Finding Genes and Family

China National Hybrid Rice Research and Development Center
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The World Food Prize Foundation
2015 Borlaug-Ruan International Internship

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ABSTRACT:

"There is no national boundary in science." Professor Yuan Longping, director general of the China National Hybrid Rice Research and Development Center and the lead scientist behind the development of heterosis in rice, could not have spoken truer words. Today, humanity as a whole faces a challenge that spans across the lines etched in a map: hunger. Hunger fuels wars and economic instability. Hunger hinders advancement in every country it effects. There is an ever increasing need to produce more with less land and resources while the human population continues to grow. Scientific research, however, could be the light that tears through the darkness that is the reality of hunger. No matter the nation, science must focus towards the ever present battle of hunger.

The aim of this study was to determine the location of the narrow leaf gene present in rice. The narrow leaf phenotype of rice results in a decrease in photosynthetic productivity and biomass production, thus a decrease in overall yield. Through the extraction of DNA of two parent lines and narrowing of effective Simple Sequence Repeat (SSR) DNA markers, the analysis of a multitude of polyacrylamide gel electrophoresis (PAGE) gels produced the exact location of the recessive narrow leaf gene in the rice genome. Through field trials, a narrow leaf male-sterile line and a wide leaf restorer line would reveal a heterosis in which a wide leaf F1 generation would increase the overall yield of the rice plant when compared to a wild-type variety. The continued increase of rice yields through the utilization of heterosis technology can provide an avenue in which to fight hunger across national borders.

ACKNOWLEDGEMENTS

Before I begin to tackle describing the vastness of my experiences in China over my two-month internship, there are a few people I would like to extend my deepest gratitude towards for making this experience one that will not soon be forgotten. First and foremost, I would like to thank the **World Food Prize Foundation** for dedicating resources not only to the leaders of today, but those of tomorrow as well. Namely I have more thanks than can be expressed for the tireless **Lisa Fleming and Keegan Kautzky**, two people who dedicate their lives towards seeing a new generation of Norman Borlaugs rise up and take the helm in the fight to end food insecurity.

Furthermore, two people who also deserve as much gratitude as I can express are **my parents, Jeff and Katrina Bohlin**. Not only for their amazing support this summer, but throughout my entire life as well. I would never have even taken the chance had they not instilled in me during my life a dedication towards pursuing my passions. Without them, I literally would not be here today. Nevertheless, I would not be the individual I am today had they never taught me how important it is to dream but also to work towards those dreams with an uninhibited passion.

My mentors, **Yao DongPing** and **Dr. Bai Bin**, require some of the most praise. My thankfulness for Ms. Yao lies in her dedication towards ensuring that my time in China was unforgettable. Her "Chinglish" and kind words always brought a smile to my face. From ensuring I had chocolate to fighting with a mosquito net, she was with me every step of the way to guide me in learning the culture and even some of the language of China. Dr. Bai Bin, my patient mentor during experiments, deserves an award for answering and re-answering my endless questions. From discussing movies to politics, I found I had acquired a Chinese brother in Dr. Bai and I could not be more grateful for his easygoing attitude and helpful guidance.

In addition, I would like to thank all the **graduate students** in Room 408 for their warmth and acceptance of me during my two months inhabiting a desk in their office community. Among feeding me watermelon and playing volleyball with me, you all made me feel like I had been accepted into a family I never knew existed and for that, I will always be grateful.

Thank you to **Professor Yuan Longping** for opening his center to me and being a fantastic volleyball coach. I can never express how much of an honor it is to say that I have met the "Father of Hybrid Rice." He never held back a smile when he saw me around the center and always cried my name whenever I would serve the volleyball when we were playing. His warmth is unprecedented and he became one of my heroes during this too short summer.

Dr. Xin, many of my thanks go to you for being my supervisor this summer and always ensuring that I was safe and taken care of.

Finally, I would be remiss if I did not thank **Ms. Patty Keating** of LaPorte County Indiana 4-H for coming to me all those years ago and asking if I wanted to spend my summer writing an essay about food security. Little did I know that one essay would be the spark that would change my life and lead me to switch from wanting to study Veterinary Medicine to now studying International Agronomy. So thank you Patty, for providing me the opportunity to find my true calling in life and making all of this possible.

1. INTRODUCTION

1.1 My Story

"Never give up on your dreams." These words of guidance have been spoken to me by my parents throughout my life in a variety of ways. When I was young, I used to think of them as permission to daydream about being a famous singer or artist or veterinarian. Now, I still dream of large goals but with a mindset of the reality that is food insecurity and the epidemic of hunger.

Growing up in the Midwestern town of LaPorte, Indiana, I had never considered myself anything other than a "city kid." I had, however, always had an interest in agriculture due to my life long involvement in the 4-H Youth Development Program. I can remember constantly telling my mother that I would one day become a veterinarian because of how much I loved the animals I would show come fair time. In 2011, that all began to change.

One day, my mentor and 4-H Extension Educator Patty Keating approached me with an odd proposition. While at a conference, she had heard about an essay contest called the World Food Prize Youth Institute and wondered if I would like to participate. At the time, my deep love of writing and researching was being to flourish, so I readily agreed. I wrote an essay over the food insecurity of Syria and the various issues the country was facing and attended the Indiana Youth Institute in the spring of 2011. There, I was not selected to go to the large meeting in Des Moines.

