Identification and Development of Single Nucleotide Polymorphisms linked to Rpk-1k Resistance Genes in Soybeans for Marker Assisted Selection of Varieties resistant to *Phytophthora Sojae*

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My Background

My name is Eric Chen, and I am a student in San Diego, California. I currently attend Torrey Pines High School, and I am deeply interested in science for its potential to impact other people. When I first heard about the World Food Prize program through my AP Chemistry teacher, I was intrigued by the mission of the organization as well as the opportunities that it offered. The World Food Prize creates a platform for open dialogue between world leaders to end the global food crisis. This unique vision prompted me to submit a research paper about the impact of communication and infrastructure development on rural Bangladeshi farmers. As I wrote the paper, I became increasingly interested in the social and economic impacts of proper communication and access to resources.

From rural Bangladesh, I made my way to Iowa. After being selected as a California delegate to the 2013 Global Youth Program, I was exposed to a community of extremely motivated and creative people. It was through the GYI that I was first introduced to the work of Dr. Norman Borlaug and the fundamental role that he has played in the green revolution. Dr. Borlaug was undaunted in the face of difficulty, and improved the lives of many people. At the GYI, I also met people from all walks of life: government workers who were focusing on agricultural policies, farmers who were interested in ending world hunger, and people like President Grímsson of Iceland, Tony Blair, and Howard G. Buffet. Probably the most outstanding element of this conference is that people from diverse careers gather together for a week to focus on a common issue, to reach a common goal—ending world hunger. Being able to listen and contribute to the ongoing dialogue provided an enriching experience for me. After talking to the previous Borlaug-Ruan interns, I was inspired to personally contribute to this global effort. The opportunity to perform important research in another country and be immersed in a new culture was something that I could not resist.

After reading several research reports and hearing back from previous interns, I decided that I would like to work with biotechnology and agronomy. Together with my desire to discover a new culture, Brazil was the perfect fit. I worked at the Brazilian Enterprise for Agricultural Research (Embrapa Soja), paired with a Master's Student from the biotechnology lab. I would spend the next two months working to help identify a soybean resistance gene to *Phytophthora Sojae*, a pathogen that decreases crop productivity in Brazil and the rest of the world. In the time between experiments for my main research project, I assisted other researchers in the lab to get a global view of the biotechnology program at Embrapa.

On June 15, 2014, I embarked on a journey that would change my life.

Londrina, Brazil

When I landed in Londrina, I picked up my belongings by the baggage claim, and I was greeted by warm hugs from Marcelo and Denyse, my host parents. As we drove through Londrina to the apartment, I noticed was that many of the sidewalks, buildings, and other structures had rusty hues. I soon discovered that this was because the soil has a rich, red color, and it tends to get blown everywhere. The rich soil is also a contributing factor to the great agricultural productivity of Londrina. Londrina's economy is largely based on agriculture, as it was historically a coffee producing region. Now soybean, wheat, corn, and sugarcane are the major crops, though there are many other cultivated crops.

While in the car, I asked Marcelo about the work schedule at Embrapa Soja, and he said that we would normally work from 8:00 AM to 5:00 PM each day. However, my internship coincided with the FIFA World Cup, so whenever Brazil had a game, work would stop at 12:30 PM and everybody would go celebrate. When I entered the apartment, Denyse immediately showed me some Brazilian hospitality. She had already prepared a pan of freshly baked pão de queijo (cheese bread) and a glass of Guarana, a traditional soft drink. I was grateful for the warmth and kindness shown to me from the moment I stepped foot in Londrina. Overall, Londrina is a calm and diverse city, with immigrants from Portugal, England, Japan, and Italy among other countries. I grew to love the people, food, and culture during my two months there.

Embrapa Soja and the Biotechnology Lab

Embrapa, the Empresa Brasileira de Pesquisa Agropecuária or Brazilian Enterprise for Agricultural Research, is a network of state-owned companies in Brazil. There are 46 units throughout Brazil, all devoted to research with different agricultural areas such as livestock, sugarcane, and the environment. Because agriculture accounts for 5.5% of Brazil's GDP, many research facilities are dedicated to increasing agricultural quality and productivity [1].

Figure 1 Field for developing technology at Embrapa

The branch of Embrapa that I worked in was Embrapa Soja (Embrapa Soybean), located in Londrina, Brazil. At Embrapa Soja, research is focused on the improvement of the soybean, with some research on sunflower and wheat as well. The overall mission of Embrapa Soja is to "facilitate, through research, development, innovation, and solutions, the sustainability of production chains of soybean and sunflower for the benefit of Brazilian society." [2]

On my tour of Embrapa's facilities, I had the opportunity to visit many of the research fields and the Embrapa lake.

