What I Happened to Stumble Upon

My Experience as a 2008 Borlaug-Ruan Intern in Brazil

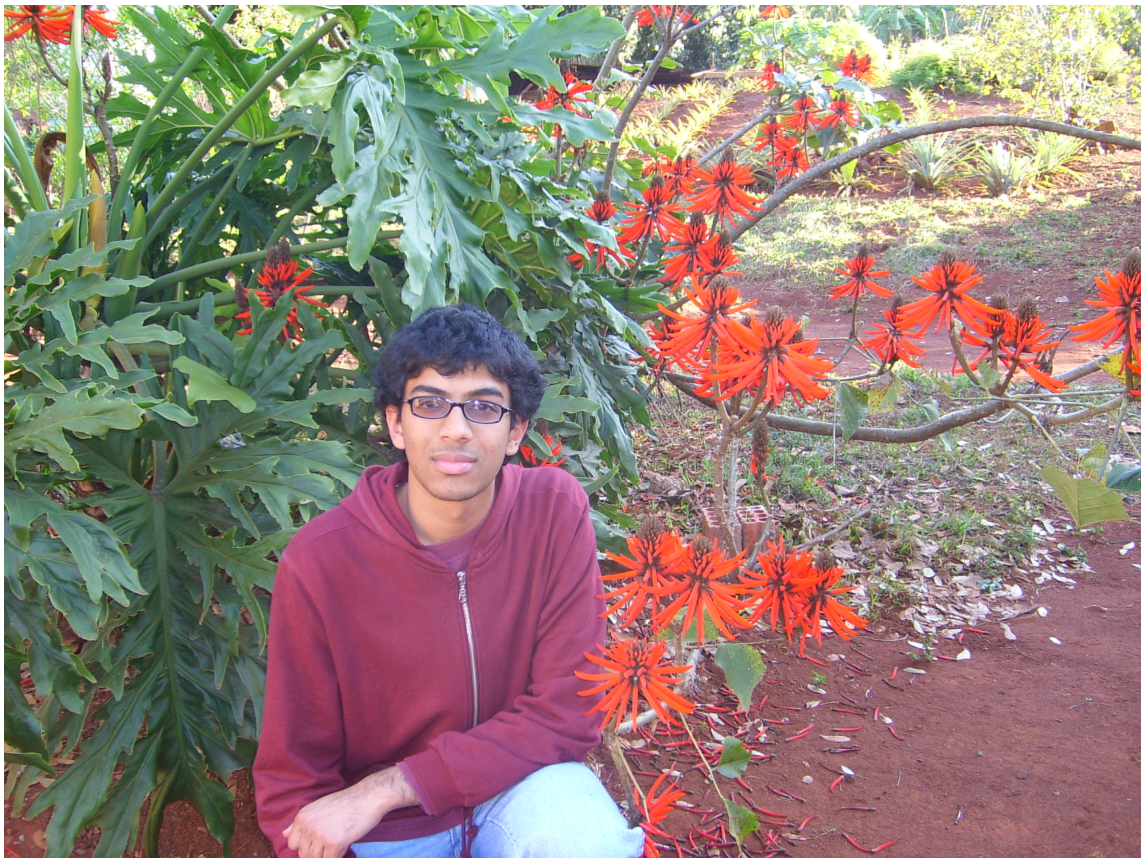

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Table of Contents

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I want to thank all my friends and colleagues at the Biotechnology division of Embrapa-Soja for all their guidance and companionship in and out of the lab. They made me feel right at home in my new surroundings and exposed me to their wonderful culture. They even took me to their homes and families. I would especially like to thank Rodrigo Poeta, Amanda Rusiska Piovezani and her family, Valeria Lopes and her family, Renata Stolf, Renata Fuganti, and Lizandra Cattelli for their guidance during my project and close friendship.

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I thank Ms. Karen Downing, my teacher at Valley High School, for introducing me to the World Food Prize and this opportunity in particular. I also want to thank her for her continued support of my educational endeavors.

Lastly, I want to thank my family for their love and encouragement for whatever I do.