I was crushed but not deterred. The next year, I wrote my essay over Nepal and their current social hierarchy. The long hours of editing and revising my essay resulted in me being selected to go to Des Moines in the fall of 2012. There, my heart was swayed from my youthful dreams of veterinary medicine. As the then head administrator of USAID was speaking to my peers and I about our responsibility to step up as the next generation to continue the fight towards a food secure world, I found myself growing to love the idea of spending my life working to further international agriculture development.

As a result, when my time came to apply for university, I found myself actively seeking international agriculture programs. This search led me to where I am now, a proud Boilermaker at Purdue University majoring in International Agronomy. Now, after completing my internship with the China National Hybrid Rice Research and Development Center, I am even more assured of the path I set out on during the World Food Prize Global Youth Institute those few short years ago.

1.2 An Unexpected Welcome

The solid jolt as my plane touched down shocked me out of my half dazed sleep. Finally, I had reached Changsha. After thirty hours of travel and a two-hour delay, I was in the city that would be my home for the next two months. Swallowing around a ball of nerves lodged in my throat, I disembarked from my plane and gathered my luggage. At two thirty in the morning, I found my

gaze falling over the people lined up to welcome various individuals into the city. But who was there for me?

It did not take long for me to hear my name being shouted from the mouth of a short Chinese woman. She smiled and introduced herself as Ms. Yao Dongping, my mentor for the summer. Dr. Bai Bin welcomed me as well saying he was Ms. Yao's husband and a researcher at the center where I would be working. Our driver stayed mostly silent in the car ride to the center. I felt myself relaxing more and more as I chatted with Ms. Yao and Dr. Bai though my mind was mainly focused on finding a bed to fall asleep in. After helping me with my luggage and showing me all the food they had bought me in case I was hungry, I was left alone in my room and preceded to fall asleep without further preamble.

The next few days would be a blur of jetlag and various new faces. Ms. Yao informed me that we would be going to a BBQ and to play Counterstrike just two days after I had arrived. Though I had no idea what either of those entailed in China, I was excited to go and meet so many new people. During that day, numerous people welcomed me to the Graduate Student Room 408 at the research center and to China. As it turns out, Chinese BBQ is not hamburgers and hotdogs like an American BBQ would be. Instead I found myself munching away on eggplant, fish, chicken feet, and various other foods cooked over a grated fire. Counterstrike, or CS as they normally call it, ended up being a rather enthusiastic game of laser gun tag. While the other team kept winning by somehow managing to always shoot everyone on our team, I will always stand by the good old Union Team.

Overall, I was welcomed into China with warmth and laughs and smiles. To be completely honest, it had not been what I was expecting. Growing up in America, you learn this stereotype of Chinese being distant and serious. I may have never openly acknowledged such as belief, but it had still taken some root in the confines of my mind. Of course my surprise came when I learned that while they can be serious when the situation calls for it, they are also some of the mostly lively people I had ever had the pleasure of getting to know. Lunch and various dinner parties had proven that to me. So while the way my welcome was carried out might have broken quite a few stereotypes I had been led to believe, I could not be happier that it did so.

2. BACKGROUND

2.1 CNHRRDC

This summer, I found myself at the Hunan Hybrid Rice Research Center. In 1984, the Hunan Hybrid Rice Research Center (HHRRC) was established in Changsha, China as the first institute in the world to specialize in the development of hybrid rice. Years later, in 1995, the China National Hybrid Rice Research and Development Center (CNHRRDC) was founded within the previously established HHRRC in order to promote the continued research into the production of hybrid rice varieties (Hunan Hybrid Rice Research Center, 2012). From its inception in 1995, the CNHRRDC has been under the leadership of Professor Yuan Longping, otherwise known as the "Father of Hybrid Rice" for his development of commercial heterosis of rice utilizing male sterile line, as the acting director general (Ramon Magsaysay Award Foundation, 2007).

Since its foundation, the center has persisted in "developing hybrid rice for the welfare of the people all over the world" (Hunan Hybrid Rice Research Center, 2012). Such a goal has led towards the development of various breeding-oriented research from developing new hybrid rice varieties to studying technology used in high yielding cultivation to evaluating seed purity. Research is far from the only goal of the center however. Training people who come to the center from across Asia and Africa to learn about hybrid rice technology is also a task that plays into the center's goals. Currently, more than two thousand individuals from over thirty different countries have visited the center to either lecture or attend trainings on hybrid rice technology (Hunan Hybrid Rice Research Center, 2012). This dedication towards the development of research but also of intellectual understanding for hybrid rice technology has led the center to be a global leader in hybrid rice advancement.

