The Brazil - US Soybean Connection: A Study in Cooperation

The World Food Prize Foundation

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On June 5th, I woke up at 5:30am, an hour before my plane would arrive at the São Paulo airport and I would step foot in a country where the land, culture and people can only be described as beautiful. Through my window, my first glimpses of Brazil were tainted with a layer of brown haze that covers the city of São Paulo, the third largest city in the world. Despite this pollution I could see accents of vivid reds and oranges that became more intense the more we descended. When the plane emerged from the haze I was breath-taken. The textured land was a marvelous green that contrasted with the red and orange roofs, plastic and scrap. It was apparent that the area we were flying over was impoverished, a *favela*. Yet the vivid colors signaled life and vibrancy. I was not yet aware that those contrasts I first saw from the plane window would harbinger the many contrasts that came to define the country in which I would spend my summer of 2004.

I got off the plane in São Paulo and made my way to customs early in the morning, surrounded by quickly moving people who, unlike me, appeared to have some notion of where they were going. It was a blur. I felt excited while I tried to look collected and blend in. Through all of the movement and foreign noises, I saw something that sobered me. In front of me was an older Brazilian woman in a tattered jacket and worn out shoes weighed down with a heavy bag. She limped along. We were passing a plywood wall emblazoned with the Brazilian emblem. Walking about 5 inches from the wall she slowly and discretely turned her hand and let her fingers brush over one of the emblems as she passed. It seemed being in Brazil offered her some sort of peace. It was then that I realized this experience was going to be deeper than excitement for me. It took me several months, but now I am beginning to understand her action and the feeling it generated in me.

INTRODUCTION

I have lived my entire life in Iowa, surrounded by agriculture, but it was not until I had the opportunity to attend *The World Food Prize Symposium 2003* that I understood the connection between agricultural and humanitarian efforts. I was impressed listening to the vast array of research presented and discussed. Research topics ranged from rice production, AIDS, women and water quality to overpopulation. Each topic held great

importance to defeating hunger and famine within the world. Clearly, in order to better the lives of those in need many types of work, research and interest were imperative.

In the paper I prepared for the *2003 World Food Prize Youth Institute* I addressed the economic and social problems that face Brazil and contribute to hunger and poverty present in many areas. While 10% of Brazil's estimated 184 million citizens enjoy affluence similar to that of the wealthy of First World countries, 20% are surviving at the middle class level, and an astounding 70% face poverty. Within that 70%, exist a special 20% - the miserables, the poorest of the poor in Brazil. During the discussions of our papers at the *Youth Institute*, former World Food Prize Laureate Dr. Nevin Scrimshaw suggested that Brazil's situation currently could be likened to that of the United States' during the Great Depression. With the right economic and social program implementation, there is hope for defeating this depressing poverty. Currently a large foreign debt, inflation, and reminders of past economic chaos haunt Brazil, hindering it from shedding its ranks as a Third World country.

Dr. Scrimshaw's words resonated with me as I thought of the extremes and contrasts I had been introduced to while writing my paper. Brazil, although categorized as a Third World Country, is in an unusual position. The largest country in South America, rich in natural resources, Brazil is rapidly overtaking the US in soybean production – helping to feed Western Europe and Japan even while experts estimate that nearly 32 million Brazilians are going hungry within their native land of plenty. However, writing about these contrasts and seeing them first-hand while living in the country were two very different things. It was one thing to know that the poorest 40% of Brazilians have only 7% of the country's income. It was quite another to watch little boys no older than 9 years old, walk up to BMWs waiting at stop-lights and try to sell fruit to the drivers. This highly uneven income distribution cannot be ignored when beggars mingle among expensively dressed shoppers in downtown Londrina – a city considered well-off by Brazilian standards. This depressing situation of hunger sparked by poverty for the poorest of the poor in Brazil may seem hopeless. Because I spent two months at Embrapa Soja, I know that there is hope.