Introduction

There exists a computer program called Stumble Upon, whereby you click a little button and the program directs you to all sorts of random websites based on predefined interests. I've used this program for the past couple of years, and through it I've found some real gems. The way I came to learn of the youth programs of the World Food Prize is analogous to this method. It's as if a button was clicked and life led me to stumble upon the gem that is the World Food Prize Youth Institute and the Borlaug-Ruan Internship. Had my parents not been delayed in picking me up from the state Mock Trial tournament, I would not have encountered my friend, Hemali Batra, and would not have heard how she just got accepted into some program that was going to let her do agricultural research with some of the world's brightest people in Taiwan, and would not have readily agreed to be Valley High School's representative at the following fall's World Food Prize Youth Institute, and would not have been able to apply for the Borlaug-Ruan Internship, and would not have been accepted, and would not have undergone the most engaging experience of my life. It's truly amazing what one "click" can do.

So after I stumbled upon this information Hemali gave me, my goal was to be one of those lucky thirteen or so kids who would comprise next year's group of Borlaug-Ruan Interns. I had always wanted to do some sort of research before I graduated from high school, and if I could do it for free in another country and culture to boot, I was definitely going to try. These were my primary motivations going into the 2007 World Food Prize Youth Institute. But, during the course of the Symposium, I listened to speeches by various government and private sector experts on the issues of food security and biofuels. As part of the Youth Institute I participated in a hunger banquet, which simulates the plight of millions around the world every day. I also got to listen to the past summer's interns talk about their experiences in places like China, Kenya, and Peru. After those three days, my motivations for pursuing the internship weren't just to get research experience. I wanted to *help* people. I wanted to experience a different way of life and become a part of a new culture. I wanted to be in on that secret of just how amazing and diverse this world and its people are.

With those thoughts in mind, I set about applying for the Borlaug-Ruan Internship. I somehow managed to get through each successive step until one winter day, I opened my mailbox and saw an envelope from the World Food Prize Foundation. I was to be one of those lucky thirteen. For the remaining months of the school year, all I could think about were the two months I was going to be living in Londrina, Brazil and working at Embrapa-Soja-the soybean division of Brazil's national institute for agricultural research. So on June 22, after six flights and two days of travel I arrived at my destination. It was a beautiful, sunny day with a light breeze. Everything looked so crisp and vibrant. As I walked out of the airport I took a big whiff of my surroundings, and I remember thinking to myself, "This is going to be amazing."

Setting the Scene

The backbone of my life in Brazil was working at Embrapa-Soja. The word “Embrapa” is a Portuguese acronym for “The Brazilian Agricultural Research Corporation”. “Soja” is Portuguese for “soybean”. It is an organization run by the government, founded in 1973. It consists of a network of 38 research centers, three service centers, and thirteen central divisions. The research centers are spread throughout Brazil, with each one focusing on a particular area of agriculture. The combined efforts of these centers has led to the development of over nine thousand technologies for Brazilian agriculture, thus lowering production costs while increasing output, ensuring the conservation of national resources and the environment at the same time (About us-Embrapa).

Embrapa-Soja’s mission is “to provide competitive technological solutions for sustainable soybean development through generation, adaptation and transfer of knowledge and technologies, for the benefit of the society”. Among their major accomplishments are the development of germoplasm adapted low latitude areas, the natural control of pests, and techniques for soil conservation (Embrapa-Soja). Along with soybeans, Embrapa-Soja also engages in sunflower research for all of Brazil and assists other Embrapa centers focused on wheat, corn, and bean research in applying their work to the state of Paraná, where Embrapa-Soja is located. Research is conducted by a mix of professional researchers and undergraduate and graduate students. My work was completed in the Biotechnology lab of the soybean center.

My first day in the lab was quite eventful. During the course of the day I was given a tour of the center as well as the lab I would be working in. I met with my fellow labmates (mostly undergraduate and graduate students going to the nearby universities) and just observed them during the course of the day. Then as the lab was closing, one of the people who was to become a good friend of mine, Rodrigo Poeta, asked me if I’d like to stay back after closing and help him and some others inoculate some soybean plants with a fungal disease called Asian soybean rust. These plants and this disease eventually became the focus of my main project. I had not really done any work the whole day, just observations, so I readily agreed. I also thought it would be a good way for me to become better acquainted with people in the lab. That day ended up being the most strenuous day of

my whole internship! There were over a hundred plants that needed to be sprayed with solutions of the fungus and some with plain water solutions (to serve as a control group). Only Rodrigo did the spraying though. The rest of us (four people) were left with the more labor-intensive task of maneuvering large, plastic bags over the plants and ensuring they adequately covered each plant and its pot-which were rather heavy. By the end of it all, four hours had past and it was 9:00 p.m. All throughout I had a pleasant interaction with the other three people and I felt content about my contribution for my first day. I had been of use. I got back to my host family about an hour later, had a delicious meal complete with a salad and desert, and went right to bed.