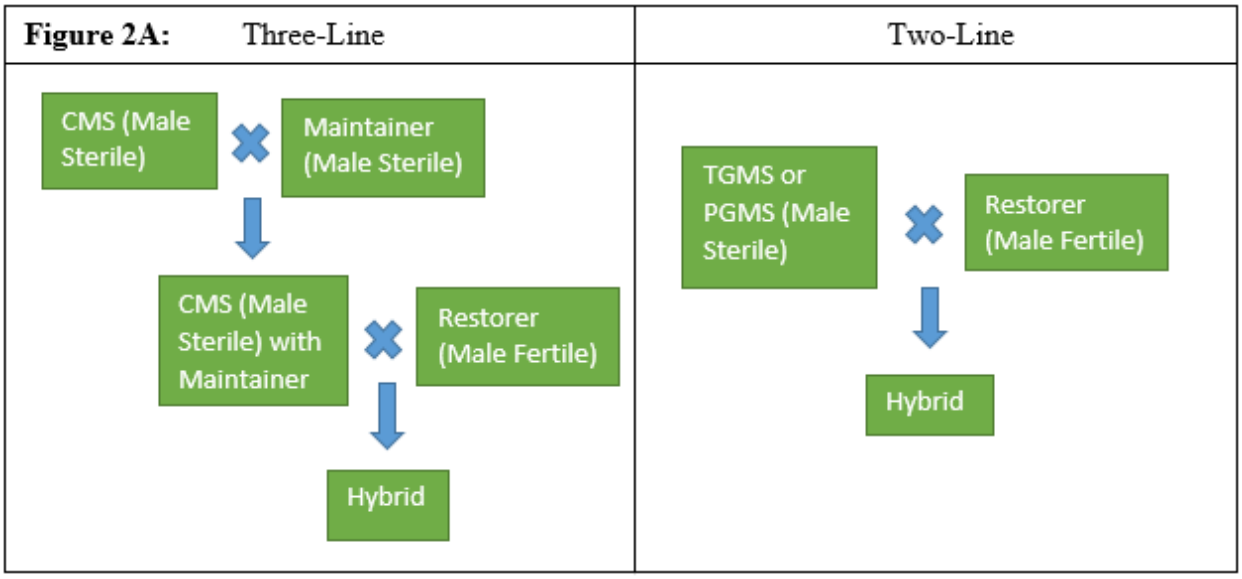
2.2 Hybrid Rice

Feeding over half of the world's population, rice is currently the main grain consumed by the human race (Yuan, Developing Hybrid Rice for Food Security, 2015). Its value, therefore, towards aiding in the fight to end hunger and malnutrition cannot be understated. The International Rice Research Institute (IRRI) has stated that the same hectare of rice which today feeds on average twenty-seven people will need to feed forty-three people by the year 2050 (Yuan, Developing Hybrid Rice for Food Security, 2015). This is coupled with the reduction of arable land for producing rice and other crops both in China and around the world. Rice has become a key player in food security development. This status has resulted in ever increasing yield goals being placed on rice development.

None of these goals would be within the realm of obtainability, however, without Dr. Yuan Longping and his development of hybrid rice technology. At the time he began his research in the 1960's, commercial hybridization was considered to be a poor tactic for rice yield increases (Ramon Magsaysay Award Foundation, 2007). This was due to the fact that rice is a self-pollinating crop, thus the crossing of two genetically distant parent lines was seen as a laborious and futile exercise. Since the effects of "hybrid vigor" or heterosis are only prominent in the first generation, or F_1, it was a pointless effort to save seeds for the next year. Therefore, efforts to produce hybrid seeds only proved to be too arduous of an effort for a single generation. In order for large amounts of production to occur, a "male sterile" line must be utilized but none existed in mass quantity at the time (Ramon Magsaysay Award Foundation, 2007). "Male sterile" lines are rice plants that have ineffective anthers and thus cannot produce fertile pollen in order to self-pollinate (Yuan, Wu, Liao, Ma, & Xu, 2003).

This reality did not deter Dr. Yuan Longping. For years he sought to increase the production of hybrid rice seeds. Through methodical research and strenuous field work, he bred a special "male sterile" line of rice which he then planted rows of between pollen-bearing rice plants (Ramon Magsaysay Award Foundation, 2007). This produced large quantities of F_1 seeds, thus making commercial hybrid rice seed production feasible where it had not been before. It would be his paper, *A Preliminary Report on Male Sterility in Rice*, published in 1966 that would come to be considered the launching point of hybrid rice research (Ramon Magsaysay Award Foundation, 2007).

Following this report, in 1973, a three-line breeding system involving a cytoplasmic male sterile line (CMS), a CMS maintainer line, and a CMS restorer line was established. By 1976, the commercial production of rice hybrids had begun utilizing this system (Cheng, Zhuang, Fan, Du, & Cao, 2007). This process did not dominate the hybrid rice field of technology for long. In 1987, a two-line system, one that does not require the utilization of a maintainer line, was developed by Dr. Yuan (Ramon Magsaysay Award Foundation, 2007). This system utilized TGMS or PGMS lines which are lines that have their male sterility controlled by photoperiod-sensitivity (PGMS) and thermos-sensitivity (TGMS) (Yuan, Wu, Liao, Ma, & Xu, 2003). With these varieties of male sterility, the ineffective anther phenotype could be controlled by exposing the developing plants to specific temperatures or photoperiods in order to induce sterility in the panicle. **Figure 2A** demonstrates the difference between the three-line and two-line hybrid rice breeding system. This advancement to a two-line system led to a yield increase of five to ten percent over the three-line system which had already increased conventional yields by fifty percent (Ramon Magsaysay Award Foundation, 2007). Therefore, the benefits of a simpler and more effective breeding system has resulted in a flourishing of the commercial production of hybrid rice.