THE SOYBEAN IN BRAZIL AND EMBRAPA SOJA

"I can see you are here as a part of the American invasion." I froze as I heard those words uttered in the faintest Portuguese accent. My first day at Embrapa Soja and I was failing to represent my country well – was I an "invader?" Fortunately, the man who said this to me then offered me relief with a comforting smile and handshake. He introduced himself and was eager to know specifically why I was in Londrina. He was a researcher at another Embrapa research station located in Brasilia. He, along with many other Embrapa employees, were at Embrapa Soja in Londrina to hear presentations about research being conducted at many of the 40 research centers that make up the Embrapa organization, The Brazilian Agricultural Research Corporation. He repeated that I was an American "invader," but added he was overjoyed that I was "invading" Brazil. He said he prayed there would be more exchanges between Brazil and America, as he explained these are necessary not only for the future of agriculture and soybean production in the United States and Brazil, but for world food security as well. While at Embrapa Soja I learned that these "invasions" were actually a cooperative venture that had already played a key role in development of the soybean as a major crop in Brazilian agriculture. Annually, there are numerous exchanges with American farm groups, agricultural researchers and business people visiting the Embrapa research stations. These groups come to learn about Brazilian agriculture and exchange information vital to the common cause of food production. This man was letting me know that seeing an American in the lab was becoming almost commonplace.

The soybean was introduced to Brazil in 1882 in the northeastern state of Bahia. The germplasm was imported from southern United States of America. This germplasm could not survive in the tropical environment and low latitudes of Bahia. In 1900, the same germplasm was tested in Brazil's southernmost state, Rio Grande do Sul. The climatic conditions of Rio Grande do Sul approximate those in the southern part of the United States and the result was successful production of the different varieties introduced. Starting in 1900 the soybean was a minor crop of Brazil, only grown on a small-scale, being used solely for hay production to feed dairy cattle and bean production to feed pigs. It was not until 1960 that Brazil realized the future benefits of large commercial production of the soybean. In the mid-1950s a government decision was

made to drastically increase wheat production. A summer crop was needed to follow the wheat-growing season. The soybean was the obvious choice for a legume to follow a grass, meanwhile allowing farmers to use the same farm infrastructure and machinery for production.

The positive effects and real increase in national production of soybean was witnessed during the 1970s. The soybean emerged as Brazil's leading agricultural crop, still primarily produced in the Southern Region. New growing areas were rapidly being developed and there were increased demands for the use of soybean as a protein source in animal feed and human consumption. A great boost that helped contribute to the increase from 1.5 million MT of soybeans produced in 1970 to 12.5 million MT in 1977, a relatively short period, was the use of American technologies and varieties in Brazil. Being able to capitalize on the similarities in climate and methods of production, Brazilian soybean producers were greatly aided by American agricultural expertise. Brazil's expanded soybean production called for its government research organizations to create more research centers dedicated to understanding and creating technologies specifically for soybean production in Brazil.

In 1975 Embrapa Soja, (Embrapa Soybean) was established as one such research center. Its 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." Its objectives are: "To provide technological solutions that contribute to decrease social unbalances; To provide technological solutions that contribute to improve nutrition quality for human population."

I observed the practice of the mission and objectives every day I worked at Embrapa Soja. I worked in the Laboratory of Biotechnology and Bioinformatics. Under researcher Dr. Alexandre Nepomuceno, the lab researches environmental stresses and diseases that plague Brazilian soybean producers.

MY WORK AT EMBRAPA SOJA

I helped work on several different research projects during my two month internship at Embrapa Soja. This served as a broad orientation and gave me the chance to see the scope of research conducted there. After this orientation, my supervisors had me

concentrate on two specific projects of global importance, soybean drought tolerance and soybean rust disease. Both areas are of paramount importance for soybean producers because the soybean is a major protein source for the world food supply.