2 Embrapa's lake and research fields

At Embrapa Soja, there are many researchers, ranging from undergraduate students to professional researchers. I worked in the Biotechnology lab, and I had the opportunity to interact with many of these researchers. My mentor for the internship was Dr. Francismar Marcelino-Guimares, and she familiarized me with the different lab units. During my two months in the biotechnology lab, I worked closely with Adriano on his Master's project, and I helped out other people in the free time that I had in my own project.

Research Results

Soybeans

Soybean cultivation has historical roots in East Asia that predate many written records [3]. In Asian cuisine, the soybean was mainly used for fermented products like soy sauce, natto, and miso. Eventually, cultivation of soybean spread to other continents, including South America. Cultivation of the soybean first began in Southern Brazil in 1882, with seed from the United States of America. However, the seeds were not suited for the Brazilian environment, so initial progress was slow. Substantial increase in importance of soybeans in Brazil started in 1960, with the adaptation of better seeds, technology, and farming techniques [4].

The soybean is an incredibly important crop worldwide. As a food source, soybeans are healthy and nutritious because they contain alpha-linolenic acid, isoflavones, and all the essential amino acids [5]. The two main components of soybean crop include soybean oil and the soybean meal. The majority of the soybean is composed of the protein-rich soybean meal, which is used for raising livestock, which ultimately produces poultry, pork, beef, dairy, and fish. In the United States, the poultry and livestock markets consume 98% of soybean meal. The international market plays a substantial role, consuming nearly half of the soybean crop of the United States [6]. Of the international market, China imports about one-fourth of the soybeans produced in the US. Thus, the soybean crop is essential for sustaining the worldwide food supply.

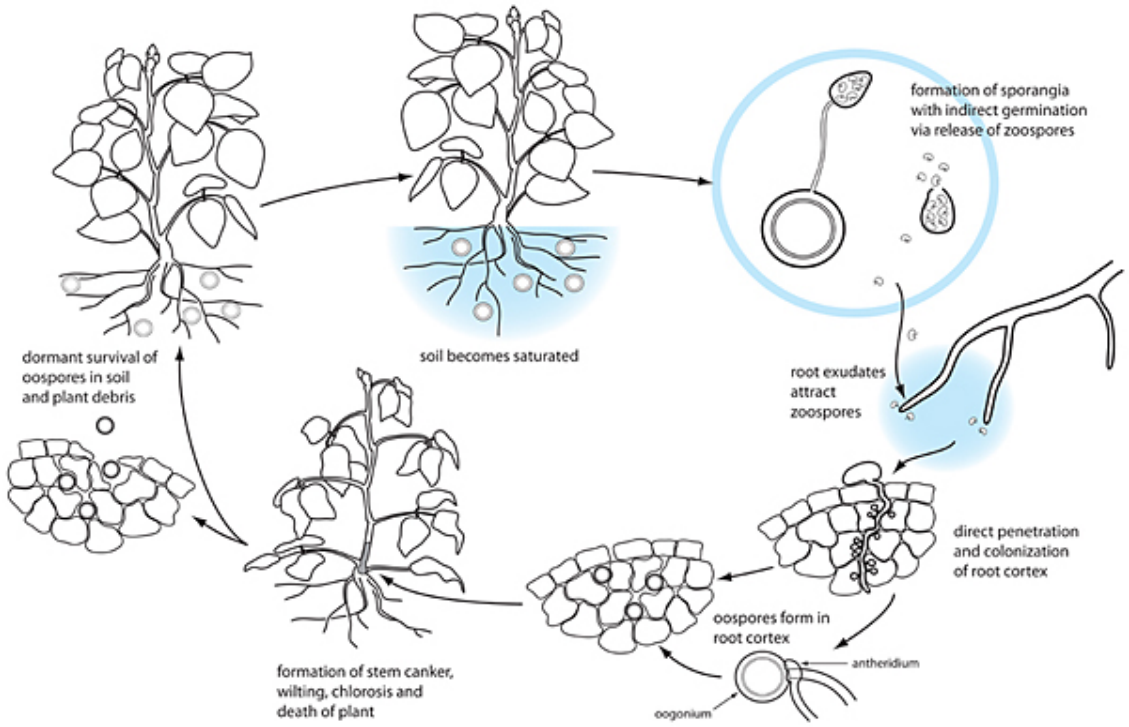
Soybean oil is currently used mainly for human consumption, although there are some secondary purposes. The soybean protein has also been integrated into international cuisine, with possible end products like soy milk, tofu, and soy sauce. However, soybeans are increasingly being used for purposes outside of human and animal consumption. Researchers around the world are finding ways to use soybeans to make oil, plastics, and many more products. Currently, the United States is the largest producer of soybeans, accounting for 32% of worldwide production. Brazil is the second largest producer of soybeans after the United States, accounting for 31% of production [7].

