

# Minha Historia do Brasil

# (My Story of Brazil)

# **Table of Contents**

- Prologue -- 3
- Introduction -- 4
- The Brazil Experience -- 5
- The Biotechnology Lab and Embrapa Soja 8
- The Supervised Practical Training -- 10
- Brazil and World Food Security -- 17
- Epilogue -- 19
- Acknowledgments -- 21
- Appendix 1 22
- Appendix 2 -- 23
- References 24
- Photos -- 25



### Prologue

"Is that all? Can we just leave now?"

"I think so..." came the reply.

I was asking two Mormon missionaries just outside the customs inspection in the São Paulo International Airport, engaging in what would be my final conversation with Americans for eight weeks. I had just gotten off the plane after a sleepless night and felt tired, excited, and very confused at the same time as I just passed inspection. From what my parents and other sources told me, long lines and intensive baggage verification was routine; however, I was able to pass through in about a minute without a second glance.

"But isn't Customs supposed to be a bit more careful and need to inspect my luggage?" I asked the missionaries again.

One of the missionaries put his hand on chin in the thinker pose. "hmmm... you're right." He said thoughtfully, "But after all, this is Brazil..."

This is Brazil... all of the sudden it hit me. For the next two months, this was home. Unlike home though, I did not have the slightest idea what or who I would find in this place. I immediately had second thoughts. What am I doing in this country? I had an internship here in an unknown lab, but I didn't have any lab experience or communication skills in a different language. I had just graduated from high school and I would be by far the youngest and least experienced person to work in the lab. Outside the lab, I would be living with a host family. However, I was not able to see them outside the picture and the info-blurb that the World Food Prize gave me, I would be going into Brazil blind-date style. My first few minutes in Brazil felt like a strange dream. After all, just three days before, I was arguing with my friends about which Adventureland ride we should go on. Unfortunately, unlike a dream, I would not be able to wake up for another two months.

Half confused, and half expecting that customs would come and confiscate my baggage, I said goodbye to the missionaries and trudged onwards to the front desk, where I needed to catch a bus to get to the rest of my flight. After a few minutes of absent-minded wandering, I found the line to the bus, when I felt a tap on my shoulder.

"@\*\$Q\* @(^\*# @#I!#?" What?! It was then when I came to the realization that the Portuguese language was much more different that I could have expected. Before now, I naively assumed that four years of another romance language in high school, French, and a night of studying Portuguese on a plane would be enough to understand some basic phrases in Portuguese. Now, I concluded that I had absolutely no knowledge of the language; I just hoped that I could get through two months in Brazil using English.

"Você fala îngles?" (Do you speak English?) This was about the height of the Portuguese I learned from the ten hour plane ride.

The man shook his head. Meu Deus, this was going to be a long eight weeks. I suddenly felt disdain and shame for coming to Brazil and not learning the language beforehand. It was then when vowed to learn as much Portuguese as I possibly could in two months. As the line got shorter, I began to feel more and more excited and anxious with each passing step. In just a few moments, I would be alone in a foreign country, whose language that I did not speak, whose people I did not know, and whose experiences I would never forget. As I arrived at the bus, I would encounter one of my life's most important turning points in ways I still could never imagine. I would make the memories and friends of a lifetime during simply two short months living and working in Londrina, Brazil. My first few hours in Brazil were some very strange ones. Not only because I set foot in a nation that I never thought I would have the chance to go to, but also it was the last time I would doubt whether or not I ought to travel and study abroad.

### Introduction

Looking back, my experience in Brazil still felt like a dream. I still wonder how I landed a spot with the Borlaug-Ruan Internship and eventually Brazil, because even now, it feels too phantasmal to be true, yet in my mind, the memories are still vivid. I guess the story all started with a French girl, Chloe Vansoeterstede. It was summer two years ago, and I was no more than wallowing in suburban Ankeny with almost nothing to do. One day, my dad arrived home with some interesting news. Apparently his co-worker's wife was hosting her friend's daughter from France, and like me, she was also in need of activities to do in Des Moines. My father suggested that I probably should take her out for a good time in Des Moines, a feat in itself. Not having anything better to do, I decided to accept. I was extremely glad that I decided to take her. After a day in Des Moines, I was able to meet the host. Her name turned out to be Lisa Fleming, and she directed a very interesting program for high school vouths called the World Food Prize Youth Institute. Students would write a five-page report about a current world food and nutritional information. The issue for my year was about the paradox between hunger and obesity. Then they would be able to attend the World Food Ceremony and eventually be able to apply for a summer abroad through the Borlaug-Ruan internship to a developing nation to research a project related to world. To apply for the internship, all I would need is a school and mentor sponsor.

"Are you sure you want to do this?"

"Yes I do, this may be my chance to finally gain that opportunity; to see the world, to make myself marketable to the business world. It's your move now."

I was challenging my chess coach, Charles Lierman (also known as Chuck) to a game on an uneventful Tuesday afternoon (I was winning of course). A retired and accomplished teacher and my chess coach and history day judge, Chuck and I had developed a friendship from the last two years. Although I never had him for any class, he was mentor I admired and wanted to be just like. Conversing with him was almost like asking a sage for advice, so I was all the more ecstatic when I found out that he had already taken two other high school students from years past, both went on to win the John Crystal Award for excellence in the Borlaug-Ruan internship. From that time, I knew that I simply needed to complete the Youth Institute and then the internship, under the guidance of Chuck.

"Well then, you had better get started writing then..."

At first, I had to admit that my true motivation for entering the institute was slightly less than noble. After all, with only two years left into college, I would have to start making myself competitive to match some of the other student applicants in the pool. I came into my essay thinking with the mentality that it would be good for my resume; however, it by no means would stay that way. I chose Brazil as my topic because I was curious about the country and believed that there would be much information about the subject. Almost as soon as I wrote my first words, I realized that if I were going to continue, I needed to build up a genuine interest about world food security. I soon became fascinated with both the hunger and obesity, and Brazil.

My new interest was enhanced during the Youth Institute. I simply remember Norman Borlaug's inspiring words to us, "One of you will soon become the next Norman Borlaug." That seemed impossible for me to grasp. This great man, credited for saving a billion, believes that we also have the potential to change the world in such a titanic manner. I was certain to apply for the Borlaug-Ruan Internship.

Unfortunately, during the spring of my junior year, I applied for the internship and failed to be accepted. I felt a lost opportunity flutter away. No internship meant that even if I was accepted the next year, I could not add it to my college resume. This thought was going through my head near the

application deadline for my senior year. However, my parents were able to encourage me to apply for it. As immigrants from China, they believe that I should have a global view on the cultural and professional world since I was very removed from it growing up in the United States. I felt very fortunate for listening to my parents. I reapplied and was accepted. I was paired with the Embrapa Soja research station. It suddenly dawned on me. I was going to Brazil! And after a few ticket inconveniences that Lisa was able to help me solve, on June twelfth, I embarked on the greatest summer of my life.