Today, hybrid rice accounts for fifty-eight percent of rice planting area and sixty-three percent of rice production in China (Yuan, Developing Hybrid Rice for Food Security, 2015). While China is far from the only country that has benefited from the development of hybrid rice, its impact has been extreme in a country whose populace is dependent on rice for daily nutrition. Arguably the most important result of the development of hybrid rice has been that seventy million more people are fed annually (Yuan, Developing Hybrid Rice for Food Security, 2015). However, the reality remains that the world's population is continuing to grow and crops must continue to increase production. While Dr. Yuan's research was the foundation of an entirely new possibility in rice production, current research must continue to build on and surpass the knowledge of former days in order to meet the ever growing demand for more food.

2.3 Genomic Research

One such way that scientific research has continued to advance in terms of hybrid rice development is the focus on broadening the understanding of the rice genome. Rice functional genomic research progresses with the goal of increasing understanding of how the genome functions in order to produce a plant's phenotype as well as how to apply this knowledge for the betterment of the crop (Jiang, et al., 2012).

3. RESEARCH

3.1 Introduction

Photosynthetic activity greatly impacts the amount of yield that a crop plant can produce as well as the amount of resources it utilizes in its lifetime. This photosynthetic activity is greatly impacted by the surface area of leaves present on the plant. One recent study demonstrated how increasing the leaf size by inserting *NARROW LEAF1* (*NAL1*), one of the several genes that have been located which results in the expression of a narrow lead phenotype in *japonica* rice, into an *indica* cultivar resulted in a leaf surface area increase and, consequently, a yield increase of 13 to 36% (Fujita, et al., 2013). This understanding of gene expression was the direct result of gene

mapping with the hybrid rice genome. Within my project, I worked closely with Dr. Bai Bin as well as other graduate students within the center in order to locate the narrow leaf gene of a specific cross between Nipponbare and Y58S mutant line. Their phenotypes are depicted in **Figure 3A**.

FIGURE 3A

Y58S mutant line was the two-line male sterile, otherwise known as the female, parent. This rice plant acted as the receiver of the pollen from the male parent, Nipponbare. Y58S expressed the recessive narrow leaf phenotype while Nipponbare is the dominant wide leaf phenotype. Due to Y58S's narrow leaves and decreased photosynthetic activity, it also expressed a decreased biomass as a dwarf plant. This expression was explored in research which demonstrated the decreased amount of photosynthetic development a rice plant undergoes when containing the narrow leaf gene (Qi, et al., 2008). Y58S mutant line is also an *indica* variety while Nipponbare is a *japonica* variety. These vastly different genetic backgrounds would provide less similar genes between the two parents so that when crossed, there would be less risk of the F_2 generation expressing similar genes which could be mistaken for the narrow leaf gene (Bin, Gene Mapping , 2015).

3.2 Methodology

There were a number of steps that had to be taken in order to find the location of the narrow leaf gene within the F_2 mapping population. **Figure 3B** provides a brief overview of the process of gene mapping. The F_2 generation was planted and grown before my arrival at the facility.

SSR Markers: SSR, or Simple Sequence Repeat, markers are small pieces of DNA, normally between six and eight base pairs, that repeat themselves several times, anywhere from nine to thirty times (Bin, Gene Mapping , 2015). These small segments of DNA are developed from the existing genetic knowledge of the genome of the organism.

PCR: PCR, or polymerase chain reaction, is a fast and inexpensive method in which many copies of DNA are made. It consists of three steps: denaturation, annealing, and extension. In denaturation, the double helix of an organism's DNA is deconstructed into single strands. During the annealing process, these single strands then bond to the complementary SSR markers that were mixed with the organism's DNA. Extension then occurs to outspread the DNA chain and finish constructing the new complimentary strand (PCR Protocol, 2015).