Background

Unfortunately, the soybean crop is threatened by a number of different factors such as drought, Asian soybean rust, and nematodes. Because the soybean is an internationally important crop and has roles in many different industries, it is important to develop methods to increase crop resistance to the many threats.

This research project specifically addresses P*hytophthora sojae*, a particularly devastating cause of crop loss in *Glycine max*, the cultivated soybean [8]. *Phytophthora sojae* is a pathogen that causes stem and root rot, seed decay, and damping off in young plants. When plants are more developed, *Phytophthora Sojae* causes root and stem rot, and wilting of the leaves. Eventually, the lesions caused by pathogen can extend to the rest of the plant and cause death [9]. Overall, *P. Sojae* drastically reduces yield per plant, regardless of the time of pathogen introduction.

The following figure illustrates the general disease cycle of oomycetes, the general classification of *P. Sojae* [8].



The first instance of *P. Sojae* in the US was in Indiana, in 1948, just as soybean production was taking off [10]. During the late 1970's, there was little done to manage this disease, so Ohio lost 300,000 acres of soybeans in one year. Soybean infection by *P. Sojae* is exacerbated by poor drainage techniques, high clay content, reduced till practices, and absence of crop rotation [9]. The pathogen is present in many soybean production areas, but the problem will worsen as it continues to spread to other regions. Even at this stage, *P. Sojae* is estimated to cause losses of about $300 million annually in the United States [11].

The current methods of coping with the pathogen include fungicide application, field drainage, and soil tillage. Metalaxyl, a fungicide used to kill oomycetes, can also be used to control *P. Sojae*. The fungicide is used as a preventive measure to stop the spores of *P. Sojae* from entering the plant tissues [12]. However, proper soil management is not entirely effective, and fungicide application is only temporary and may have adverse effects.

Thus, the most promising route for crop viability lies within developing genetic resistance to *Phytophthora sojae*. There are currently 15 single resistance *Rps* genes (*Resistance to*

Phytophthora Sojae), each of which provides some measure of resistance to *P. sojae* [13]. *Rps* genes exist in a gene-for-gene relationship with the corresponding genes of *P. Sojae*, and activate the plant's immune system [14]. For instance, one soybean plant response is induced apoptosis in the infected plant tissues. This prevents the spread of the pathogen to the rest of the plant by localizing the potential infection areas [15].

Identification and Development of Single Nucleotide Polymorphisms linked to Rps-1k Resistance Genes in Soybeans for Marker Assisted Selection of Varieties Resistant to Phytophthora Sojae

Though there are currently 15 *Rps* genes that have been identified, there are more to be discovered [16]. These 15 genes include all the different alleles, and they map onto four different chromosomes and molecular linkage groups. These are Chromosome 3 (MLG N), Chromosome 13 (MLG F), Chromosome 16 (MLG J), and Chromosome 18 (MLG G) [17]. This report specifically studies *Rps*-1k, which is located on Chromosome 3. A single *Rps* gene is not sufficient to provide resistance to all the strains of *P. Sojae*, so in order to ensure resistance to multiple strains and create a viable crop, the overarching goal of the project is to help stack multiple *Phytophthora sojae* resistance genes for the cultivated soybean.

The more specific goal of this project is to develop genetic markers to be used in marker-assisted selection (MAS) to speed up incorporation of *Rps* genes into new soybean cultivars. These molecular markers must be closely linked with the *Rps* genes in order to confer resistance to *P. sojae*. This project studies the relationship between *Rps*-1k and single nucleotide polymorphisms (SNPs), which serve as a specific type of genetic marker. A SNP is a variation at a single nucleotide base in the DNA, and is highly conserved in a population, which makes it an ideal genotypic marker. Thus, this project identifies SNPs linked with *Rps*-1k for use in future MAS of resistant cultivars. Specifically, primers and probes can be developed to select cultivars that contain the desired sequence containing the SNP. This would allow researchers to determine a cultivar's resistance to *P. sojae* in a much smaller timeframe, before there are phenotypic manifestations of resistance.