The next few weeks in the lab I spent following and assisting different people on their specific projects. There were three projects the majority of people in the lab were working on. Some were working on identifying genes that would make soybeans less susceptible to drought. Others were working on a project to identify what types of genetic characteristics are conducive to soybean resistance to a nematode pathogen called *Meloidogyne javanica*. And others were working on a project to identify molecular markers called single nucleotide polymorphisms (SNPs) in order to identify genes responsible for soybean resistance to Asian soybean rust (ASR). My primary focus was on this project. It's important to note that all of these studies are ongoing and won't be completed for months. As I said above, before I focused on a single project, I followed various people learning and performing the various lab protocols. These included, RNA extraction, DNA extraction, polymerase chain reacting (PCR), transforming of soybean embryos via gene gun, planting soybean embryos, gel electrophoresis, DNA quantification, and by far the most laborious (inoculating the soybean plants with ASR excepted)-restocking the pipette tip cartons. Several of these techniques were ones I ended up using for my work on the ASR project.

Soybeans, Fungus, and SNPs

Asian soybean rust (ASR) is a disease caused by the fungus, *Phakopsora pachyrhizi.* It's characterized by gray, tan or reddish-brown lesions with raised pustules on the leaves of the plant. Lesions initially start on the underside of the leaves, but as the disease progresses they cover most of the leaf (Where did soybean rust come from? Will it get to South Dakota? 2). This defoliation inhibits photosynthesis, and consequently the terminal growth and productivity of the plant. For the 2007/2008 harvest, estimated economic losses amounted to $204.5 million dollars. It cost $1.97 billion to apply the fungicides necessary to control the rust. This was a comparatively

good season thanks to the predominantly dry weather, which prevented the intense amount of sporulation that occurs with extended rains. For the 2008/2009 harvest, the situation does not look. The rains lasted through June 2008, providing the moisture needed for fungal development and spread. If left untreated, ASR can lead to yield losses of up to 80%. The common range is between 10 and 50%. In the 2002/2003 growing season, losses totaled approximately $1.3 billion (Garcia). With this kind of devastating potential, the control of ASR via fungicidal spraying is vital. Yet, this is not the most cost effective or environmentally sustainable solution. The development of continually, resistant cultivars is a primary research goal.

Project

Essential to reaching that goal is the mapping and cloning of resistance genes using molecular markers. SNPs are the most advantageous marker because one, they are the most abundant form of genetic variation within genomes, and two, there are many technologies available for high-throughput SNP analysis.

So far, four genes have been identified as conveying resistance to ASR, *Rpp1, Rpp2, Rpp3,* and *Rpp4 (*Where did soybean rust come from? Will it get to South Dakota? 2). The aim of the project is to map *Rpp1* and *Rpp3*, by using mapped SNPs near the *Rpp3* and *Rpp4* genes, to identify SNPs linked to the *Rpp1* and *Rpp3* genes.

Procedure

DNA was extracted from five different soybean cultivars, Abura, Kinoshita, Shirauni, R141, and R175 by grinding the seeds for each type, taking one gram of a sample, crushing it again with liquid nitrogen and then immersing it in a CTAB extraction buffer. The sample was then purified using a chloroform-isoamyl alcohol (24:1), precipitated with 70% ethanol, dried, then stored overnight in a freezer at -20 degrees Celsius. *(See Appendix A for detailed protocol)*

PCR was performed on each of the five samples, eight times, each time with a different Barc SNP primer. The eight primers used were:

1. 04633 (33)
2. 02618 (18)
3. 07498 (98)
4. 08076 (76)
5. 06198 (98)
6. 01160 (60)
7. 00640 (40)
8. 05872 (72)