Research into drought tolerance of soybean varieties is an ongoing area of study because the soybean is such a valuable crop. Because of its high protein content, there is no area of the world that potentially could not benefit from growing soybeans. However, areas of the world that are arid or have unstable climatic conditions are unsuitable for growing today's varieties. Research into soybean rust disease is an area of urgent concern because there are no known soybean varieties with resistance to rust. Rust has existed for a century in Japan and China. Further, the disease has spread rapidly throughout Brazil during the past few years and now threatens to enter the United States. Yield losses in Brazil have been significant, and in some cases, devastating.

My previous experience in biotechnology was working in a laboratory in the Plant Sciences Institute at Iowa State University. At Embrapa Soja, methods of bioengineering are being used to study both of these problems. My lab experience at Iowa State was very enjoyable to me. That, and my interest in biotechnology may have helped me obtain these two assignments

The soybean plant is sensitive to environmental stresses. When a situation such as drought strikes, yield and economic losses can be devastating to growers. The first project I worked on employed the bioengineering approach of plant transformation to try to solve problems of drought tolerance and herbicide resistance in soybeans. I worked under the guidance of Noélle Giacomini Lemos. Noélle earned her undergraduate degree in Biology from Londrina State University. She is at Embrapa Soja, completing research for her Master's Degree in Genetics.

Plant transformation is a process by which DNA coding for a specific trait is introduced into a plant whose genome does not code for that trait. The product is a transgenic plant expressing that trait. For this experiment the method we used for the actual transformation was the gene gun method of plant transformation. The gene gun method was chosen over the older method of agrobacterium because the specific transformation process can be completed much more quickly, speeding up the entire experiment. The experience in the lab at Iowa State confirmed for me the sophistication

of the Embrapa laboratory, equipment and researchers. I was fortunate to be allowed to participate and assist in all aspects of the procedures described below.

SCIENTIFIC PROCEDURE

From start to finish, the entire process of plant transformation takes about two months. To begin, 70g of soybeans must be used for one entire transformation process. The seeds, of the variety Conquista, were sterilized with a 70% alcohol solution and then placed in a 1:1 solution of H_2O and sodium hypochlorate. After the sterilization procedure the seeds were placed in a container of water and allowed to sit overnight. The next day embryo extraction was performed. The embryos were extracted from the seeds using sterilized tweezers and a scalpel, simply separating the seed and removing the embryo. The cotyledons - the primary food-storing tissue of the seed – must be separated from the embryo in order for transformation to take place. They were removed from the embryos in the same way the embryos were extracted. These extractions are performed in petri dishes in the presence of water. It is a slow process as it is vital the embryos are not damaged during transformation.

The embryos were dried on filtration paper in an autoclave machine, and placed in small glass plates containing phytagel, an agar substitute used as a growth medium for plant tissue. The embryos were positioned in the phytagel in such a way that when placed in the gene gun they would not be subject to pressures great enough to kill the embryos. After this step is finished, the DNA coding for the desired traits, or 'DNA of interest' must be prepared.

This project is attempting to develop transgenic plants containing the drought-tolerance gene DREB, or the herbicide-resistant gene AHAS. AHAS is resistant to specific herbicides in the Imidazolinone family, including Arsenal and other commercial herbicides. Tungsten microparticles are used by the gene gun to transport the DNA into embryos. The tungsten microparticles, which tend to stick to each other tightly, are prepared by placing them for 15 minutes in a sonicator to spread and separate them. After these 15 minutes, 50uL of DNA, 50 uL of $CaCl_2$ and 20uL of Spermidem are placed on the microparticles. The $CaCl_2$ and Spermidem assist in binding DNA to microparticles. The mixture is centrifuged for 10 seconds to eliminate the supernatant. Afterwards, the

microparticles are washed with 150uL of 100% alcohol solution. The centrifuge process is then repeated and 24uL of alcohol is used.

Now the prepared mixture containing the DNA microparticle complex is spread on sterilized membranes, as 3.2uL of mixture is spread on each. Four membranes are placed together in isopropanol. Membrane thickness is critical to the process with the gene gun because it allows for the pressure to be secured within the gun until the embryos are shot.