Materials and Methods

To identify SNPs for *Rps*-1k, 12 different samples (6 resistant and 6 susceptible) were studied. The Resistant samples were Williams 82, Kingwa, PF 11314, PF 11326, Alvo, and Resnik, while the susceptible samples were MG 752S, 268, 133, 284, 360 RR, and Invernada.

The initial step is to extract and purify the DNA from the leaves of the soybean samples. Ideally, the young leaves would be selected and grinded and processed for the DNA extraction, because they contain the highest concentration of DNA. While the DNA is being extracted, the primers are designed to flank both sides of the sequences of interest. These primers are designed based on information from the soybean genetic databases.

The next step is to amplify the target sequence through the polymerase chain reaction, or PCR. Below are the materials necessary for the PCR.

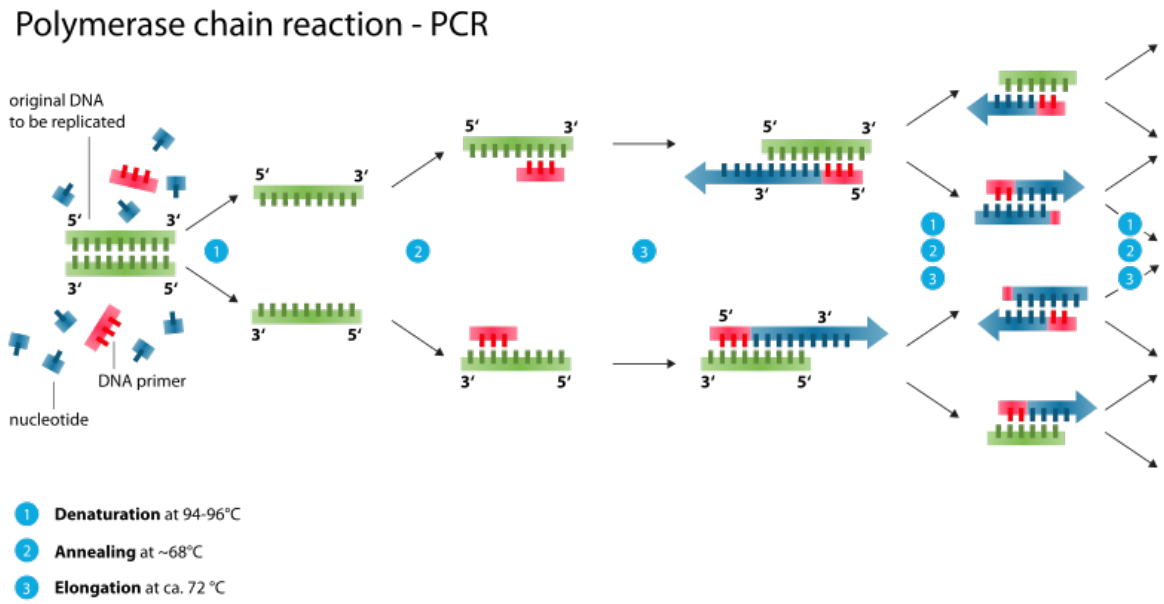
PCR reaction sample		
Material	Quantity	Unit
Primer F	2.5	µL
Primer R	2.5	µL
H2O	29.7	µL
Buffer	5	µL
MgCl2	3	µL
dNTP	3	µL
Taq P	0.3	µL
Total Volume of Mix		
DNA	4	µL
Volume of mix per well	46	µL
Volume of mix + DNA per well	50	µL

The quantities described above are for one single DNA sample, so for multiple samples, the quantities are simply multiplied by the total number to be completed. The samples are then mixed with a pipette tip, and placed into a thermocycler.

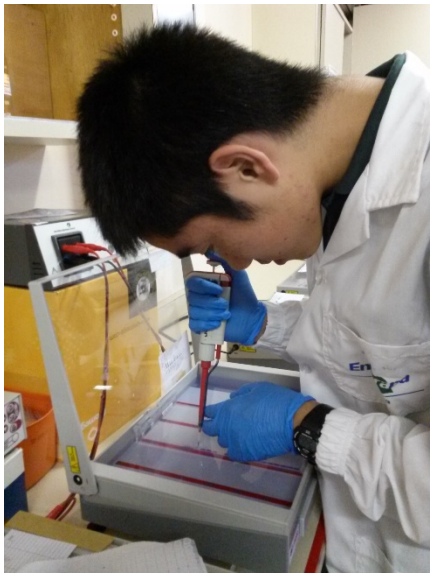
The PCR for amplification of the target DNA sequence was performed in the order as shown below:

PCR Cycle		
# cycles	Temperature	Time per stage
1x	95 ^{O}C	2 min
35x	95 ^{O}C	30 sec
	50* ^{O}C	30 sec
	72 ^{O}C	1 min
1x	72 ^{O}C	5 min
	4 ^{O}C	∞

For each cycle, the target sequence is replicated once, so if n is the number of cycles, there will be 2^{n+1} times the original DNA sample. The following diagram briefly illustrates the process of PCR, which amplifies the target DNA [18].