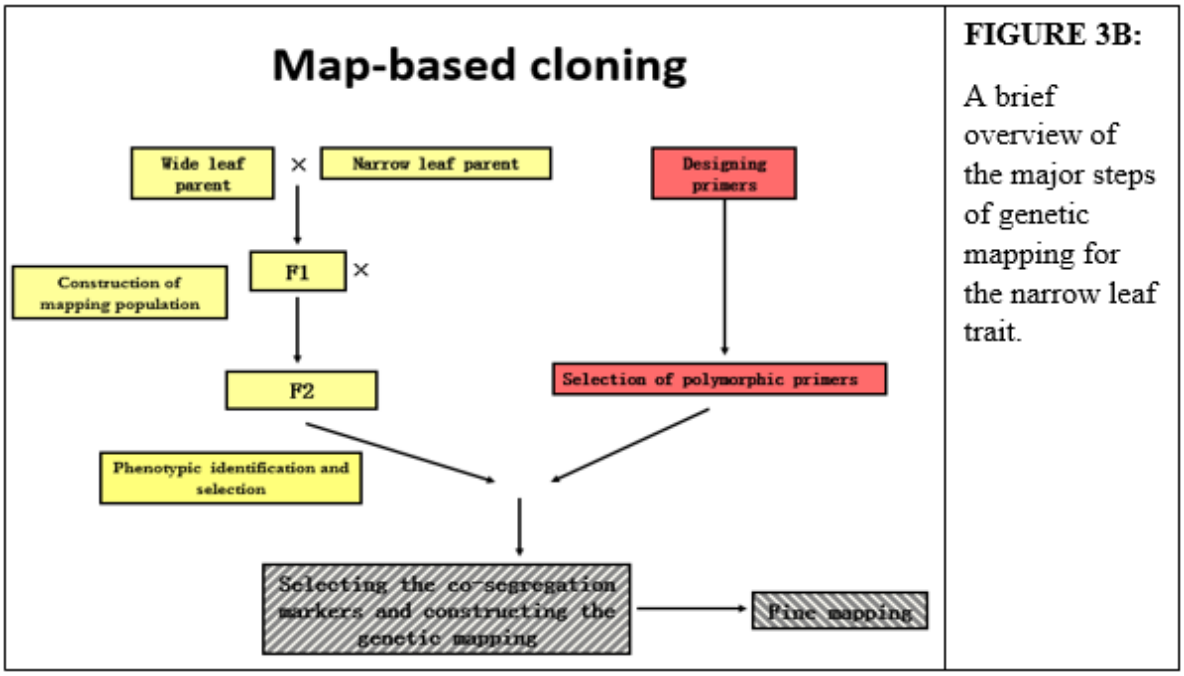


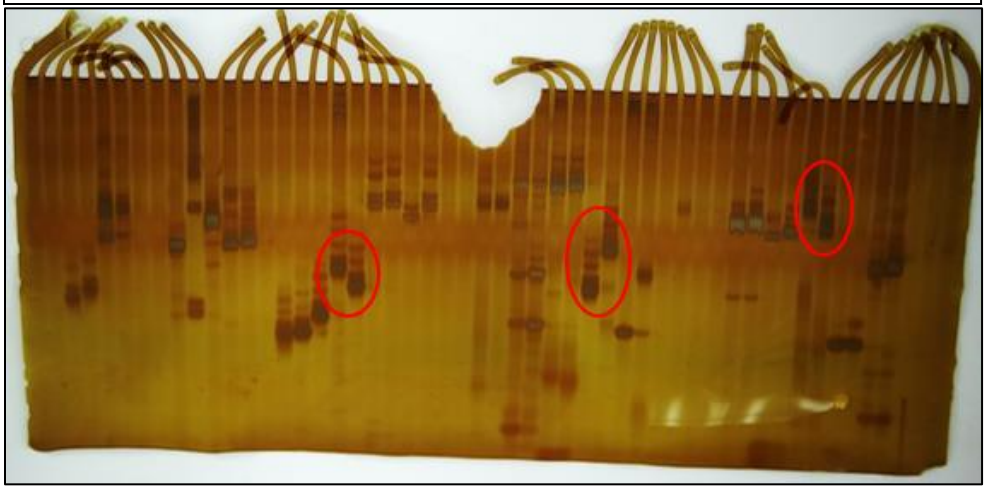
FIGURE 3B:

A brief overview of the major steps of genetic mapping for the narrow leaf trait.

PAGE: PAGE, or DNA polyacrylamide gel, is utilized to demonstrate a physical distance between pieces of DNA. The products of PCR are placed within individual wells of the gel and an electric current is run through the gel. This current causes the PCR products to move through the gel according to their physical size (Yao, 2015).

SSR Marker Selection: My first responsibility was specific SSR marker selection. In order to select SSR markers for gene location, Dr. Bai and I looked for SSR markers in the twelve chromosomes of rice that demonstrated a distance between the dominant male and recessive female gene. (For a full list of the SSR markers used, please refer to the SSR Marker Index at the end of the paper.) In order to do this, we utilized PCR and PAGE technology. After this process, the finished PCR products were then placed into individual wells of a PAGE to demonstrate a physical distance between the varying genes. In the SSR marker selection, our specific goal was to locate markers that demonstrated a physical distance between the two parent lines. **Figure 3C** depicted below shows the second half of the first PAGE gel for chromosome 9 SSR markers to demonstrate the process. The markers within the

FIGURE 3C



red circles are SSR markers that showed a physical distance between the female (left) and the male (right) PCR product. This meant that there was a difference between the linkages of the SSR marker to the specific gene in the parent line. These specific markers were then run through PCR and PAGE again to verify the presence of a physical distance between the parent lines.

F_2 Generation Plant Selection: In order to locate the narrow leaf gene within the F_2 backcross, we collected 107 individual rice plants that expressed the recessive phenotype. Specifically, plants with a leaf width of 0.6 centimeters or less were selected. From these samples, 94 were utilized for individual DNA extraction. Nanodrop technology was utilized in order to test the DNA solution concentration of the extractions. Since the plant had to only contain narrow leaf alleles within its genome, we were able to assume that these samples would express the recessive gene when mixed with the previously selected SSR markers and run through PCR and PAGE.

Finding Chromosomal Location: From the previously created F_2 generation DNA extractions, two DNA mixes were created. By mixing together 30 microliters from 10 individual DNA samples for both mixtures, we were able to create a DNA mixture in which we could assume that the recessive genetic sequence was highly concentrated. These mixtures were then used in finding the chromosomal location using PCR and PAGE. **Figure 3D** represents a 96 well PCR plate which is utilized in the PCR machine. In well A1, the female parent Y58S mutant line DNA was placed while well A2 contained the male parent Nipponbare DNA. A3 and A4 held F_2 DNA Mix 1 and 2 respectively. To each of these four wells, a single selected SSR marker from the first step was added. This process was repeated in every four wells for each of the selected SSR markers. After performing PCR and PAGE with these samples, the PAGE plates were analyzed for an area SSR markers that expressed only the recessive female band. **Figure 3E** demonstrates this desired outcome. The four SSR markers which are located on Chromosome 3 of the rice genome are highlighted by the red boxes. SSR markers RM 1284, RM 14772, RM 282, and RM 3134 all demonstrated the recessive female band.