PROCEDURE WITH GENE GUN

I have never had the opportunity to work with a gene gun and I was excited to learn to develop the skills to use it. The embryos are "shot" with the DNA of interest on the microparticles. When transforming the seeds the DNA-particle complex is accelerated in a partial vacuum. Placed in the path of the accelerating particles are soybean embryos. The pressure needed for successful bombardment of the embryos is 1,200PSI. A vacuum is created within the path of the embryos and the shooting takes place. The microparticles bombard the embryos at 1,500km/h. Before reaching the embryos, a perforated plate prevents the shell cartridge from reaching the embryos. If performed correctly, only the slivers of metal with DNA will pass through the plate. They enter the embryos at the nucleus and are introduced into the genome.

After being shot, the embryos are placed in a phytoregulator called BAP (benzene aminopurine) in the presence of cytocine, a growth inducer. The embryos are kept within the phytoregulator overnight while the cytocine induces growth of the meristematic section of the embryo. The following day the embryos are transferred to cups containing MS (Murashige skoog basal medium salt), agar and imazapyr. The cups with the embryos are placed inside a growth chamber where they remain for 45 days. When this 45-day growth period is over, embryos are removed from the agar growth medium and placed in different cups containing sand and a nutritive medium, Vermiculita. After the growing plants are placed in the sand and Vermiculita mixture, they are put back in the growth chamber for15 days. Throughout the duration, plants are watered with a nutritic medium.

Following this time in the growth chamber, plants are transferred to the greenhouse where they remain for 15 days with plastic bags covering them. After the first

15 days, the plastic bags are removed and replaced with bags containing holes. These bags stay covering plants for 7 more days, then are completely removed and the plants are kept growing in the greenhouse for a month.

After this one month period, Polymerase Chain Reaction, PCR, was the first molecular analysis we performed to determine if the plants were transformed. To perform these and other analyses, leaf samples are taken from the mature plants in the greenhouse and DNA extraction performed. After the PCR analysis is conducted, results are viewed by using gel electrophoresis, which separates DNA fragments into bands according to size. The resulting bands can be compared with known bands of AHAS and DREB and their presence within the samples determined. The results are viewed under UV light. If the plants were successfully transformed, then a Southern Blotting analysis can be conducted.

The Southern Blot determines the relative amounts and molecular weight of a specific gene present in a sample. The amount of inserted DNA present and its correlation to drought or herbicide resistance is of great significance. To conduct a Southern Blot, the gel containing the DNA of interest from the electrophoresis test, is placed in an alkali solution. A nitrocellulose paper is placed on top of the gel. A stack of paper towels is placed atop that. This creates a capillary action, allowing DNA to be sucked up with solution and transferred onto the nitrocellulose paper. After this occurs, the nitrocellulose paper can be treated with a probe specific to the DNA. The probe will bind with the DNA of interest, and through autoradiography the amounts of product can be determined. Real Time PCR is used to get an accurate quantification of PCR products.

RESULTS

At the end of my internship, the soybean plants that had been sent through the transformation process were analyzed using PCR to see if any had been transformed. Unfortunately, none of the plants were positive for AHAS or DREB. Obtaining no results after two months of work was disheartening. The reason for the failure of the results is not known. This may demonstrate some lack of precision inherent in the gene gun technology. There is ongoing research to improve the precision of this process.

During my final week, I participated in preparation of a new batch of seeds for transformation. The batch went through the same process with AHAS and DREB. I continue to correspond with Noélle and I learned that, at the end of September 2004, PCR analyses were run and 47 plants were successfully transformed with AHAS. As of early October 2004, Southern Blotting is being performed with the plants positive for AHAS, and cDNAs being prepared for Real Time PCR analysis. The researchers hope they will continue to have positive results.