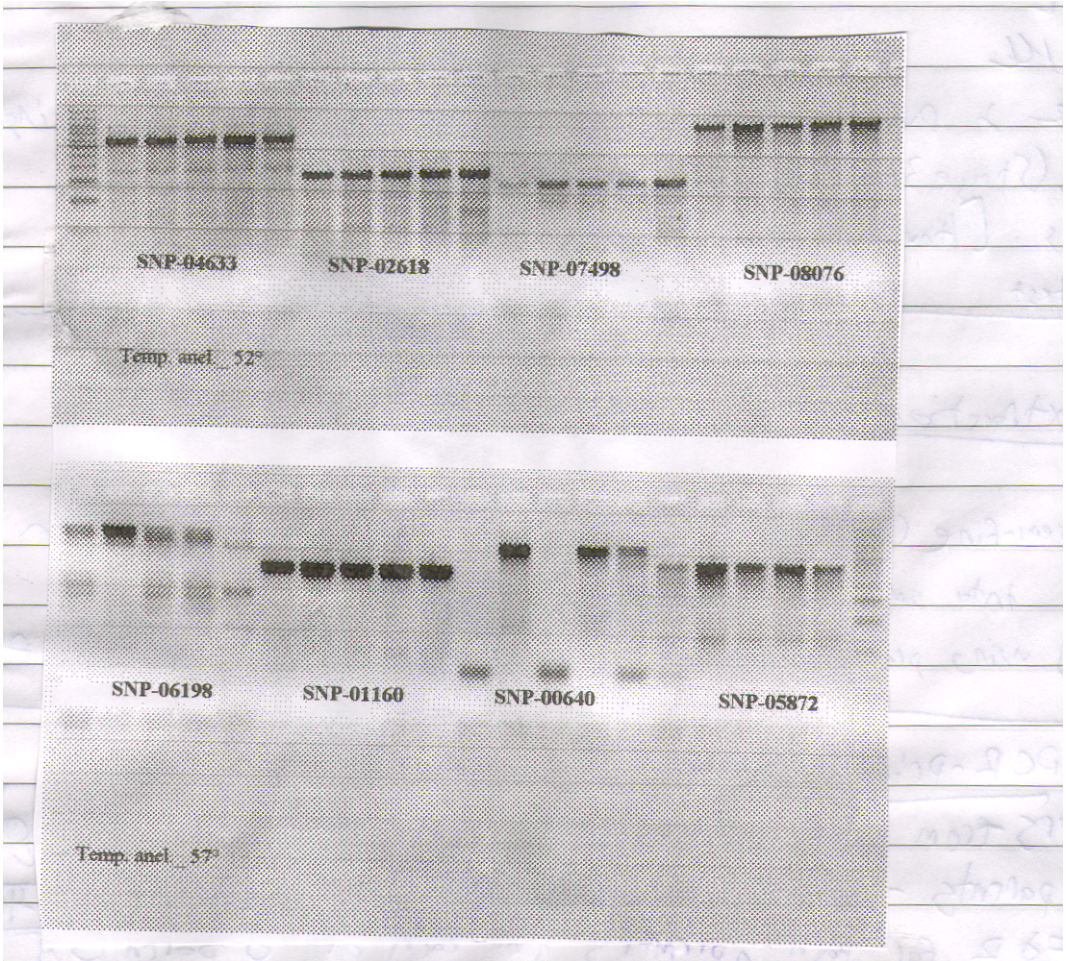
First the primers were diluted, then 20 nanograms of DNA from each of the five samples was placed into a different eppendorf. This was done 8 times, resulting in 40 different eppendorfs being filled. Then 1 microliter of forward and reverse primer for each of the 8

primers was added to each eppendorf. Then 5 microliters of “mix” consisting of water, Tampão, $MgCl_2$, dNTP, and Taq, was added to each eppendorf. Each sample was centrifuged at 23ºC for about five minutes. Then the samples were put in a thermocycler and the following PCR program was run:

1. 92ºC for 5 min
2. 92ºC for 45 sec
3. 52ºC for the 1st 4 primers)/ 57ºC for the 2nd 4 primers for 45 sec
4. 72ºC for 45 sec
5. 72ºC for 5 min
6. Repeat steps (2-5) 39 more times. Then have it rest at 4ºC until picked up.

During the PCR a 1.5% agarose gel was made by taking 3.75 grams of agarose, putting it in a 350 mL Erlenmeyer flask, then adding 250 mL of TBE 1x. The solution was swirled in the flask gently. The top was covered with a taught, piece of saran wrap, and three small holes were poked in it. The flask was microwaved in 45 sec intervals, until the agarose was completely dissolved. The flask was taken out of the microwave between intervals and swirled a few times. When the agarose was completely dissolved, 3.5 mL of Ethidium Bromide was added to the solution. The agarose solution was poured into the gel tray, and a well comb put into the indicated spot. The gel was given two hours to set at room temperature.