# **The Brazil Experience**

"Let's see... Eu tenho que...um... I give up." I finally raised my hands in exasperation. However, the faces I was staring at were just as quizzical as ever.

It was my first night in Brazil, and it would be one that I would never forget. Earlier that afternoon, I met my host family just outside the baggage claim of the Londrina Airport. They seemed nice enough; however, a rush of terror came upon me as I looked at the woman standing next to them. She was the only person who was speaking to me in English. Evidently, my host family had arranged a translator. The next two months were going to be some long ones. Now, the translator had left, we had finished dinner, and I was alone trying to communicate with the "Learn as You Fly" Portuguese with my host father, Emidio; my host mother, Fátima; and their twenty-one year-old son, Felipe. Felipe had taken two months of English prior to June; however, I still found communication a nightmare.

"É difcíl, não?" Felipe said.

"Sorry, é muito dificíl."

We all laughed as we continued through the night playing language charades.

My first night in Brazil seemed awkward. I felt like a helpless fish out of water, entirely at the mercy of my host family. However, in hindsight I now cherished those moments as valuable learning experiences. After the cultural shock in my first, I began to slowly pick up the habits of my host family as well as the language and culture of Brazil, and although I wrote my original Youth Institute report on the Paradox of Hunger and Obesity in Brazil, I soon found out that I knew next to nothing about the real Brazil.

In my perspective, Brazil and the United states are not very far apart as I had originally thought.



A Brazilian Soda-pop, Guarana

At first glance, I immediately recognized plenty of American companies such as Pizza Hut, Ford, and iPod. Brazil seemed to a country invaded my American products and culture. When I first flipped television channels at my new Londrina home, I instantly found American networks such as the Discovery Channel, Cartoon Network, and MTV. In fact, almost all the music I found on Brazilian radio or MTV were the same songs I would have listened to in the United States such as Akon or Justin Timberlake. It did not take me very long to realize the economic and cultural value of the United States.

The people of Brazil did not seem to be very different as well, especially for the younger generation. Night outings for both Brazilians and Americans included the same activities: movies, malls, nightclubs etc. However, the general atmosphere in Brazil simply felt different. The sociability of Brazilians is higher than Americans. Unlike the United States, all greetings in Brazil are accompanied by a kiss on the cheek (between females, or male-female), or a handshake (between males), or an embrace (any gender combination). In Brazil, the people are likely to greet strangers as if they were old friends, which helped me greatly when meeting other people. Nevertheless, there were some grander differences issues of Brazil as I observed. Prior to my voyage to Brazil, I was advised to look at three things: corruption, slums, and soccer. Through my paper for the Youth Institute, I was astounded to find out that the poorest 20% owned 2.2% of Brazil's income, while the richest 20% owned over 60% (Meade et al., 2004)! In the city in which I lived, Londrina, with many richer inhabitants, I observed a wall complete with concrete and barbed wire guarding the well-to-do from the rest of the city. After speaking to some working Brazilians, the greatest complaints were always the enormous tax rate (50%) which the government uses very inefficiently and the elevated import taxes in a fruitless attempt to protect Brazilian businessmen. This often opens the gap between rich and poor. The high prices and high taxes make the atmosphere rich for pirated multimedia and pirates struggling to make a living. In fact, Londrina, located in a key smuggling route, was under an economic lockdown by the police for several days during my stay.

Another interesting observation I made in Brazil was the relationship with the middle and upper classes with the slums. With the gap of wealth of the rich and poor, poverty is still a rampant problem in Brazil. Brazilians in the bottom quartile can only purchase a dismal 80-90% of the recommended 2200 calorie diet (Meade et al., 2004). This lurking in hunger as well as poor living conditions makes the poor very real and the slums, as described by Brazilians, muito perigoso (very dangerous). Those who hosted me and those who I worked tried hard to have me avoid seeing the slums. However, despite that and despite the fact that Londrina was a relatively rich city, I still saw the slums, where the houses were made from pieces of scrap metal, and where the local church was nothing more than a tool shed. As I rode past those places, I simply had to wonder: how can Brazil, with plenty of natural resources and financial backing, have so many slum dwellers? After spending two months in Brazil and speaking to a wide range of people, I have concluded that the inefficiency, corruption and relative apathy of the Brazilian government makes it so, and in order to quell the disparity, the government must undergo reform.

As many of my friends in Ankeny told me, it would be a sin to go to Brazil and not learn about soccer. Soccer, as I found out, is much more than just a game, it is a way of life. In a way, it is the great equalizer in Brazil. Almost every prince and almost every pauper in Brazil grows up by the ball and comes together during the Brazil national games and tournaments such as the World Cup or Copa de America. Soccer is an ideal form of entertainment for people of all social classes. Requiring virtually no coaching or equipment, only a ball (or an improvised form of one) and an open area, soccer has reached insurmountable level especially with the poor. Soccer has provided hope for those in the impoverished rural and inner city kids for the one chance to be discovered and leave their world behind forever. Soccer has also provided a temporary escape as well. When one plays, all pain, all hunger, and all adversity simply disappear for ninety minutes.

My host family soon turned out to be a very interesting group of people as well. Felipe and I were able to become great friends, almost like brothers. As a massage therapist, his schedule required many long hours, yet he was always able to find time to show me a good time, especially during those nights where I had almost nothing to do even if our ideas of fun were greatly different. One of my more memorable outings with him was when he asked if I wanted to see a movie. I decided to come, however, his idea of a movie ended up being a sentimental "Chick-Flick" that we saw with his girlfriend. Neither one of us realized it until it was too late.

My host mother, Fátima, as I soon found out, could not communicate in English, which at first resulted into a rather detached relationship. However, as I learned more Portuguese and as I started to play volleyball with her, we were able to bridge the gap. She was the quiet, caring mother figure that I knew I couldn't live without in Brazil, as well as helping me take care of many of the home-economics activities that I struggled to do such as cooking, laundry, and cleaning. During the weekends, she and my host father, Emidio, also took me to see their families. Longing for my own family, I could not thank him enough for showing me his. The new family connection helped me cope with the isolation

from my own.

Perhaps the most influential member of Emidio's extended family was his five-year-old niece, Rafaela. She instantaneously dubbed me her "grande amigo" when we first met, and indeed, she became my first friend in Brazil. I would spend every weekend looking forward to seeing her and

thinking of new activities to do with her such as drawing, flower picking, and hide-and-goseek. Rafaela and I developed a sibling-like bond, especially since she reminded me of my own eight-year-old sister, Emily.