DISCUSSION OF RESULTS

The potential for drought is always present. The significance of drought can be deceptively widespread. In 2003, for example, drought in the United States cost farmers a 12% decrease in soybean production. In 2004 60% of Brazil's soybean losses were due to weather related problems, and a large portion of that was a result of drought in the south. The world felt the effects of these droughts when soybean prices increased significantly as soybean supplies tightened.

Farmers look to the future development of drought resistant varieties as a way to allow them to grow soybeans in areas where rainfall is not as plentiful. Often countries where rainfall runs short are countries in which a protein crop such as the soybean is needed to help fight hunger and malnutrition. If this can become a reality, it will certainly improve food security and nutrition in the world.

MOLECULAR MARKERS/SOYBEAN RUST

During my time working on plant transformation I was also able to participate in a second research project studying the highly detrimental disease of *Phakopsora pachyrhizi*, commonly known as soybean rust. I worked with Rodrigo L. Brogin on this project. Rodrigo earned an undergraduate degree in Agronomy and Master's Degree in Genetics and Plant Breeding from Londrina State University. He is currently a PhD candidate in Genetics and Plant Breeding at São Paulo University. Again, I was allowed to participate and assist in all areas of the research described below.

Soybean rust is called "the worst thing that could hit a soybean farmer" by José Tadashi Yorinori, a well-known plant pathologist from Embrapa Soja. Yield losses from

rust in infected fields can be large. The disease was first found in the western hemisphere in 2001, when it appeared in Paraguay and southern Brazil. Since then this disease has been moving north and is now found in all but one northern region of Brazil's soybean growing areas. Scientists believe it is inevitable that rust will reach the United States, perhaps within the next few years.

Soybean rust is a fungal disease that reduces yield in soybean plants by preventing pod setting when the foliage is infected, causing the leaves to abscise (drop off) prematurely. This disease is very difficult to control. Spread by wind, rust spores can be airborne for up to 50 days. With the ability to quickly destroy up to 80% of a field's yield, this is a costly disease. Currently the only option to fight soybean rust is the application of fungicides. Repeated treatments are needed, adding to the grower's cost of production. As measured by lost yield and increased chemical expense, the estimated cost of rust to Brazil's economy was $2.3 billion in 2003.

Planting soybean varieties that have resistance to rust would be the most economical way to fight the dreaded disease. Today, that is not an option. There are no commercial varieties available with rust resistance. However, soybean researchers in both Brazil and the United States are screening thousands of soybean lines, trying to find genetic resistance that could be bred into soybean varieties suitable for farmers to grow.

According to Brogin it is a possibility that many genes are involved in soybean rust resistance. Presently only four specific dominant genes have been identified as resistant to soybean rust: Rpp1, Rpp2, Rpp3, Rpp4. Behavior of these genes in various cultivars and resistance to soybean rust must be determined in order to combat soybean rust biologically. It is known that the rust pathogen has the ability to break the resistance of the previously identified genes, yet there is still hope for the development of soybean rust resistant cultivars.

The soybean rust project I worked on used microsatellites as molecular markers to better understand the location of these resistant genes within the soybean genome. In order to understand the importance of microsatellites, the concept of linked genes must be understood. Linked genes are inherited together on the same chromosome. They are very important for mapping genomes. Microsatellites are linked to genes within a chromosome – in this experiment, linked to soybean rust resistant genes.

Also known as simple sequence repeats, or SSRs, microsatellites consist of repeated sequences in DNA nucleotides, or base pairs, for example, atatatatatat. These are non-coding sequences of DNA. SSRs are highly polymorphic, i.e., many different forms exist within a population. This allows them to be differentiated and identified more easily, lending them to the use of molecular marking. The researchers at Embrapa hope to use SSR markers to locate these rust resistant genes on the soybean genome.

The SSR primers being used during this research were developed by Cregan et al. (1999). These primers are striped in http://129.186.26.94/SSR.html.