When the PCR was completed the amplicons were resolved in the gel. Below is what it looked like:



The distinct bands for each primer, indicate the proper portion of each sample's DNA was amplified well. For primer 40, two of the samples reacted differently than the other three. This indicates an error in that PCR and so that primer was redone. The error was probably might have been due to the higher annealing temperature compared to the top four primers or due to human error in preparing the reaction. Or maybe that was a bad primer.

After ensuring the samples amplified correctly, they were put through a gene sequencer. Fo each sequenced sample, the sequencer gave two strings of data, one for the forward primer and another for the reverse. Using the Phred/Phrap command-line program, the two strings were assimilated into a continuous sequence like that shown below:

```
>33-Abura.fasta.screen.Contig1
AGATATCATCAATTAGTAGCTAGTTTGTACCATATTATATCCTCATTATT
GGTTCTATAGTTTATAGTTTAATTATAGACTTTTCAACCCTCATTATTAA
ATGGGAAATTGAATTCCAAGTTCTGTTATTTTTATAATCTTTCAGGAAAG
CTCTCAACTTGATCCATTACCTGTTGCATGAGAATAATTCAGACTGCAAC
ATCGTGAACGAGCTTGGGTTTCCTCGAATGTTGATGCACCTTGCCTCAAG
TGAAGATTCAGATGTGAGAGAAGCTGCCCTTCGTGGCCTTCTCCAGCTTG
CTCACAATGCGAAAGATGGCAAGGATGGCAATGAGAAAGACAGTGTGAAA
ATAAAGCAACTTCTTCAAGAACGAATAAACAACATCAGTTTAATGTCAGC
TGAGGACCTTGGTGTAGTCAGGGAGGAGAGGCAACTTGTGGACTCCCTGT
GGAGCACTTGCTTCAACGAGCAGTCTTCTCTTCGAGAGAAAGGTCTTCTA
GTGCTTCCGTGGTCGGACGTGCCCCGTACNGATGTTGCNAGATCAATTTA
GGGGAACCTCTAGATCTTGACTGCATCCATTCGC
```

After this operation, Phred/Phrap was used to cross certain samples with other samples, which would eventually be aligned for SNP discovery. The crosses that took place were:

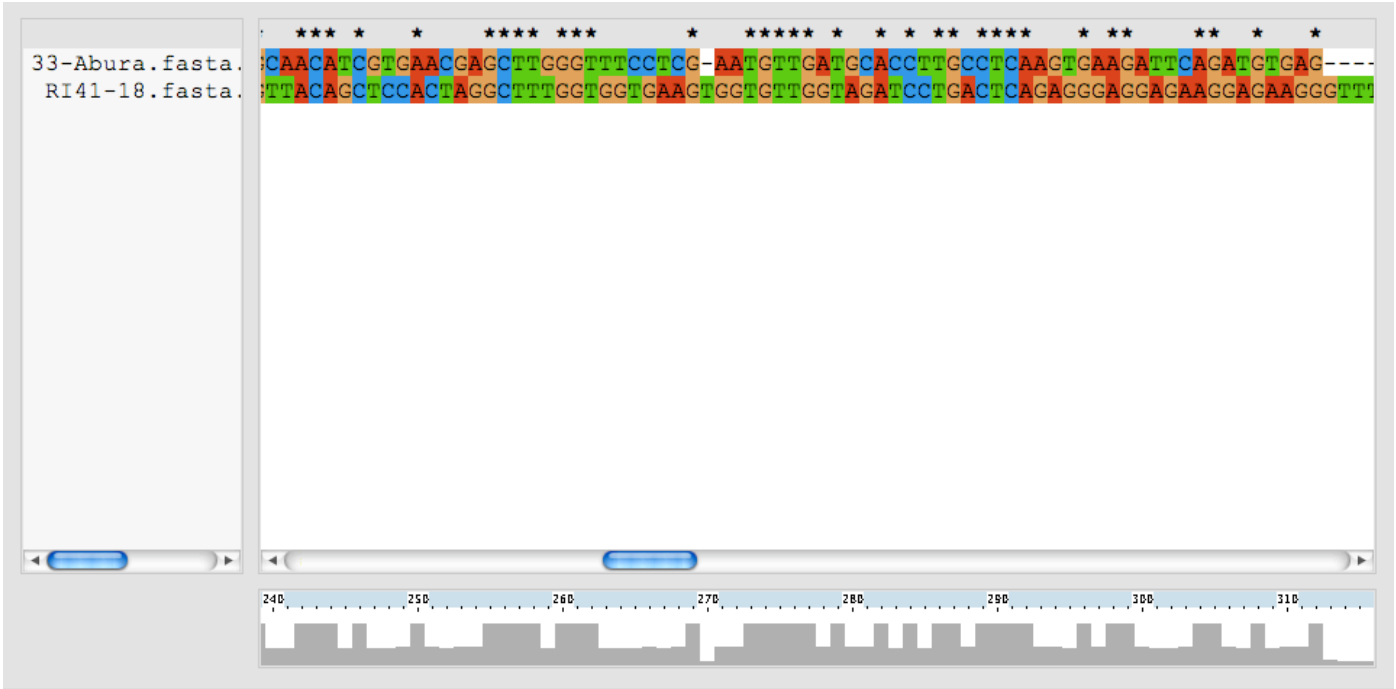
Abura 33 and Abura 98 were each crossed with each of R141 18, 33, 60, 72, and 98.
Kinoshita 18 was crossed with R141 18, 33, 60, 72, 98, and R175 33, 60
Kinoshita 33 was crossed with R141 18, 33, 60, and 72, as well as R175 33, 60
Kinoshita 60 was crossed with R141 18, 33, 60, 72, and 98 as well as R175 33, 60.
Kinoshita 76 was crossed with R141 18, 33, 60, 72, and 98 as well as R175 33, 60.
Kinoshita 98 was crossed with R141 18, 33, 60, 72, and 98 as well as R175 33, 60.
Kinoshita 72 was crossed with R141 18, 33, 60, 72, and 98 as well as R175 33, 60.
Shiranui 33 was crossed with R141 18, 33, 60, 72, and 98
Shiranui 60 was crossed with R141 18, 33, 60, 72, and 98
Shiranui 72 was crossed with R141 18, 33, 60, 72, and 98, as was Shiranui 76.