To check if the PCR reaction is completed correctly, the samples are analyzed through gel electrophoresis and imaging. The procedure for preparing a 150 mL, 1% agarose gel is described in Appendix A. After PCR, samples are mixed with dye and pipetted into the wells for gel electrophoresis. This procedure is outlined in Appendix B. This stage of the project (PCR and gel electrophoresis) was most time consuming, because the original Taq polymerase that was used for PCR did not function as expected.

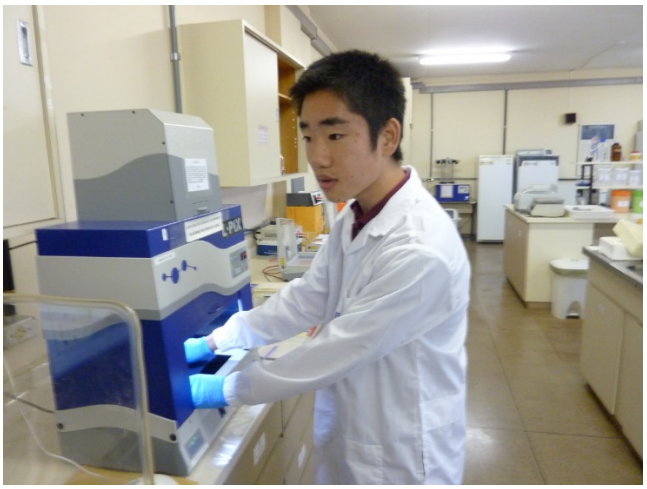


3 pipetting the DNA samples to run the gel electrophoresis

After the all the samples are identified to contain the target sequence of DNA, the samples are sent for sequencing. Once the sequencing results are received, they are parsed and analyzed with bioinformatics tools to identify the genetic marker linked with the *Rps*-1k gene.

Data

The three primer pairs used for amplification of *Rps*-1k were: 4799201 & 4799242, 4890783 & 4890785, and 4931408 & 4931379. The PCR reactions were completed, and to verify that the sequences were amplified correctly, we ran the gel electrophoresis test. I took photos of the gel after running the electrophoresis for about an hour.

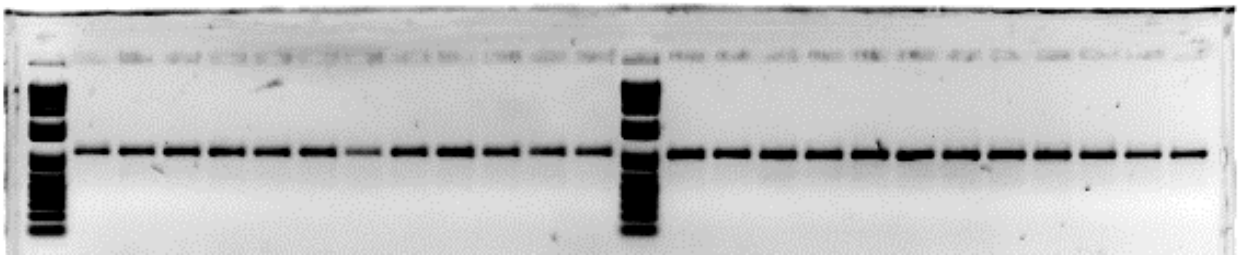


4 Taking images of the gel after running electrophoresis

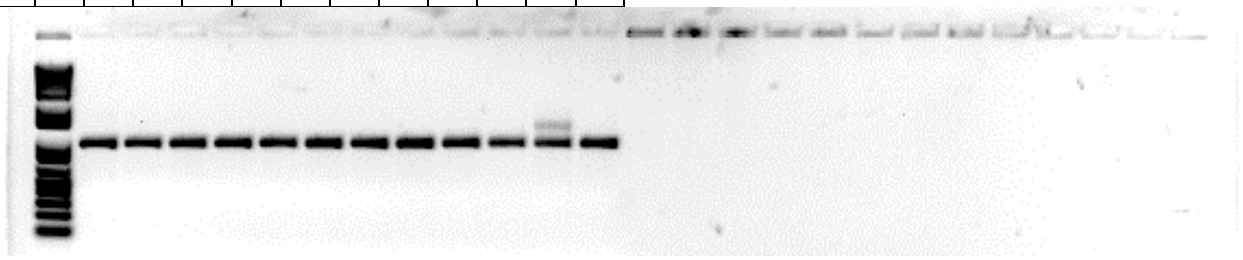
Below are the images from the successful PCR with the specified primers.