As for Emidio himself, my host father and my connection to Embrapa; he was the one arranging most of my activities and bringing me into the family. He was a one who had a grand sense of humor, yet he always worked diligently to make sure that I always received everything that I needed. Emidio also held one position that was almost vital to my stage in Brazil: he was the head of the recreational Association of Embrapa Soja (a.k.a AEE).



My Host Family: Emidio (far left), Fátima (right center, and Felipe (far right)

### The Recreation Association

"What will you do this winter vacation?"

"I don't know ... probably playing Tekken and going to this place." Vitão replied.

My newly-made friends Vitão and Henrike and I were relaxing on a lazy Saturday afternoon during my third week in Brazil after an afternoon of Capoeira, a unique Brazilian dance martial art. We were watching a pick-up soccer game at the AEE. For my new friends whom I had met in the AEE, it marked the first few days of the high school winter vacation.

"What about you Henrike?"

"Probably the same."

We decided to stay and watch the soccer game as the conversation shifted into a lesson where Vitão and Henrike attempted to teach me some Brazilian slang. It seemed that we were going to see each-other often during the next few weeks. It was then I realized just how important the AEE was to me.

The Recreational Association of Embrapa Soja is the sports and recreation complex sponsored by Embrapa Soja. It boasted of two soccer fields, a basketball/volleyball court, a tennis court, and a barbecue shelter. As head of the AEE, Emidio was able to take me to many of the club's regular events as well as some of its special parties. The AEE soon took over my life in Brazil outside of the Embrapa lab; I would spend the majority of my time. I spent my Tuesday, Sunday and Thursday nights taking up volleyball, my Wednesdays and Saturdays learning Capoeira, and Fridays playing soccer. I also was able to attend many of parties and barbecues. On the weekends, I even volunteered to help set up the many of the special celebrations.

One of my fondest memories at the AEE was during the beginning of my fourth week in Brazil, a mammoth month-long, nation-wide Brazilian celebration of the month of June. Embrapa Soja decided to host its company celebration that Saturday. Not preoccupied with anything to do, I decided to help Emidio decorate the entire recreational complex. While decorating, I must admit I was not too



enthusiastic about spending an entire Saturday working when I could have been seeing Brazil or at least the city. I was still in that petulant mood until the party was almost over. In the middle of the party, I spotted one of the graduate students working in my laboratory in Embrapa, Aguida Morales. She was with her now fiancé, Alan, and we started to talk about my experience so far in Brazil. When I mentioned that I spent most of my time at the AEE, she didn't laugh or pity me. She simply explained that I was experiencing the real Brazil and not the glossed-over tourist vision. At that moment and on, I would never again regard the time spent at the AEE as wasted.

After I had that conversation with Aguida, I recognized another phenomenon that happened to me while in Brazil. Never before, had I become so attached, so respectful, and so admiring of anybody in such a short three weeks. Aguida was not a famous researcher or untouchable saint; she was an ordinary graduate student with a very helpful and caring attitude. She attempted to her best of her abilities to invite me to a variety of the laboratory social occasions, and she treated me as her own son during the long hours of the lab. When I found out she was yearning to study in the states, I immediately offered to help her with English and finding connections in the U.S. Before that, I doubted

Soccer at the AEE

that I could have developed such a friendship in three short weeks. However, I only had two short months in the lab to know and befriend not only her, but many of the names and faces of the

Biotechnology sector of Embrapa Soja.

### The Biotechnology Laboratory of Embrapa Soja

"Do we eat dogs in China? That is a good question... I have never had dog before, but yes Chinese people eat them, and they are very expensive: a delicacy."

"But dogs are pets, not food." Maria said.

"Yes, but you must realize that the culture of China is different than Brazil."

I would spend the next several minutes trying to explain why the Chinese ate dogs, and I was on a losing side. The other interns and workers at Embrapa and I were having tea after lunch at the Break Room of the Biotechnology lab. After six weeks of working at Embrapa Soja, I became accustomed to the agenda at the lab: tea and coffee in the morning, work, lunch, tea again, work, and leave, with coffee breaks in between. However, as routine the schedule was, it never became old in my perspective. There was always someone to talk to and some interesting topics during the breaks. This was an especially memorable conversation about strange food that people ate.

"However, I did try some insects." I recalled.

"Were they any good?"

"Yes, but insects in China can be very expensive."

"Not in Brazil." Vieira joked, "Here, the bugs are very cheap, even free."

We laughed, "Haven't any of you tried anything like that?"

"Well, I think that Luana ate pigeon once." Maria told me.

"Luana, you monster, what did the pigeon do to you?" I joked.

"It's not any different than eating chicken. It even tastes like chicken!" Luana defended herself. We all laughed. Even though I have only known the employees and interns in the biotechnology, spending a work schedule with them made me feel as if we had been friends forever. I will never forget the small conversations, knowing the stories of the lives of a people half a world apart. I will never forget then events that I did with the other interns; even if it were as simple as watching a soccer game or going to a Brazilian barbecue. It was these small moments at the biotechnology lab of Embrapa Soja that I lived for in my Brazilian internship.



Social lab gathering during a soccer game

The Embrapa Foundation is the research institution managed by the Brazilian government, committed in the areas of agricultural research and food production. It has shaped itself as one of the leading agricultural institutions of the world. Embrapa divides into forty research institutions, specializing in different areas of production, such as wheat, rice, and beans.

Embrapa Soja is the main research and production head of soybeans and sunflower for the country of Brazil and the research and production head of wheat for the state of Parana. Embrapa Soja employs around 296 employees, including a research staff of 78, who head the main research

projects. In assisting the researchers with their projects and training for the job market, around 15.3% of Embrapa's employees are laboratory interns completing undergraduate, masters, or doctorate degrees (Embrapa).

The mission objectives of the institution are to provide technological solutions for sunflower and soybean production to increase Brazilian competitiveness in the world market, to help balance human nutrition, and to become more environmentally sustainable. Additionally, Embrapa has a dedicated program in order to quell the problems concerning social unbalance and unrest, by helping small farmers obtain the state-of-the-art production technology. Some of Embrapa Soja's contributions to soybean research include the development of germoplasm in low altitudes, the biological control of caterpillars and stink bugs, and a variety of soil techniques and preservation (Embrapa).