SOYBEAN RUST PROJECT

To begin this research, two cultivars were crossed, FT-2 (resistant to rust), with Davis (susceptible to rust), and an f2 generation was formed. It is known FT-2 has an Rpp gene, but it has not yet been determined which gene. In the greenhouse the f2 generation was inoculated with *Phakopsora pachyrhizi.* When the plants showed indications of infection due to rust, tissue samples were taken. DNA was extracted from these tissue samples through procedures described by Keim et al. (1988). (Appendix A).

This extracted DNA was prepared for PCR reactions, during which the SSR loci are being amplified. (Protocol for SSR Locus Amplification is found in Appendix A). The results of the PCR analyses of the f2 generation are compared to results of the parental generations that were also tested with the same SSR markers. These results give information about the distance of the SSR markers to the resistant gene. The process can best be illustrated with an example:

Two parent varieties were crossed, Davis – susceptible to rust, and FT-2 – resistant to rust. PCR analyses were completed on both the parent varieties using the SSR primers. It was determined that Davis possessed a band with 300 base pairs, and FT-2 possessed a band with 400 base pairs. The f2 generation was analyzed with PCR and tested with the same SSR primers as both parent generations. Each SSR marker amplifies just one locus in the soybean genome. Ideal results of the PCR analysis for the f2 generation in this experiment would display resistant individuals possessing a band of 300 base pairs and susceptible individuals possessing a band of 400 base pairs. This situation implies that an SSR marker is tightly linked to the resistant gene in FT-2. It can

be inferred that the closer the marker is to the gene, the more accurate it is in determining the location of the gene. When more "space" exists between the marker and gene in the parental varieties, there will be more crossing over of the markers during recombination, evidenced by changes in amounts of base pairs in bands from the parental generation to the f2. This will result in a larger distance between the SSR marker and resistance gene, causing greater inaccuracy for locating the gene.

After data from PCR analyses is gathered and SSR markers of particular interest are identified, Mapmaker software is used to analyze the data. It can process the information and return band and phenotypical data, telling researchers which bands come from which parent varieties and which bands are heterozygous. When each individual plant is classified according to the band pattern exhibited and the rust reaction of that plant is taken into consideration, the distance between the gene and that SSR marker can be inferred.

RESULTS

The results of this project are promising. Two microsatellites were identified as linked to the resistance gene and their distances from the gene determined. Thus far the results show SSR markers Satt 307 and Satt 460 as the two microsatellites linked to the resistance gene. Both are mapped in the C2 linkage group. Distance measured in centimorgans, the SSR markers can be visualized as follows:

Satt460------25cM------R gene----13cM----Satt307

Currently, more f2 individuals are being tested with SSR markers in hopes of finding markers that are even closer to the R gene (resistance gene) which would provide for an even more accurate location of the resistance gene. Ultimately, when the location is determined, this resistance gene can be sequenced and identified.

DISCUSSION OF RESULTS

As I was learning the techniques and performing the methods of bioengineering and biotechnology to deal with drought tolerance, herbicide resistance, and soybean rust, farmers were losing soybean yields and money because of these problems. Living in the United States, especially Iowa, it is understandable that the urgency of these crop threats

can be underestimated. I have seen low yields in soybean fields resulting from too little water. Never have I heard of or seen these low yields threatening our food security in Iowa. Certainly the economic situation of the individual farmer is a serious concern, but the fact remains that our overall food situation in Iowa is still secure. The past year I grew increasingly aware of reports about how soybean rust is hurting yields in Brazil and China. I learned that the disease is spreading north toward the United States. I recognized that these were problems requiring attention, but it was not until I was in Brazil that I understood the urgency with which they need to be solved.

The soybean is a naturally nutritious plant. In addition to the high protein content, scientists are beginning to document its other health and nutritional benefits as a human food. Economically, it is quite efficient to produce and has many non-food uses as well. Soybeans are used to make many products, from plastics to paint to biodiesel fuel.