Below is the result of the crosses in Phred/Phrap:

```
>33-Abura.fasta.screen.Contig1
AGATATCATCAATTAGTAGCTAGTTTGTACCATATTATATCCTCATTATT
GGTTCTATAGTTTATAGTTTAATTATAGACTTTTCAACCCTCATTATTAA
ATGGGAAATTGAATTCCAAGTTCTGTTATTTTTATAATCTTTCAGGAAAG
CTCTCAACTTGATCCATTACCTGTTGCATGAGAATAATTCAGACTGCAAC
ATCGTGAACGAGCTTGGGTTTCCTCGAATGTTGATGCACCTTGCCTCAAG
TGAAGATTCAGATGTGAGAGAAGCTGCCCTTCGTGGCCTTCTCCAGCTTG
CTCACAATGCGAAAGATGGCAAGGATGGCAATGAGAAAGACAGTGTGAAA
ATAAAGCAACTTCTTCAAGAACGAATAAACAACATCAGTTTAATGTCAGC
TGAGGACCTTGGTGTAGTCAGGGAGGAGAGGCAACTTGTGGACTCCCTGT
GGAGCACTTGCTTCAACGAGCAGTCTTCTCTTCGAGAGAAAGGTCTTCTA
GTGCTTCCGTGGTCGGACGTGCCCCGTACNGATGTTGCNAGATCAATTTA
GGGGAACCTCTAGATCTTGACTGCATCCATTCGC


>RI41-18.fasta.screen.Contig1
TTGGGAAGCAGCTAAAGAAATGTGTCATTTTTAAATATTAAAAGGAGATA
CATATCAAGATTAGATATACATCTGGATTAAATGATGAAATTCACACAAA
CTCTTAGCAAAACAATAACATATTAATAAAAAGGAGAATAAAAAAAAAAC
AGCACTGCCTCTTCCCTAGCCCATGAAACCAAACATAATTGCTGACACAA
AGAAAGACCCCACCAAAAGGCCAATGCCACTGGAAACAGTTACAGCTCCA
CTAGGCTTTGGTGGTGAAGTGGTGTTGGTAGATCCTGACTCAGAGGGAGG
AGAAGGAGAAGGGTTTGCCAAAAACCNCCCCGCNTCCCNTGTTTTTTTTT
TCTTGGCGNCCCACAACCCCGACGCCACCAGAACCCAACACCCTTTTTAG
TATAAAATGAGGGGGGGGGGGGGACCCCCCATCAAGACACACTCTGTCC
CGAATATAGCATGAAAAAGCGCGAGGGGGAGGAGAAACATACATTCACGA
ACGCCCTAAAACAAACACCACTATAGACAACTTCGACTAAAGATCAGAAC
GTCAGAGCATAAAAGAGTTACTCGCGTTGACTGAGCAGCGAGCAATAAAG
CACTACAAATATCAATTACCCGAAGAAACGTCGCAAGCAACAATATACAT
AAGACACGTATATAGCCGAGAGAACACCTAACCCTCAGAGCAGAGCATAA
CCGAGGACGCACGCGTACGGTGCGAGAAAGAAATAGAAACATAGAGAGTA
ACATATAAAGCACGATAAGAGTAAAAGGACTTAGAAACCCGACTCCGCAA
A
```