However, I could never think so highly of Embrapa Soja without meeting the helpful, intelligent, and caring people working in the institution. During my personal stage at Embrapa Soja, I was a part of a bioinformatics project under the supervision of Dr. Eliseu Binneck. It was him who was able to set me up with my training project, incorporating bioinformatics and phylogenic analysis under another professor, Dr. Alvaro Almeida. Although Dr. Binneck was a high profile and respected researcher at Embrapa, he would always find time to assist me in my project when I needed it with any problems. He would always be more than happy to show me exactly how to run my computer software for my analyses. He would also be the one arranging me with other researchers and professors, including the occupied Dr. Alvaro Almeida. More importantly, Dr. Binneck was willing to show me the concepts behind all the laboratory and computational techniques that I was participating with.

Inside the laboratory, my main instructor was Silvana Rockenbach Marin. When I first arrived at the laboratory, I had only the basic high school lab experience in the biological fields. When I first received my research project during my second week at the institution, I found that the project required laboratory techniques such as DNA extraction, PCR, RFLP analysis, and gel electrophoresis, I was very confused about where I was to begin. I could not be more thankful enough for Silvana was still able to explain to me most of the laboratory procedures. Unfortunately, her schedule was very demanding and I could not ask her for every problem, however, she made certain to address each and

every one of my concerns, despite her agenda or the language barrier. Through this experience with Silvana taught me the importance of instruction in the lab, many methods of lab communication, as well as the importance of patience.

Despite the tremendous help of Eliseu and Silvana, the bulk of my knowledge that I learned came from the graduate and undergraduate interns. It was through them where I would learn the most of laboratory and computational techniques, and it was through them where I would develop my lasting friendships. One name in particular comes into mind: Rodrigo Pereira. Rodrigo was a graduate student, the only one who pursued a discipline in bioinformatics/computational biology, and he had proficiency in English. Because we had a similarity of interests, he was the one who assisted by far the most during my stage at Embrapa. During my first week, he arranged a variety of useful laboratory and computational activities for me to see during the introductory period. It was through him where I would learn a vast amount of Portuguese for communication during those first few weeks. He has been my mentor, translator, and friend ever since. It was through him I would know virtually all the other interns at the Biotechnology.

Virtually every undergraduate and graduate intern in the laboratory proved to be a remarkable help towards my stage. Many played serious and active roles into helping me develop my professional technique, such as Aguida, my teacher for almost the entire significant laboratory exercises that I needed to accomplish. Others took on a much more candid, but nevertheless vital role to my development in Embrapa, such as João Vitor, my "Portuguese slang teacher" or Maria Thereza, an undergraduate student who became my main social connection outside the lab; however, I must thank the entire Biotechnology Lab for simply helping me and offering to be my friend in a strange land.

My duties at Embrapa included a variety of mixed activities dealing with the biological and computational sciences as well as assisting in lab maintenance. On my first week, I was introduced to the lab techniques and the people of Embrapa. After that, I started on my own project involving the phylogenic analysis of the soybean fungal infection causing sudden death syndrome, *Fusarium Solani*. The purpose of my project was to train me in the laboratory procedures as well as to make me familiar

with the widely-used programs of DNA sequencing such as BLAST, MEGA 4, and Clustal X. My next three weeks were spent largely with Aguida and Silvana managing the laboratory part of my experiment to extract, amplify, and grow the DNA of certain *fusarium* from different regions of Brazil. I was also able to aid the other interns in their own similar projects in order to further prepare myself with mine. Once the laboratory procedure was completed, the stage was set to complete the bioinformatics and computational analysis of my isolated DNA in order to compare the evolutionary stance of my *Fusarium Solani* samples.



A day in the lab

### World Food Prize: Supervised Practical Training at Embrapa Soja Student: Jack Hou Supervisor: Eliseu Binneck

Phylogenetic Analysis of Some Strains of Fusarium Genus Causing Disease in Soybeans in Brazil

This project is a part of a research program coordinated by Dr. Álvaro Manuel Rodrigues Almeida (Embrapa Soja)

### Abstract

*Fusarium solani* has become a growing problem affecting one million hectares of soybean plantations today. *Fusarium solani* f sp. Glycines is a fungal parasite living in soil that has multiple hosts. In soybeans, *Fusarium* infestations have been known to herald a deadly ailment called Sudden Death Syndrome (SDS), leading to vascular discoloration, defoliation, and usually (as the name suggests) a very swift death. In order to understand the pathogenic variability of *Fusarium* causing SDS, a phylogenic analysis was preformed based on examination of the differences internal transcribed spacers (ITS) regions, between eight different varieties of *Fusarium* collected from different regions in Brazil.

### Introduction

One of the most serious diseases concerning soybeans is the Sudden Death Syndrome. SDS has



become a major problem for farmers in Southern Brazil. It leads to rapid vascular discoloration in the roots and stem, but it is most harmful to the leaves because it is quick to cause interveinal chlorosis, necrosis, and defoliation of the soybean plant. The casual agent of SDS is a fungus known as *Fusarium solani*. Unfortunately, *Fusarium* thrives during the cool, wet growing seasons (the seasons most favorable for soybean growth) and can induce yield losses anywhere from 20-80% in large soybean growing regions of the United States, Brazil, and Argentina, depending on when the fungus begins the infection process (Freitas *et al*, 2004).

Control of this disease is possible. Two such examples include planting the field with cultivars at different times and to use improved drainage techniques. However, very little is actually known about the variations of *Fusarium* causing SDS, and whether or not control techniques

SDS in the leaves: Almeida*et al*.

for one type of *Fusarium* will work for another type. In the ideal world, newly bred soybean genotypes



SDS epidemiology map: Almeida et al.

should be screened for SDS and vulnerability to all types of *Fusarium* infections. However, little information is available to plant breeders right now about pathogen variability making this process rather difficult.

The objective work is to perform an analysis of the genetic variability on some isolates of *Fusarium* from different regions of Brazil causing disease in the plant. The analysis will be based on the Internal Transcribed Spacers (ITS) between ribosomal genes. The ITS region is the genetic space between two areas of DNA. This area is shown to have the most recent phylogenic divergence (most recent evolution) among species. Therefore the ITS region has proved very useful to determine the genetic differences between two species and within a species as well. Finding these differences will be especially important in trying to find out which genetic vulnerabilities and resistances a certain species of *Fusarium* may have to be treatment options. Therefore farmers and investors can use the best treatment protocol to increase crop

yield. This increased crop yield can do anything from feed the hungry, give subsistence farmers a decent profit, and allot more room for other soy uses such as fuel.

#### **Materials and Methods**

The materials and methods of this experiment can accurately be separated into three parts: DNA Extraction, ITS RFLP analysis and sequencing, and Phylogenic Analysis. The samples used in this experiment would be varieties of *Fusarium solani* collected from different regions of Brazil experiencing SDS. The DNA that was analyzed was mycelium DNA.