4799201 & 4799242												
ladder	Williams 82	Kingwa	PF 11314	PF 11326	Alvo	Resnik	MG 752S	268	133	284	360RR	Invernada

4890783 & 489078												
ladder	Williams 82	Kingwa	PF 11314	PF 11326	Alvo	Resnik	MG 752S	268	133	284	360RR	Invernada



4931408 & 4931379											
ladder	Williams 82	Kingwa	PF 11314	PF 11326	Alvo	Resnik	MG 752S	268	133	284	360RR	Invernada



As shown in the picture above, the results from the gel electrophoresis demonstrate that the sequences from the PCR were amplified correctly. The bar below each sample shows that the regions of interest was amplified for all the regions. Because they were amplified correctly, the samples of DNA were diluted and sent to Macrogen in South Korea for sequencing.

Data Analysis

Once the sequences were received, they were assembled in DNA Baser, a bioinformatics analysis software. Each primer pair was loaded into DNA Baser, and assembled into contiguous sequences. The following screenshot shows the sequence assembly for primer 4931408's Williams 82 cultivar.

5 Screenshot of sequence assembly in DNA Baser

The top shows the alignment of the two sequences, while the bottom shows the quality and certainty of the readings. For the regions that were studied, the quality of the reading was very high, so the results were reliable.

Once all of the sequences were assembled, the final task was to identify the SNPs in the region of interest. Because the region was relatively short, this was done by hand. Most of the sequences were identical, except a few changes in base pairs. These changes in single base pairs were the SNPs that could be used as genetic markers.

For example, below is a small sample from the genetic code. The sequences are identical, except for the 11th base pair, which is an example of a SNP of interest.

6 Illustration of a Single Nucleotide Polymorphism

Each primer pair (36 in total) was aligned and compared. The final SNPs were identified as follows:

4799201 & 4799242:
CTGCAAAATCAGAAGTCATGAAAGAAATGTC[T/A]GAGATTGAACAGACAAAAGCTATGATAA
AAACTGCTGAAA[C/T]CAGGTTGATTGCTG

4890783 & 4890785:

AAGGTTACA[T/C]AATGGTCATGATGGTTGTG[A/G]ACCGTTTCTCTAAAGCCGTATATATT
GGCACTTTTCACTTGCACTTTACAGCCTTCAGAGCAG

4931408 & 4931379:

CTGCATGACCTAGGGTTCCTTCTCTCTCTCATT[T/C]GCGGTGAACTG[C/T]GCCTCTTCTT
CCACTG[C/T]GTCGTGCACTGCCGGCGCCGCTG

Above, the SNPs are identified by the [N/N], where the two different bases form the polymorphisms of interest.

Conclusions
Through the initial DNA extraction to the final analysis of the genetic code, the SNPs that are linked to the *Rps*-1k gene have been successfully identified. Because the *Rps*-1k gene was sequenced, it is now possible to build genetic markers for faster identification of resistant cultivars in the future.

The next step in this project is to develop probes based off the identified SNPs that will enable biotechnology researchers to quickly test for the resistance gene in a new cultivar. An example would be to use fluorescent probes in a real-time polymerase chain reaction test to determine the susceptibility/resistance of a plant based on the resulting probe fluorescence. These rtPCR primers would mark resistance sequences with MGB probes for easy identification of the region.

This approach to resistance gene sequencing can save money and time, and instead of inoculating each generation manually, we can identify resistance genes through MAS. Before, the *Rps*-1k could not be identified with a simple genetic test. Now, the DNA samples from new cultivars can be quickly analyzed for the target resistance gene. It will vastly shorten the time period to help the improvement program create cultivars with high yield and resistance adapted to the soybean producer regions.

The Role of Soybeans in Brazil and the International Market

In order to put this project into perspective, it is important to consider the economic and social impact that the soybean has had in the past, and will have in the future. Specifically for Brazil, the growth in production of the soybean crop, as well as other sections of the agricultural sector has drastically changed the economy. In the 70’s, Brazil was a net importer of food, but today, it is the second largest exporter. The statistics reflect this remarkable growth, with the average yield at 1.522 kg/ha in 1990, and 3.340 kg/ha in 2013/14. In Brazil, agribusiness accounts for 20-25% of the GDP, 37-42% of the country’s exports, and 36-38% of the country’s employment

[19]. The large gains in the agricultural sector have even balanced out substantial losses in other sectors of the Brazilian economy. Thus, agriculture is clearly one of the most important parts of the Brazilian economy.