FIGURE 3D: 96 Well PCR Plate

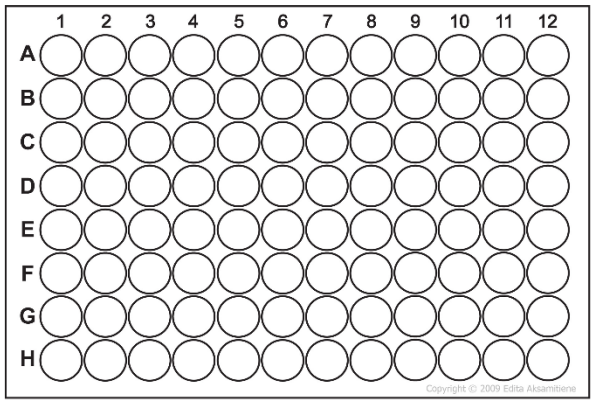
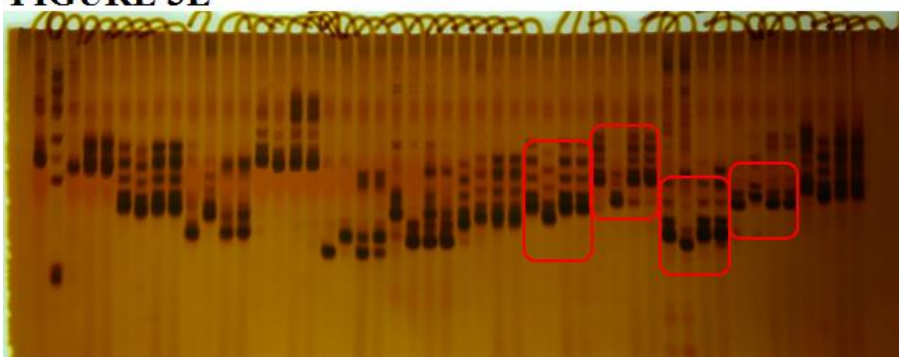


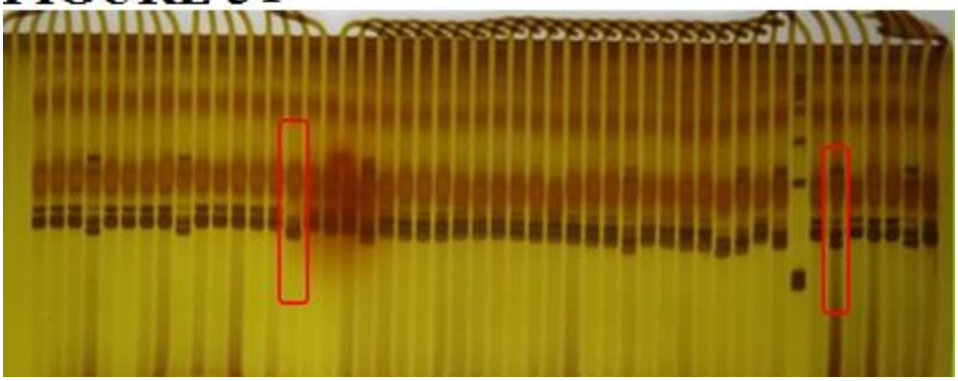
FIGURE 3E



This meant that the SSR marker DNA sequences were closely linked to the recessive gene of the female parent, and thus were close to the base pair location belonging the desired gene (Bin, Gene Mapping , 2015).

Genetic Mapping: After finding the physical distance, we were able to move forward to genetic mapping by using the four SSR markers that demonstrated linkage with the recessive female gene. By using the two parent lines' DNA and 94 individual DNA samples from the F_2 generation, we were able to find the estimated base pair location of the desired narrow leaf gene.

FIGURE 3 F



We did this by analyzing the resulting PAGE plates for the number of male and hybrid (cross between the male and female bands) that were expressed. The more male and hybrid bands expressed, the greater the rate of crossover which represents a farther distance between the SSR marker sequence and the DNA sequence of the recessive gene. This also meant that the degree of linkage was lower between the SSR marker and the recessive gene, which also equates a larger distance between the two. **Figure 3F** shows SSR marker RM 282 PAGE plate for this process. Most bands demonstrate the female recessive band. However, the red box on the left represents a male band while the red box on the right highlights a hybrid band. These bands were counted for each of the four SSR markers and then analyzed for genetic distance using the calculation in **Figure 3G**. This calculation accounted for the estimated distance between the SSR marker and the desired gene. The number of hybrid bands were counted as one since there was only one dominant allele present in the hybrid band. In the solely male bands, two male bands were counted since it is assumed that the individual has two dominant alleles. The example calculation is for RM 282's genetic distance from the narrow leaf gene. The four SSR markers' genetic distances and locations are listed in the table below (Gramene Markers Database, 2015).