#### DNA extraction:

The washed mycelium of each *Fusarium* sample was dried at room temperature. Then each isolate was wrapped in filter paper and stored a -20° Celsius. The DNA was extracted using the protocol Almeida *et al* (2001).One gram of the *Fusarium* mycelia was crushed with liquid nitrogen and then was treated with the CTAB extraction buffer (10 mM Tris -HCL pH 8.0, 1.4 M NaCl, 20 mM EDTA, 1% hexadecylrimethyl-ammonium bromide – CTAB and 0.1% 2-mercaptoetanol), followed by chloroform-isoamyl alcohol (24:1) purification, precipitated with ethanol

70% and stored at -20 degrees Celsius. See the appendix for the more detailed protocol.

#### ITS RFLP analysis and sequencing:

The ITS region, the region of DNA which had the most interspecial difference, was amplified by PCR with the Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990). PCR was preformed in 50 µL-volumes containing10 mM Tris-HCl with pH 8.3, 50 mM KCl: DNA denaturation, primer annealing, and primer extension. The temperature was set at 94 degrees celsius for DNA denaturation (7 minutes for the first cycle and one minute, 2.5 mM MgCl<sub>2</sub>, 200 µM of each deoxynucleotide triphosphate, 0.5 µM of each primer, 30ng of genomic DNAand 1 U Taq DNA polymerase. Each cycle could be divided as three parts for each the remaining cycles), 55°C for primer annealing lasting one minute, and 72°C for the two minute primer extension with the total of 35 cycles. Amplified products were resolved by gel electrophoresis in a 1.3% Agarose gel. The gel was stained with ethidium bromide (.5 µg/ml) to be visualized later. A 10 µL sample of each PCR product was purified and the digested with the restriction enzymes *Rsal*, *EcoRV*, *Dral*, *EcoRI*, *Hae*lll, *Msel*, *Pst*1, and *Taq*1, according to manufacturer's instructions. The digested DNA was then examined on a 2% agarose gel. The gel was then stained again with ethidium bromide, and the DNA bands visualized under UV light.

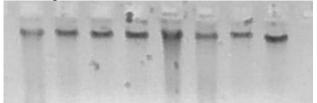
The DNA was then cloned by in a bacteria plasmid vector. This was done by using electroshock to induce bacteria to take up the DNA needing to be sequenced. The bacteria were then cultured under favorable conditions to clone the DNA. DNA sequencing was done by the chain termination method (Sanger *et al*, 1997) using the ABI Big Dye Terminator Cycle sequencing kit v 3.0 (Applied Biosystems Inc., Foster City, CA, USA) on an ABI PRISM model 3100 DNA sequencer. The reaction would analyze both the forward and reverse reactions of the sequence using the ITS1 and ITS4 primers.

### Phylogenic Analysis

Nucleotide sequences were then compared to sequences from public databases (NCBI, <u>http://www.ncbi.nim.nih.gov/</u>) using BLAST (Altschul *et al*, 1997), (using the forward and reverse reactions of the seven significant specimens of *Fusarium*). Selected homologous sequences from relevant species were then to be included in further analysis. The method of decision used was to score each sequence separately and then take the top three most proximate sequences in the NCBI Database (taken that the proximate sequences matched the original sequence with more than a 95% nucleotide agreement). Exact sequences that appeared in two different BLAST searches were not copied. Next DNA fragments were then aligned using ClustalX (Thompson *et al*, 1994) and a neighbor joining phylogenic tree was then constructed from Kimura 2-Parameter, pairwise distances using the Molecular Evolutionary Genetics Analysis software MEGA 4 (Kumar *et al*, 2004). The estimation of the consistence of phylogenic resolution was performed by a bootstrap analysis using 1,000 replicates. The pairwise differences were then calculated for the ITS 1 and ITS 2 region (rejecting indels and gaps in missing data).

### Results

The first major step point of data analysis came at the end of the BLAST search (See Appendix 2). Of the seven forward and reverse reactions, 28 different sequences were derived from the search, not counting repeats. Of these 28, eleven of these samples belonged to the belonged to the *Fusarium* solani species:



Gel Results of my Fusarium Samples.

List 1: Matching specimens using the BLAST search

- Fusarium solani
  - $\circ$  NRRL(5)
  - o F Sp. Phaseoli (2)
  - o Sp. IBL (2)
  - Others (2)
- *Chaetomium globosum* isolate (3)
- *Hypocreales* s. LM(4)
- Ascomyete (1)
- Fusarium Oxysporum f sp. Cubense Isolate
- Nectria
  - o Haematococca (4)
  - o Gliocladioides (2)
  - o Others (2)

NOTE: All pairs in this analysis have an nucleotide accuracy of at least 96% with at least one or more of the original fourteen samples.

Using these samples, a phylogenic tree was created using MEGA 4. The tree shown below is the Bootstrap Consensus Tree using 1000 replicates with the Kimura-2 Parameters.

NOTE: The numbers shown above are the percentage of trees that Bootstrap made that match this pairing. For example, out of all 1000 trees that Bootstrap calculated, 89% would have Fusarium Sp. 440 and Fus 196 M13-R.

*NOTE:* The molecular scale shown is the number of evolution differences between in the Tree. The distance was calculated with the Kimura-2 method  $d = \frac{1}{2}\ln(1-2P-Q) - \frac{1}{4}\ln(1-2Q)$  where P is the proportion of translation differences and transversional differences Q

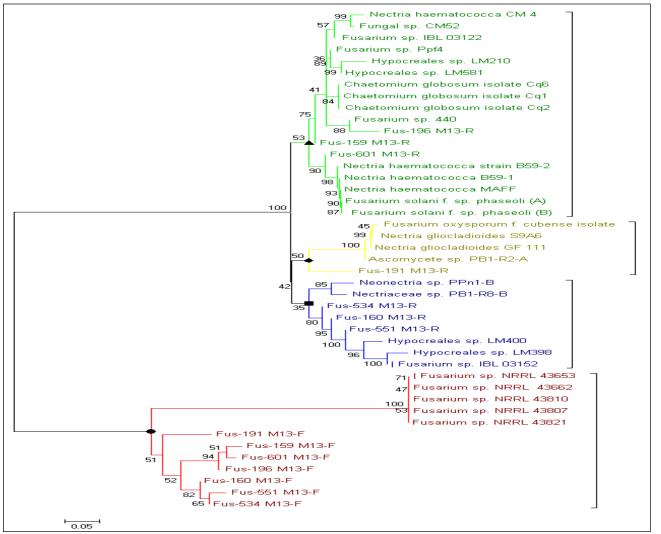


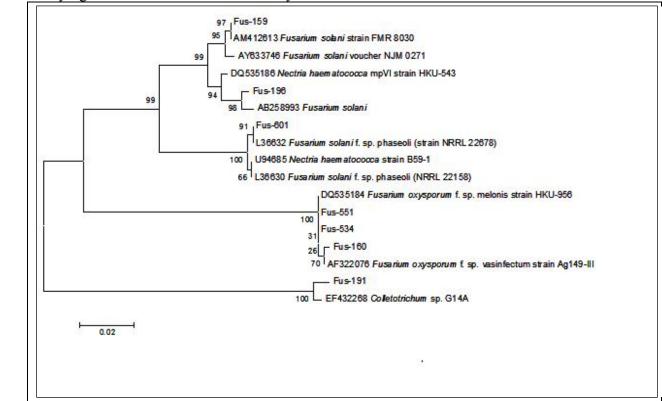
Figure 1: Full Phylogenic Analysis of the *Fusarium Solani* Samples Compared to Similar BLAST Specimens