The plant can be used as forage to feed cattle, while the soybean itself is processed and used as a protein source in feeding swine, poultry and cattle. The soybean plays a key role in Brazil's economy. Brazilian farmers lost from 10 to 12 million metric tons of potential soybean yield in their 2004 crop due to rust. The toll of this loss is economic as well as social. As global warming becomes an increasing problem, research on developing bean varieties that are more tolerant to drought is critical. Combining what I learned at Embrapa Soja with images of the poor, hungry and miserable segment of the population of Brazil I see the need for continued research on better ways to grow crops. The economy of Brazil will be impacted by the research efforts at Embrapa. The welfare of all Brazilians, most especially the poor ones, depends on this. In this light, the research at Embrapa Soybean is invaluable.

CONCLUSION

I met many people in Brazil and at Embrapa Soja, which provided a perfect setting to learn more about and discuss topics of global concern. The father in my host family is the Communications Director at Embrapa Soja. The scientists I worked for in the lab were graduate students. I lived and worked with people who are educated, curious and knowledgeable about global issues, whether they are agricultural, political or social. At Embrapa Soja, the scientists have a clear grasp of the implications of their research on

food security. I think this can be attributed to their exposure to the poverty and hunger in Brazil. During my time there, I talked with people about the problem of poverty and hunger in Brazil evidenced by the large population living in *favelas*, which I saw in São Paulo and Londrina. Several times I heard the statement about the people in the *favelas*, "Just because they are breathing does not mean they have a life."

Solving the problems of soybean rust and drought alone will not provide complete solutions to combating hunger either in Brazil or in the world. The ethnic, social, and economic diversity in Brazil creates barriers that the government will have to overcome in order to eradicate hunger and poverty within the country. Obstacles related to culture, education, equitable distribution of wealth, land, and access to food all are involved in resolving this. However, the current President of Brazil, Luiz Inacio Lula da Silva stated on his inauguration day, January 1, 2003: "If, by the end of my mandate, every Brazilian has food to eat three times a day, I shall have fulfilled my mission in life." This statement of national will offers much hope. In addition, Embrapa has committed its resources to participating in the Zero-Hunger Program which will combine government actions and policies in an effort to completely eradicate hunger in Brazil.

On my first day at Embrapa Soja, the Brazilian researcher welcomed me with a paradox. He smiled warmly and shook my hand, and called me an "invader." I have pondered the word "invasion" since that day. My first reaction was to wonder if I represented something negative or if there was something I was not supposed to know or see in Brazil. My experience with the researchers in Londrina led me to believe otherwise. I began to wonder about concerns in America about the potential "invasion" of Brazil. I thought about concerns related to competition in soybean production and resulting effects on soybean prices. I thought about the threat of soybean rust approaching, a future certainty for America. I also thought about all the people I met at Embrapa Soja with connections in America and Iowa. I think about people I am meeting in my first semester at Iowa State University who have connections to Brazil and Embrapa.

I have begun to think of the word "invasion" more in terms of "venture." The most important personal lesson I learned in Brazil was how interconnected we are in this world. The scientists understand the goal of their research. The people who lived

comfortably in Londrina speak with sorrow and compassion about the people who dwell in *favelas*, living but not with a good life. The Brazilians welcomed me, an American, and shared their homes, time, opinions, and research with me. Americans in Iowa have been very curious about what I learned and how it impacts us. Surely, our lives and fates are interconnected.

Likewise, my first images of Brazil were of contrast. Rich colors on cheap plastic, green fields covered by haze, hunger in a land of abundance. I learned that each contrasting part is truly Brazil. So what is the message of the poor woman, limping in an airport and touching the emblem of her country?

I believe her message is hope. She was old and had survived despite obvious disadvantages. Her touch seemed to convey her confidence in her country. After two months at Embrapa Soja, I think I can begin to understand why she feels this way. The energy and effort scientists at Embrapa Soja put into their work bespeaks their hope of improving crop productivity. It is energy and effort that are generated from the understanding of the interdependence involved in the struggle to defeat hunger. They are working to solve the problems of food security within Brazil, and as a result they are helping to solve the problems of food security around the world.