FIGURE 3G

Genetic Distance Calculation

$$\frac{\text{\# of Hybrid bands+ \# of Male bands*2}}{\text{Total \# of bands per primer}}$$

$$= \frac{17+ 1*2}{94*2}$$

$$= \frac{19}{188}$$

SSR Marker	Location (mbp)	Genetic Distance
RM3134	7	29/188
RM14772	9	8/188
RM1284	10	4/188
RM282	12	19/188

3.3 Results

After finding the gene location on chromosome 3 for the narrow leaf recessive gene, we were able to narrow down the location to between 10 and 12 million base pairs utilizing a genetic distance calculation. The obtained data from the last step of the methodology process was then utilized in MapMaker 3.0 software in order to create a physical genetic distance map as seen in **Figure 3HA** and **Figure 3HB**. The distance expressed in **Figure 3HB** is the distance between the marker or *na1* (narrow leaf gene) to RM 282. This was then inputted into an excel file in order to create a visual representation of the rice chromosome 3 as seen in **Figure 3HA**. As demonstrated above, the narrow leaf gene is located between RM 1284 (location of 10 million base pairs) and RM 282 (location of 12 million base pairs). This confirmed our analysis of the recessive narrow leaf trait being located between 10 and 12 million base pairs on chromosome 3.

FIGURE 3HA

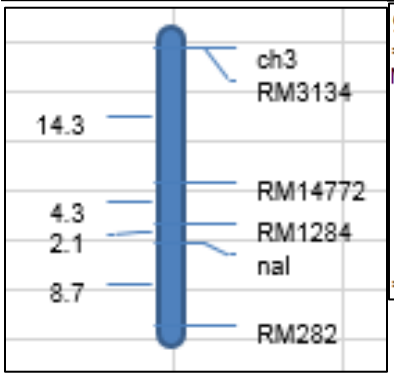


FIGURE 3HB

```
9> map
=============================================================================
Map:
  Markers          Distance
     4  rm3134       14.3 cM
     2  rm14772       4.3 cM
     1  rm1284        2.1 cM
     5  nal           8.7 cM
     3  rm282      ----------
                     29.4 cM   5 markers   log-likelihood= -123.18
=============================================================================
```

3.4 Discussion

This specific location for a narrow leaf gene had yet to be located within the rice genome before this experiment (Gramene Gene and Allele Database, 2015). The data found through this experiment will now be utilized in fine mapping in order to find an even more exact million base pair location between 10 and 12 million base pairs for this specific narrow leaf gene. Through this further research, the extrapolated data can be utilized in order to expand the understanding of the rice genome as well as ways to selectively breed and alter the hybrid rice plants in order to increase yield. One such method will be the selection for narrow leaf phenotypes in the male rice plants. The pollinating male rice plants do not to be as large as the female plants since the female's seed is the desired goal of hybrid rice breeding; therefore, the male plants do not need to perform as much photosynthesis and intake as many resources in order to produce a large yield. The female plants, however, are desired to produce large quantities of the F_1 generation hybrid seed. In order to do this, they must be able to perform large amounts of photosynthesis

and intake more resources than the male plants. By increasing the leaf width of these female plants, a higher photosynthetic productivity and thus a larger overall biomass including yield becomes possible (Bin, Narrow Leaf Gene, 2015). In conclusion, it will only be by understanding the rice genome through experiments such as these that such yield advances can take place within hybrid rice production in order to establish food security and meet the growing demand for staple foods.

4. PERSONAL REMARKS

As a high school student, the Global Youth Institute ignited a passion within me to dedicate my life towards working to build a more food secure world. Now, as a sophomore in university, I can say with complete honesty that I never imagined experiencing such an amazing introduction to what that goal represents so soon in my barely begun professional life. While in China, I not only grew as an individual but I grew as a professional. I experienced culture in a way I never had before. From acquiring a Chinese name and family to learning how to ride a bike for the first time (I am not kidding about that.), I have experienced something that many people would wish for if only they knew the opportunity existed.

My summer in China in Graduate Room 408 will not be one I soon forget. It reminded me of the passion and love I have for the people of this world and the desire I have to see every person have the equal opportunity to food security. I know the journey will not be an easy one. Regardless, it is one I am all too excited to take.

I am forever grateful to my Chinese friends who taught me more than they will ever know. I cannot deny that coming home was a bittersweet moment. As I said my goodbyes in the Changsha airport, I could not help but feel saddened that I was saying goodbye to these people who had become a family to me in just two short months. However, I am reminded of the words Dr. Bai Bin spoke to me just before we parted. He told me, “This is not goodbye for forever. Only for a short time.” I think that is the beauty of this battle for food security. That people across cultures can find such a common passion to see humanity prosper even in the hardest of times that they will view the world with such an optimism that even half a world away we still see each other as part of this collective family of human beings that will one day see each other again.

5. SSR MARKER INDEX

This represents the SSR markers that were utilized in the genomic mapping as well as the physical distance location experiments. The markers highlighted in green are those which were selected for physical distance location within chromosome 3.