This tree does provide the basic overlay of the phylogenic roots of the similar sequences; however, this tree cannot be the most accurate method for analysis as many of the bootstrap results are under 50%.

The above chart shows the phylogenic tree when the *Fusarium solani* samples are treated as different samples. However, in the phylogenic tree above, one can see that there the forward and reverse reactions are considered to be different, because the forward and the reverse reactions sequence for different parts of the DNA duplex, thus showing different sequences. It would be more accurate to combine the sequences of the forward and reverse reactions to obtain a more precise reading (Campbell, 2006). The second BLAST was preformed in the same manner as the first search, except combining the DNA sequences of the forward and reverse reactions.

List 2: Matching specimens using the BLAST search (Combined Sequences)

- Fusarium Solani (5)
- Fusarium Oxysporum (2)
- Nectria Hematococca (2)
- Collectotricium (1)



#### Figure 2: Phylogenic tree for the combined analysis of the Fusarium Strain

Substitutions to Include : d: Transitions + Transversions Pattern among Lineages : Same (Homogeneous) Rates among sites : Uniform rates No. of Sites : 517 No Of Bootstrap Reps = 1000

The combined sequences, as one would expect, provides a narrower reading of the strains most related to the *Fusarium* samples, and it also cut down the number of specimens needed to make an analysis. Also note that the vast majority of each branch is around 90-100% accurate.

#### Discussion

In order to better understand how the *Fusarium solani* f sp. Glycines infections in different regions in Brazil operate and link together, one should study the closest genetic counterparts to discover the most efficient treatment for each kind of *Fusarium solani* infection. One should compare the combined analysis of the fungal DNA sequences to obtain the most proximate readings from the Kimura 2 parameter tests. In order to study an infection of FUS 159, one should look into the behavior and properties of the *Fusarium Solani* FMR 8030. The same can be said about AB2 58993 *Fusarium solani* and its relation with FUS 196, *Fusarium solani* f sp. Phaseoli (NRRL strains) and *Nectria hematococca* for the FUS 601 strain, *Fusarium oxysporum* f sp. Melonis for FUS 551 and 534, *Fusarium oxysporum* f sp. Vasinfectum for FUS 160, and *Collectotricium* for FUS 191.

However, the data above does show some interesting validations. The first area of analysis is how different the separate and combined analyses are. Ideally, the two trees should pair the same fungal strains to their respective fungal samples on the second figure. However, the two trees have deviations. For example in Figure 2, FUS 191 is paired with *Collectotricium*, but in Figure 1, FUS 191 has no paired strain for at least .05 molecular pairings. This is due to the process of PCR in DNA sequencing. PCR begins when two designated primers from either end of the desired replication region replicate the sequence going both forward and backwards, hence the forward and reverse reactions. However, alone, the forward and reverse strands alone will provide more arbitrary primers once the two primers meet (Campbell, 2006). Therefore, it is necessary that one finds the common, combined region to analyze, thus Figure 2 should be more accurate than Figure 1.

The second area of analysis is that many of the specimens studied are phylogenically not *Fusarium solani* f sp. Glycines infections. The FUS 196 strain, for example, is actually a member of the formal species, Phaseoli, and its anamorph, *Nectria haemotococca*. It was also found that FUS 551, 534, and 160 are more similar to the cousin of *Fusarium solani*, *Fusarium oxysporum*, a strain of *Fusarium* that is also pathogenic and shares many of the same traits and symptoms as *solani* (Arruda *et al.*, 2005). FUS 191, shown as a blank in Figure 1 turned out to have a counterpart in another species, *Collectotricium*. The difference in species and causing SDS leads to two conclusions: *Fusarium Solani* f sp. Glycines is not the only cause of SDS in soybeans, and looking at the different strains that appear in the phylogenic tree may be beneficial to finding genetic preventions and cures for Sudden Death Syndrome.

The results of this experience probe deeper into disease that until now, has not been studied for variation. Ideally, farmers, scientists, and businesses should screen and treat seedlings for malicious *Fusarium solani* infections. However, many strains of *Fusarium* may fall under the genetic radar as the variation information is still lacking. By searching for more strains in more areas of Brazil, more sequences and variations can be discovered, giving a broader scope to protect soybean farmers. In order to better understand how the *Fusarium solani* f sp. Glycines infestations in different regions in Brazil operate and link together, one should study the closest genetic counterparts to discover the most efficient treatment for each type of *Fusarium solani* infection.

"Good work, Jack, I am proud of you." Eliseu said to me.

It was at the end of the seventh week of my internship. I was walking with Eliseu after having just completed a thirty minute laboratory presentation to Embrapa. After this, I would participate in a series of social trips, resulting in the end of my Embrapa internship. I decided to incorporate the variety of scientific research along with its relation with world food security and social issue. However, what made the report memorable to me and everyone else in the lab was that I presented the entire statement in Portuguese. This moment represented the pinnacle of my learning. Looking back on this experience, I am still very surprised about what I was able to learn in two short months scientifically, socially, culturally, and linguistically.

"Thank you, very much," I said "I could not have done it without you."

"Yes, but I am still surprised about how much you learned."

I don't believe that I have ever felt more pride or more of a sense of accomplishment than completing this project. I walked back to the lab with a skip in my step and a more confident stride.

"Jack, I must say, I loved it," I felt a tap on my shoulder. It was Veria, the caretaker of the lab.

"So did I," Said Luana, an undergraduate intern of Embrapa.

"Yes, Luana cried during it."

As Luana tried to silence Veria, I realized just how attached I was to the people and how mutual the feeling was. I used to joke with Luana that since she did not speak English to me and I could not understand her Portuguese accent that I could only be fluent if I were able to understand her and vice-versa. With the presentation, she said told me she understood every word, thus this moment would be one I would never forget.