Each of these crosses was then sent through ClustalX alignment software, which aligned both sequences in the cross and determined SNP sites. Below is what the above cross looks like in ClustalX:



The stars are points of congruency between the two sequences. The spaces between the stars are SNP sites. The white spaces with dashes are points where one sequence doesn't have a base pair in the other sequence.

At this point I had to leave, but what they have continued to do is try and link the identified SNP sites near the mapped *Rpp3* and *Rpp4* genes to identify sites that could convey ASR resistance. These crosses will actually be carried out and the segregant population of plants will then be genotyped using these SNPs. The crosses will also will be evaluated for susceptibility or resistance to try and establish a correlation between the SNPs and a certain phenotypic condition. Then the SNPs will be mapped using GQ-Mol software. The study should be completed within the next year.

Developing productive, soybean cultivars that have a sustained resistance to ASR is a task has been going on for many years now. This research hopes to bring scientists closer to such a development. Soybean as crop isn't just vital to people as a source of food, but also as a source of income. Many farmers in Brazil, especially the poorer ones cannot improve their station in life if they have to continually be spending massive amounts of money spraying their crops with fungicides, or risk losing up to 80% of their yield if they don't. If sustainable, resistant varieties were to be created and made available for cheap, these farmers would be able save money, and increase yields. This would increase their income, and as a result their ability to be adequately nourished. Higher yields also means more people can be fed nutritious food high in protein for a lower cost. Many people around the world wouldn't have to go hungry every day if a robust soybean crop resistant to ASR was developed. Hopefully, this research takes takes one more step to making that a reality.

Beautiful Brazil

I went into Brazil a broken, defeated person. I came out renewed and reinvigorated. Over the course of my junior year, I had felt increasingly frustrated and disgruntled with what I saw around me. I sensed in the people that surrounded me a general ignorance and disregard for the world outside their own, self-serving bubbles. There was also a lack of authenticity. Every thing was for show and under some pretense. I was dismayed at the lack of any true, inclusive community of people who genuinely cared about others. The past five or so years up to that point finally took their toll on me. By the time it came for me to board my plane to Brazil, I couldn't leave fast enough.

When I arrived, things just felt different. My host family welcomed me with warm embraces and kisses on the cheek. From that moment on, I was one of their sons and one of their brothers. I went to the lab and I saw how happy and friendly everyone was. I never saw a mean glare or heard an insidious comment. The atmosphere felt open and free. The beautiful, temperate weather didn't hurt either. Everybody was so outgoing and willing to introduce me to their culture and lives. It was there I finally understood

what “companionship” feels like. For the first time in months my mind felt clear and in focus.

People there also knew how to genuinely enjoy life. I never witnessed anxiousness or uneasiness, even in tense situations. When people would arrive at the lab, there would always be a group sitting in the small, worn break room drinking some coffee or tea, maybe having a biscuit, and just chatting away. After maybe ten or twenty minutes, they would go in to the lab proper and start doing their work. Between this time and lunch, there would be several more breaks to be had, but this time much shorter. Then came lunch. People would lazily stroll down to the cafeteria, chatting and looking out at Embrapa’s gardens that line the open walkways. The cafeteria was a place buzzing with activity and conversation. Rarely could you see anybody eating alone. After lunch they would come back, and sit in the break room yet again, drinking some coffee or tea and just chatting away, possibly continuing the conversation from lunch. This break might last as long as an hour. Then they would get back to work, and from that point until closing time they would again take several, smaller breaks.

It was a steady pace of life, a pace I enjoyed immensely. Being surrounded by such a mood did much to mend my psyche and outlook on people and life in general. It renewed my faith in humanity, and I returned with a reinvigorated spirit. I look around and still see the things that broke me down before, but it is like I got immunized when I was in Brazil and so now I can see what is possible and what exists in this world. I cannot get discouraged.