The substantial boosts in productivity in recent years are due in part to better soil management and planning. For instance, the cerrado land in Brazil is the tropical savannah, and it was once considered completely unsuitable for major agriculture. This was due to the poor chemical composition of the land, which prevented substantial plant growth. However, in the late 20th century, the cerrado soils were developed by several groups, including Embrapa. Large quantities of lime were poured onto the soil to reduce acidity, nitrogen fixing bacteria were added into the soil, and crops were adapted to the tropical climate [20]. Now, the cerrado accounts for 70% of Brazil's agricultural output – very different in comparison to the distribution several decades ago.

Biotechnology work has also made an impact in Brazilian agriculture as well. Especially helpful with developing genetic resistance to diseases, genetic engineering has also helped make plants adapt to new agricultural climate zones. The combination of management techniques and biotechnology has allowed Brazil to show substantial growth in the past decades.

The reason that the issue of agricultural production is so important is that there will continue to be a very high demand for produce. Especially with the rapidly growing middle class in developing countries such as India and China, the increase in population directly drives up the demand for crops like soybeans. As developing countries rapidly increase their GDP, the characteristics of the demand for food also begin to change. The IMF projects that in 2018, the GDP of the US will be $21.6 million, compared to China's $20.7 million, and India's $7.4 million [21]. Specifically, as per capita income grows, there is shift toward greater demand for meat. Not only are soybeans important for direct consumption, but they are also key for raising livestock. Thus, the pressing question is if we will be able to meet this surging demand for food. It is then important to consider all possible options, from scientific to social, to ensure that we will be ready to meet this future demand.

Cultural Experience

Before going on this internship to Brazil, I talked with one of my Brazilian friends to get a sense of what I might experience in Brazil. He taught me some basic greetings and conversational words, and told me about his family. With this information and some basic pamphlets about Brazil, I embarked on this journey, excited to visit a new place and conduct important research.

One of the first cultural differences that I noticed was the way of greeting people. For girl-girl and girl-guy greetings, it is customary to give a kiss on the cheek and a firm hug. Between guys, the traditional exchange is a handshake or a hug. I learned to appreciate the openness that Brazilians tend to show, even toward strangers.

Brazilians learn to speak Portuguese as their first lanugage, though most people understand a little bit of English and Spanish. I was able to understand some of the conversation in Portuguese because of the four years of Spanish that I took, but I was still not conversational. By the end of

two months, I was following along with approximately 50% of what people said in general conversations. People from the lab at Embrapa also taught me some slang terms, like “estou picando mula” and “vazar na braquiara.” Both are slang terms for saying goodbye, and “estou picando mula” literally means “I’m spurring the mule.”

Aside from the people and the culture, one of the most memorable parts of Brazil was the food. Brazilians absolutely love to eat meat, and luckily, I was brought along to several barbeques. Although almost everything from electronics to clothing is very expensive in Brazil, the meat is fairly cheap. In addition, it’s always very fresh. In my opinion, the barbeque with salt and spices was the best food that I had. Another traditional dish is the feijoada, a black bean dish with sausage and beef served over rice. I soon found myself eating much more that I normally eat, trying to taste all the different food available.

Of course, I can’t leave out the excitement of the 2014 FIFA World Cup. This summer, Brazil hosted the World Cup, and there was a lot of anticipation before every Brazil game. There were Brazilian flags ubiquitous, and there were pictures everywhere of Neymar, the Brazilian superstar. Here’s a cardboard cutout of him in a restaurant:

Government organizations closed at noon on all Brazil game days, and all other stores (except bars) closed down about few hours before game time as well. In Brazil, people really know how to celebrate - the streets would be packed with cars, and people would honk and blast airhorns in celebration. After each goal scored by Brazil, there would be loud cheering and singing. If Brazil won a game, people streamed into streets, playing music and celebrating.

Unfortunately, Brazil’s run for the title ended against Germany, with a saddening score of 7-1. Brazilians took the loss rather well, and even cheered for Germany in the final game against Argentina.

My time in Brazil was filled with exciting events, wonderful people, and delicious food. I really value the opportunity I had to experience a new culture, meet new people, and do research in another country.

Travels in Brazil

During my time in Brazil, I was lucky enough to spend some time traveling with my host family and my coworkers. The first time that I traveled with my host family, we went to Balneário

Camboriú, a beach city. I played Frisbee with Igor, and some other people walking by, and we had a lot of fun there.