NAME	CHROMOSOME	POSITION	SIZE
RM3627	1	10,307,848-10,307,999 bp	116
RM529	1	40,670,383-40,670,655 bp	273
RM5389	1	35,732,311-35,732,554 bp	132
RM6073	1	13,540,292-13,540,742 bp	96
RM6333	1	38,008,586-38,008,769 bp	102
RM6340	1	1,058,498-1,058,646 bp	149
RM6777	1	4,216,111-4,216,252 bp	142
RM207	2	35,369,336-35,369,671 bp	118
RM213	2	34,652,316-34,652,502 bp	139
RM240	2	31,497,147-31,497,256 bp	132
RM2634	2	20,495,111-20,495,503 bp	154
RM3512	2	27,314,922-27,315,116 bp	194
RM290	2	10,806,902-10,807,358 bp	142
RM492	2	7,285,639-7,285,862 bp	224
RM530	2	30,532,198-30,532,386 bp	161
RM5472	2	30,637,202-30,637,353 bp	151
RM555	2	4,305,685-4,305,907 bp	223
RM7082	2	5,100,903-5,101,063 bp	130
RM71	2	8,760,433-8,760,557 bp	149
RM1284	3	10,620,093-10,620,291 bp	150
RM14772	3	9,948,802-9,948,971 bp	169
RM282	3	12,407,382-12,407,510 bp	136
RM3134	3	7,240,409-7,240,586 bp	178
RM3525	3	30,386,575-30,386,931 bp	179
RM426	3	27,588,613-27,588,762 bp	150
RM520	3	30,912,691-30,912,804 bp	247
RM6266	3	23,821,943-23,822,102 bp	160
RM280	4	34,989,558-34,989,727 bp	155
RM307	4	N/A	174
RM335	4	688,353-688,466 bp	104
RM3471	4	6,310,055-6,310,203 bp	147
RM348	4	32,650,358-32,650,527 bp	136
RM5414	4	N/A	116

RM5687	4	15,742,285-15,742,442 bp	158
RM7279	4	13,748,372-13,748,573 bp	201
RM163	5	19,189,417-19,189,547 bp	124
RM2422	5	7,022,676-7,023,094 bp	125
RM267	5	2,881,317-2,881,455 bp	156
RM289	5	7,807,745-7,807,830 bp	108
RM3345	5	2,011,716-2,011,916 bp	116
RM405	5	3,073,406-3,073,516 bp	110
RM501	5	N/A	179
RM5140	5	13,484,209-13,484,464 bp	190
RM548	5	2,818,504-2,818,762 bp	259
RM162	6	24,035,491-24,035,615 bp	229
RM253	6	5,425,408-5,425,602 bp	141
RM2634	6	20,495,111-20,495,503 bp	154
RM276	6	6,230,045-6,230,185 bp	149
RM3	6	19,499,320-19,499,437 bp	145
RM3183	6	28,469,081-28,469,279 bp	105
RM3353	6	435,582-435,697 bp	116
RM340	6	28,599,181-28,599,319 bp	163
RM412	6	30,327,854-30,328,051 bp	198
RM508	6	441,616-441,850 bp	235
RM541	6	N/A	158
RM5314	6	24,842,796-24,842,949 bp	154
RM11	7	19,256,914-19,257,039 bp	140
RM1134	7	3,592,604-3,592,890 bp	144
RM1279	7	21,613,944-21,614,125 bp	182
RM1377	7	12,783,530-12,783,710 bp	181
RM320	7	18,693,223-18,693,635 bp	167
RM3691	7	19,225,143-19,225,259 bp	117
RM560	7	19,582,983-19,583,222 bp	239
RM5623	7	23,109,505-23,109,688 bp	184
RM6697	7	1,186,674-1,186,980 bp	229
RM1235	8	1,208,467-1,208,914 bp	118
RM22786	8	11,061,283-11,061,467 bp	184
RM3120	8	27,817,526-27,817,645 bp	120
RM331	8	12,294,124-12,294,755 bp	176
RM337	8	152,299-152,485 bp	192
RM339	8	N/A	148
RM547	8	5,591,403-5,591,685 bp	235

RM447	8	26,546,992-26,547,102 bp	111
RM6845	8	27,560,145-27,560,304 bp	143
RM6990	8	14,360,120-14,360,247 bp	130
RM7049	8	20,812,376-20,812,534 bp	159
RM72	8	N/A	166
RM201	9	20,174,289-20,174,430 bp	158
RN205	9	22,720,624-22,720,811 bp	122
RM24545	9	18,219,861-18,220,006 bp	145
RM257	9	17,719,660-17,719,823 bp	147
RM3808	9	20,547,384-20,547,502 bp	119
RM3912	9	10,826,210-10,826,472 bp	205
RM410	9	17,642,699-17,643,295 bp	183
RM566	9	14,704,764-14,704,906 bp	239
RM6174	9	22,268,515-22,268,709 bp	108
RM222	10	N/A	213
RM228	10	22,243,157-22,243,349 bp	154
RM258	10	18,014,265-18,014,633 bp	148
RM330	10	25,153,466-25,153,681 bp	177
RM342	10	N/A	141
RM5666	10	21,407,378-21,407,562 bp	151
RM6673	10	23,010,698-23,010,897 bp	147
RM167	11	4,073,024-4,073,313 bp	128
RM21	11	N/A	157
RM229	11	18,407,879-18,408,009 bp	116
RM286	11	383,711-383,945 bp	110
RM287	11	16,767,319-16,767,617 bp	118
RM479	11	7,692,726-7,692,978 bp	253
RM6965	11	24,637,594-24,637,781 bp	187
RM1246	12	19,086,651-19,086,837 bp	162
RM1337	12	11,933,306-11,933,452 bp	210
RM17	12	26,954,657-26,954,947 bp	184
RM247	12	3,185,384-3,185,581 bp	131
RM270	12	N/A	108
RM6296	12	3,200,571-3,200,724 bp	155

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