In terms of my perceptions of the issue of food security, they weren’t as drastic as I thought they’d be. I lived with an upper-class family in a progressive area. The people I interacted with were not as wealthy, but were well situated. I worked in a biotechnology lab all day, extracting and replicating DNA, making gels, and using fancy equipment. When you’re involved with things at such a fundamental level, it is easy to forget about the context that all of that is in. It is easy to feel removed from the world outside the lab. Initially, I found this conflict hard to resolve. Here I was on the pretext of getting a better understanding of food insecurity in the world, yet I hadn’t seen anything like that firsthand. People weren’t even really talking about issues of hunger or poverty. How could I get a better understanding like this? Then as time progressed I began to realize that a great portion of the solutions that will be created to deal with issues of food insecurity will come from labs just like this. The problems and struggles that happen in that lab are a vital part of the struggles that exist to ensure everyone in the world can be adequately fed. Whether you realize it or not, what you do is a part of that. So by just doing what you do, you help. In this manner I was able to put everything back into a larger perspective.

With that I realized that what I thought I wanted to do (which really wasn’t that definite to being with), maybe not be the path I take. After having this experience with the science of agricultural development and seeing the diversity of people and the types of cooperation that take place in the field, I feel a greater and greater urge to pursue such an area for my college studies. From the Youth Institute through the internship, I’ve

been better able to detect the urgency of so many million's situation, and this knowledge has instilled in me a desire to help.

Since I was a kid, when I thought about possible careers, the one standard I always used was how challenging the career was, and as a result, what kind of intelligence does it require. Now, I can't see any challenge more vital to overcoming, or more in need of the brightest humanity has to offer. The issues of food insecurity and poverty are ones I'm intent on combating in my eventual career. Let's see what I stumble upon.

Appendix A

Extração de DNA de Plantas - MINIPREP (Portuguese)

- Triturar cerca de 1g de folha em presença de N_2 líquido em almofariz e transferir para tubos de microcentrífuga e adicionar tampão de extração na proporção de 4 vezes o volume da amostra.
- Incubar a 65 ^oC por 60 minutos, agitando de vez em quando (cada 15min)
- Resfriar e centrifugar a 6000 rpm por 10 minutos. Transferir a fase aquosa (superior) para outro tubo.
- Adicionar igual volume de clorofórmio-álcool isoamílico (24:1) e agitar por suaves inversões por cerca de 5 minutos
- Centrifugar a 6000 rpm por 15 minutos e transferir a fase superior para outro tubo.
- Repetir os passos dois passos anteriores
- Precipitar os ácidos nucléicos pela adição de isopropanol gelado (2/3 do volume). Misturar por inversões até os ácidos nucléicos tornarem-se visíveis e deixar over night a 4 ^oC ou 2 hora a -20 ^oC
- Centrifugar a velocidade máxima por 10 minutos. Descartar o sobrenadante e adicionar 500ul de etanol 70%. Centrifugar novamente por 5 min., descartar o sobrenadante e secar o pellet (O pellet pode ser sêco a vácuo ou deixar o tubo invertido sobre a bancada por cerca de 2 horas)
- Ressuspender o pellet em 400ul de TE pH 8,0. Se necessário, pode-se aquecer a 65^oC por 5 minutos para ajudar a ressuspensão.
- Adicionar RNAse A, na concentração final de 40ug/ml e incubar a 37^oC por pelo menos 30 minutos.
- Re-precipitar o DNA pela adição de 1/10 do volume de NaCl 5M e 2 vol. de etanol 95% gelado. Deixar over night a 4 ^oC ou a -20 ^oC por 2 horas
- Repetir os dois passos anteriores a adição de RNAse A . Quantificar o DNA em espectrofotômetro ou gel de agarose 0,8%.

Tampão de Extração

- **Componentes**
- **Conc. final**
- **Vol. Final (10ml)**
- CTAB 5%
- 1%
- 2,0 ml
- NaCl 5M
- 1,4 M
- 2,8 ml
- Tris-HCl 1M pH 8,0
- 100 mM
- 1,0 ml
- EDTA 0,5M
- 20 mM
- 0,4 ml
- Mercaptoetanol
- 0,1%
- 0,01 ml
- Água
-
- 3,7 ml

OBS.: O mercaptoetanol deve ser adicionado somente no momento do uso do tampão

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