"It is OK." I will miss you too. Luana gave me a hug. My last week would involve much traveling outside the lab for the field studies part of my internship, so this was the last real day I had with her.

### **Brazil and World Food Security**

During the final two weeks of my stage in Brazil, I was fortunately to participate in a variety of social projects sponsored by Embrapa. Although I could study the DNA sequences of the soybean infection, I had yet to see the importance of my research in the small agricultural committee. I traveled with Carina Gomes, a woman from the journalism sector of Embrapa Soja, and Amélio Dal'Agnol from the economics and business department. Both Carina and Amélio had already hosted other interns from Brazil, so they knew the places I would need to go in order to discover the social situation in Brazil concerning soybeans. Carina arranged a series of travel related trips related to visit small and subsistence soybean farmers in Brazil to hear their input on the tribulations of growing soybeans.

After interviewing several farmers, I found out that their greatest obstacle in soybean farming was almost unanimously the money. Money was needed to buy the most viable soybean varieties; it was needed to buy farm equipment to aid in the planting, growing, and harvesting of soybeans; it was also used to obtain the varieties of fertilizers to grow and sustain a field of crops. The cost to grow soybeans was generally expensive compared to other crops, so many small and subsistence farmers often go without one or more of the growth aids to grow soybeans, yet the importance of soybeans was nevertheless substantial. As a legume, it is used to replenish the soil with nitrogen every other year in order to pave the ground for other crops such as corn, wheat, or barley. Amélio also told me that soybeans, although not used often in direct consumption, was regarded as a cash crop. Subsistence farmers, especially, would grow one or more crops for nourishment such as corn or wheat. In addition, the farmers would also grow soybeans for currency in order to fund the growth of other crops and to generate an income.

Sudden Death Syndrome also has a more indirect impact on the consumer. Amélio told me that soy was perhaps one of the most nutritionally balanced crops sold in the market. Those who suffer from malnutrition, mainly the impoverished, would need soybeans to help complement their diet. However, such disastrous events such as SDS decrease crop production of soy, thus raising prices on an already expensive crop. The price greatly limits the access of soy to the poor, labeling the soybean as a rich man's food. Thus, research to combat disease is imperative for us to make soybeans affordable for the poor.

Because of the necessity of the soybean plant to small and subsistence farmers in Brazil, I could



A market stall for subsistence farmers, sold are various arts and crafts made by the women and children to supplement the farm's income

see why the protection of soybeans from disease would be so important. Sudden Death Syndrome is a devastating disease to the small farmer. An infection of *Fusarium* can cause SDS to achieve a mortality rate of around 51%, often leaving large patches in a farmers' field and making the land inviable for months (Fronza, 2003). To the small farmer, an SDS infection may be disastrous. The estimated cost of a SDS infection is around \$220 per ton (Fronza, 2003). Unfortunately, for rural Brazil, where 32.6% of the population lives in poverty, and much of the population is unable to afford some of the even most

basic amenities, the loss of the soybean means a drastic cut in the income needed for shelter and equipment for the next year (FOA, 2006). This leads to a deteriorating cycle in which the sustenance farmers eventually lose the

ability to produce food or support their families in providing an adequate nutrition or living standard. Thus malnutrition caused by a SDS infection may cause a very real danger.

However, there still hope for the small farmer. Many companies are stepping up to help the subsistence farmers. Embrapa Soja has recognized this threat and has started to support a small rural village of 180 farmers called São Jeronimo, providing them the technical and financial support to train them as leaders of Brazil's food production (Embrapa). In addition, Embrapa is also supporting a philanthropic society called Humanitás, to allow small farmers to gain extra income producing organic foods through soybean and sunflower technology. Also, Embrapa has started an initiative to expand the soybean market to make growing soy more profitable, called "Soybeans on the Table", a campaign to educate the ordinary consumer and corporation of the advantages of soybean use in consumption, production, and fuel (Embrapa).

Farmers themselves have decided to commune to deal with soybean complication including disease and economics in the form of Cooperatives. I was able to visit cooperative in the state of Paraná, called Carol. In Carol, I learned from interviewing the leaders that this was an innovative business to help farmers, no matter how small. Through the payment of 1% of a farmer's capital, Carol offers much that an individual is often unable to receive. With more funding, the buying power of Carol allows the cooperative to buy in bulk and sans the middleman, thereby reducing the cost of farming. The cooperative also aids the farmer in selling collectively, helping increase crop prices and landing more income for the farmer's pocket (Carol).

Brazil still has a long way to travel in order to deal with the world food security issues caused by the income gap and crop diseases. Thus the research, in which I participated, in the broad perspective, is one step to solving the crisis of Brazilian world food security. When more is known about the factors threatening soybean growth, soybean production can rise, allowing Brazil to become a world leader in food production.

## Epilogue

"I have arrived."

I looked back and saw the smiling face of Maria Thereza Bazzo de Martins. I instantly lit up. This was on the Saturday of my last week in Brazil. I would be leaving for home the next day. Rodrigo had arranged a goodbye party for me. At this time, the party was almost over, and the only ones remaining was Rodrigo and his wife and daughter; my host father, Emidio; and André Mateus, a new intern at Embrapa Soja that I instantly befriended and was proud to be the one showing him around Embrapa Soja.

"You arrived just on time; we were just about to leave." Rodrigo said.

"I wouldn't leave Jack without coming for a "thauzino" (little goodbye). Anyways, this is my dad. Father: this is Rodrigo and Jack."

"Nice to meet you, and it was just before I was about to leave." It was this little story that I remember almost as if it were yesterday. On my last day in the lab, amidst the tears, Maria promised me that she would catch me before the weekend since we could not go out that night for a party. Maria's father was visiting, and she did not know if she could make it to Rodrigo's party. Maria, as my main social connection, made the most effort to acquaint with Brazil outside the lab despite her demanding schedule, taking me to most of the social gatherings that I attended to Brazil. For that, I am eternally grateful. Maria's presence that day, even though it was only for a short time, was important to me and would cap my leaving on a very high note.

"Jack came to Brazil with very little knowledge about the culture or the language of Brazil. Now he is almost a Brazilian, and he does not even need speak English here. He can survive here."

"Without Maria or Rodrigo, never." I said.

"I am surprised" Rodrigo said, "I don't believe that I have seen anyone learn so much in such a short time."

"Thanks to all of you." Pondering back to my experience in Brazil, I realized my progression since I arrived in Brazil. I learned almost everything that I know now about laboratory procedure. I become more aware of the food security issues affecting the world. I became more conscious of my social cultural surroundings. I became conversational in a language I knew nothing of before this trip. I was able to make friends who will hopefully be around forever. The collective impact added great strides to my maturity and identity.

