04-95	14.2	28	142	2.02	13.86	BN	
19	02-25-99	10.5	16	121	1.46	10.96	BN	
20	09-26-98	10.9	24	143	2.04	11.76	BN	
Grade VI								
1	09-17-97	11.9	28	147	2.16	12.96	BN	
2	11-26-97	11.7	28	138.5	1.92	14.58	BN	
3	04-03-95	14.3	28	149.5	2.24	12.50	BN	
4	11-30-96	12.7	22	141	1.99	11.05	BN	
5	01-19-98	11.6	30	141	1.99	15.08	BN	
6	08-02-96	12.3	27	136.4	1.86	14.52	BN	
7	10-05-97	11.8	27	141	1.99	13.57	BN	
8	11-03-97	12.7	27	140.2	1.97	13.57	BN	
9	05-24-98	11.2	29	145.8	2.13	13.62	BN	

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