A DIVERSITY OF CROPS IN BRAZIL

Banana Trees

Grazed Lands

An Embrapa Researcher examining wheat in Embrapa's fields

Coffee Plants

Examining soybeans for rust spores in greenhouse

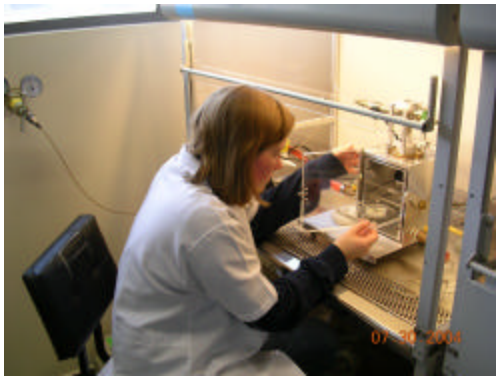
Wheat, peas, red earth of Embrapa Soja

A DIVERSE LEARNING EXPERIENCE

With advisors Rodrigo L. Brogin, Noelle Giacomini Lemos in the Biotechnology Lab

Transferring transgenic plants to the greenhouse

Transferring transformed embryos



Working with the Gene Gun

Progression of rust in soybeans

Symptoms of soybean rust

APPENDIX A

Works with soybean rust (*Phakopsora pachyrhizi*) and Brown Spot (*Septoria glycines*)

DNA Extraction

The DNA extraction of the plants was accomplished according to the procedure described by Keim et al. (1988), with some modifications. Approximately 1g of frozen leaf tissue was sprayed, being proceeded by the transfer of a small part of the sample to microtubes with capacity for 1.5 mL and addition of the extraction buffer [100 mM tris, pH 8,0, 1,4 M NaCl, 20 mM EDTA, 1% (m/v) trimetil N-cetil ammonium bromide (CTAB), 0,1% (v/v) 2-mercaptoetanol] in the proportion of four times the volume of the sample. The solution was incubated at 65º C for 60 minutes, with agitation every 15 minutes. After the incubation, the samples were centrifuged at 2940 g for 10 minutes and the aqueous phase were transferred to another microtube. Equal volume of chloroform-isoamilic alcohol (24:1) was added to the samples, being proceeded the homogenization by inversions and centrifugation at 2940 g for 15 minutes. These last steps were repeated. DNA was precipitated with the addition of isopropanol (2/3 of the volume) and, later, the solution was centrifuged at 16000 g for 10 minutes, being discarded the aqueous phase and added 0.5 mL of 70% etanol. It took place a new centrifugation and the aqueous phase was discarded. The precipitated DNA was diluted in 0,4 mL of 10 mM tris/1 mM EDTA, pH 8,0. To finish, it was added the enzyme RNAse A in the proportion of 40 µg/mL, with incubation at 37º C for at least 30 minutes.

Amplification of SSR Locus and Fragments Visualization

All the SSR primers used in the studies developed at Embrapa Soybean were described by Cregan et al. (1999) and they are striped in http://129.186.26.94/SSR.html.
The amplification of the SSR locus was accomplished in agreement with the methodology described by Akkaya et al. (1995). The PCR reactions were composed of 10 mM Tris, pH 8,5, 50 mM KCl, 1,5 mM Mg2+, 130mM dNTPs, 0,2 µM of each primer, 30 ng of genomic DNA, 1 unit of Taq DNA polymerase, in a total volume of 10 µL. The amplification conditions consisted of an initial DNA denaturation stage for one minute at 94º C, followed by 35 thermal cycles, each one composed by the stages of DNA denaturation for 35 seconds at 94º C, annealing of the primers for 35 seconds at 55º C and DNA extension for 35 seconds at 72º C. After the thermal cycles, a final period of extension at 72º C for one minute was accomplished. The eletrophoresis of the amplified fragments was done in 10 % poliacrilamide gels (29:1 acrilamide-bisacrilamide). The gels were stained with ethidium bromide, visualized under UV light and recorded using the Kodak digital system.