"One last picture!" Emidio exclaimed after the conversation, "Come Maria, Rodrigo, André, and Jack."

As we lined up, I felt thankful for everything for everyone that I met in Brazil. Had it not been for Emidio, Rodrigo, Maria, André or anyone else, I surely would not be as attached to Brazil as I was then or am now.

"Good bye Jack, my friend." Maria said at last, "Remember, do not feel sad or cry, for this goodbye is not forever. We will see each other soon."

"You have a good daughter." I told Maria's father, "See you very soon."

We all laughed, as Maria and her father left for the horizon.

"Is this goodbye for you too?" I asked Rodrigo and André.



Rodrigo and his wife, Thais, Me, Maria, her father and André

"No, I will say goodbye to you tomorrow, just before you leave." André said. He would.

"No, the afternoon is still young. Let us see some more of Brazil." Rodrigo said. If I could repay Rodrigo back for anything he did for me, whether it was for his computer help, biology lessons, Portuguese training, small conversations, or simply being my friend, I would immediately jump to the cause. It was hard to believe that I would leave him until who knows? "Now, are you up for Pizza tonight?"

# Acknowledgments

I would like to dedicate my work to the following, for without them, my experience in Brazil would not be possible:

### From the World Food Prize

**Norman Borlaug and John Ruan:** for founding the World Food Prize's Borlaug-Ruan Internship and making the international experience possible.

**Ambassador Kenneth Quinn:** for allowing me to partake in this wonderful opportunity. **Lisa Fleming:** for coordinating the Borlaug Ruan internship, and providing me with guidance and notices during my stay in Brazil.

### **From Brazil**

**Emidido, Fátima, and Felipe Casagrande:** for taking care and nurturing me, in a land I was a stranger to. For providing me with a family when I was so far from my own.

**Dr. Eliseu Binneck: f**or entrusting me with a research project and mentoring me through the scientific world.

Carina Gomes: for arranging my social projects during my stage at Embrapa.

**Drs. Alvaro Almeida, Amélio Dal'agnol, and Vanoli Fronza:** for providing me with the valuable information and guidance.

All the friends that I made in Brazil: for leaving me with countless memories.

**The Biotechnology Lab of Embrapa Soja:** for their help and kindness to make my internship experience unique. A special thanks goes to:

Silvana Rockenbach Marin: for providing me with guidance and assistance with the biotechnology laboratory.

**Rodrigo Pereira:** for being my teacher and friend in all areas I would need.

Aguida Morales (Now Dr. Aguida Morales): for aiding me countlessly in my laboratory procedures.

Maria Thereza Bazzo de Martins: for being there for me both in and out of the lab.

### From Back Home

**Charles Lierman:** for mentoring me through the youth institute and eventually through the Borlaug Ruan Internship.

**My friends in Iowa:** for maintaining a strong bond before, during, and after my internship in Brazil.

**Zhenglin Hou, Yumei Sun, Emily Hou, and Eric Hou: my family**: for supporting and encouraging me to become active in the world food issues. Also for trusting me to pursue the BR-Internship

Special thanks to John Deere for sponsoring this experience.

## Appendix 1: DNA Extraction Preparation (Portuguese) Extração de DNA de Plantas - MINIPREP

- Triturar cerca de 1g de folha em presença de N<sub>2</sub> 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 °C 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 °C ou 2 horar a -20 °C
- 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°C por 5 minutos para ajudar a ressuspensão.
- Adicionar RNAse A, na concentração final de 40ug/ml e incubar a 37°C 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 °C ou a -20 °C por 2 horas
- Repetir os dois passos anteriores a adição de RNAse A . Quantificar o DNA em espectrofotômetro ou gel de agarose 0,8%.

Tampao 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

### Tampão de Extração

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

## Appendix 2: DNA Sequence Results for the Fusarium Strains

>Fus-159

#### >Fus-160

#### >Fus-191

#### >Fus-196

#### >Fus-534

#### >Fus-551

#### >Fus-601

## References

- Almeida AMR, Abdelnoor RV, Calvo ES, Tessman D, Yorinori JT. Genotypic diversity among Brazilian isolates of *Sclerotium rolfsii*. J. Phytopathol. 149:493-502. 2001.
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. 25(17):3389-3402. 1997.
- Arruda GMT, Miller RMG, Ferrira MNSV, Café-Filho. Morphological and molecular characterization of the Sudden Death Syndrome pathogen of soybean in Brazil. *Plant Pathology*. 54: 53-65. 2005.
- Campbell NA, Reece JB. Biology: Seventh Edition. 2006. Pearson Education inc. San Francisco.
- Corol (Cooperativa Agroindustrial). Nossa Gente. 2007. < http://www.corol.com.br/>.
- Embrapa Soja. Mission Statement. 2007. < http://www.embrapa.br/soja>.
- FOA Statistics. June 2004. Food and Agriculture Organization. 3 Aug. <a href="http://www.fao.org/faostat/foodsecurity/countries/EN/brazil">http://www.fao.org/faostat/foodsecurity/countries/EN/brazil</a> e.pdf>
- Fronza, Vanoli. Distribuição geografica e importânca econômica da PVR. Tese (doutorado) Escola Superior de Agricultura Luiz Queiroz, 2003.
- Freitas TMQ, Meneghetti RC, Balardin, RS. Yield losses due sudden death syndrome in soybean. *Cienc. Rural* 34(4):991-996. 2004
- Kumar S, Tamura K, Nei M. MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. <u>Briefings in Bioinformatics</u> 5:150-163. 2004.
- Meade, Brigit, Valdes, Constanza, and Rosen, Stacey. Brazil's Food Security and Food Assistance Programs to Reduce Poverty. May 2004. <www.ers.usda.gov/publications/GFA15/GFA15h.pdf>.
- Sanger F, Nicklen S, Coulson AR. DNA sequencing with Chain Terminating Inhibitors. *Proc. Natl. Acad. Sci.* 74:5463-5467. 1977.
- Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680. 1994.
- White TJ, Bruns T, Lee S, Taylor J. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. *In*: Innis MA, Gelfand DH, Sninsky JJ, White TJ. (Ed.). PCR Protocol, a guide to methods and applications. Academic Press, San Diego, CA. p. 315-322. 1997.

# **Photo Collage**



Vitão and me in the AEE trophy room

Visiting a Farm Expo



André and me preparing for field Work

Karaoke Party with Rodrigo and André



The Final lab day, with Maria Thereza

Luana and me at the Festa Julina



Saying Goodbye

A Brazilian Barbeque



Me and Aguida Morales

Eliseu (left) and two other researchers



Me and Rafaela

The Capoeira club at the AEE