Because there is a fairly large Asian population in Brazil, people assume that I am Brazilian. As a result, when people start to talk to me in Portuguese, I have to say “Eu sou Americano e não falo português,” or “I am American, and I don’t speak Portuguese.” However, sometimes I had fun and conversed in Portuguese for as long as possible, until they realized that I was not Brazilian.

I also had the opportunity to visit a couple of farms with my host family and a coworker. Marcelo, Denyse, Iago, and Igor took me to their farm in Floraí, a small farming town. It was actually the first time that I spent much time on a farm, and I found the experience very enjoyable. I had lots of barbeque, and I spent some time in the cornfields and with the animals.


I went to another farm in Santa Mariana with Adriano, who showed me around his hometown. We participated in a lot of the local activities, including a festival where there were performances and food.

I really enjoyed my time in Brazil, and I never could have imagined how much I would do, both inside and outside of the lab. Overall, this really was an unforgettable time, and the experience as a whole has given me a greater understanding of the research environment as well as international culture. I hope to visit Brazil again someday!

Acknowledgements

First, I express my gratitude toward Dr. Norman Borlaug and Mr. John Ruan for founding the World Food Prize organization. Due to their continual compassion and determination, there is now an incredible platform for world leaders, researchers, and innovators to come together and address global food security problems. They also recognized the important role that young students would assume in the battle against world hunger. It is through the Borlaug-Ruan international internship that I have had the opportunity to travel to Londrina, Brazil and engage in important biotechnology research. This experience has opened my eyes to the enormous international effort that is being put forth to increase crop productivity, quality, and availability. I have been inspired to make a personal contribution toward this progress.

I greatly appreciate the dedication of Lisa Fleming and the rest of the World Food Prize team. Without their hard work every day, this opportunity would not have been possible. Lisa's work as the WFP youth programs director has truly allowed me to grow, and I am sure that all the current and past Borlaug-Ruan Interns feel the same way. Caring for and communicating with 23 interns all over the world is certainly no easy task.

I would like to extend my thanks to Dr. Francismar, Dr. Norman Neumaier, and Dr. Amélio Dall'Agnoll for helping me get settled in at Embrapa. Your advice and information was very important for understanding the relationship between biotechnology and the soybean.

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Thank you to the Favoreto family for welcoming me so warmly into your home. Marcelo, Denyse, Iago, Igor, and Elena. Thank you for showing me Brazilian hospitality. I enjoyed visiting Balneário Camboriú, your farms, and other places in Brazil. Your warmth made the transition to Brazilian life much smoother, and I will always remember the time that I spent with you.

I am grateful for my AP chemistry teacher, Mr. Belyea, for introducing me to the World Food Prize organization and being my Global Youth Institute teacher mentor.

Lastly, my family has given me continual support and advice. Thank you for encouraging me with my pursuits, and for always being there for me, just a call away.

Photos

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Appendix A: Preparing agarose gel

These instructions are for a 1%, 150 mL agarose gel, so if other concentrations are desired, the quantity of agarose can be adjusted accordingly

1. Measure 1.5 grams of agarose gel in the chemical preparation room
2. Place agarose powder into a clean Erlenmeyer flask
3. Measure 150 mL of TE buffer using a graduated cylinder, and add to the flask
4. Cover the top of the flask with plastic wrap to prevent evaporation of liquid, and poke small holes on top to prevent dangerous pressure buildup
5. Microwave the mix for approximately 2 minutes, or until the agarose powder is completely dissolved, and the solution is clear.
6. Mix the solution well
7. Place flask in cool water, and circulate gently to reduce the temperature until comfortable to touch
8. Add in .1% the total volume of the solution of ethidium bromide. For a 150 mL solution, add 15 μL of ethidium bromide
9. Mix well, and pour into a level electrophoresis tray
10. Place in comb of desired thickness and quantity
11. Spray tray with alcohol solution to remove surface bubbles
12. Allow gel to polymerize, and place into electrophoresis machine.

Appendix B: DNA quality test preparation

1. Pipette 5 μL of each DNA sample from completed PCR reaction into respective tubes.
2. Add 2 μL of dye to each microtube
3. Mix all of the tubes briefly, and centrifuge all of the samples
4. Pipette the samples into the wells of prepared gel electrophoresis tray, skipping the first well
5. Add the DNA ladder into the first and last wells, for future comparison
6. Run the gel for about 1 hour at 100 V
7. View and capture images of gel under UV light, and save images