

Embrapa

Soja

**Brasil: The Land of Red Soil and
Warm People**



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Acknowledgements

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Without all these people, this trip would not have been possible, and I thank all of them from the bottom of my heart.

Experience

When I was approached by my biology teacher, Mrs. Kacia Cain, about attending the World Food Prize Youth Institute, I was a little hesitant to be honest. Was it really for me? What would I really learn from it? I finally decided to give it a shot; after all, I didn't have anything to lose. At the YI I was amazed by what I experienced. I found it to be more than I expected. It was such an incredible sight to see so many people from different backgrounds come together to try to defeat world hunger. The speeches I heard were inspiring and made me want to make a difference. When I heard about the Borlaug-Ruan International Internship program, I was determined to be a part of it.

The day the letter came was the beginning of a new chapter of my life. That was it, the letter I had been working so hard to get for the longest time, and it finally happened. I can still feel those words in my bones: *You have been selected to be a 2005 Borlaug-Ruan International Intern*. I knew that this would be the start of something completely different, something that I have never experienced before, but I never expected for it to change my life.

I was born in Bangkok, Thailand on May 9, 1987. My parents had fled Vietnam and were staying at the camp until we had permission to go to the United States. I was too young to remember anything from the camp, but I know that it was a hard time. Although I was there physically, I wasn't aware of all of the poverty and hardship that was present. Two years later my family and I immigrated to the United States. From then on, I have grown up in Des Moines, Iowa in a sheltered world, not knowing anything outside of my protected little circle.

When I was informed that I would be interning at Embrapa-Soja in Brasil, I was ecstatic. I heard all the stories from the previous intern, Catherine Swoboda, and they sounded amazing. She had learned and experienced so much; would I be able to do all that too? I looked up Brasil on the internet, looked at numerous pictures, found out as

much as I could on Embrapa, and tried to learn a little bit of the language and culture. I asked Catherine about what to expect, but when I arrived in Brasil, nothing could have prepared me for the breathtaking experience I was about to embark on.

The night before my flight was hectic. I was leaving for two months. Everything that was familiar to me was going to be 6,000 miles away. I looked around my room to make sure I didn't forget anything. After I made sure I had everything that I needed, I slept in my bed one last time before I left it for the next two months. As I drifted off into a deep slumber, my thoughts wandered to the land that I would be living in for the summer and the fantasized about the experiences I would have.

The next morning was a bittersweet one. As I loaded all of my things into the car, I was excited, but also nervous. I was looking forward to this wonderful adventure into a foreign land, but I would also miss all of the comforts of home. I had already been contacted by my host mother, Ivania, and we exchanged a few emails, not knowing what to expect of each other, but looking forward to the day we would meet in person. Will we be able to communicate? Will her family be able to understand me? These questions and more were circling in my head non-stop.

The ride to the airport was an emotionally charged one. I made my last round of phone calls to say goodbye to all of my friends and I paid more attention to all of the landmarks in Des Moines that I usually don't even notice and take for granted. I've lived all my life in Des Moines and now I was venturing to a land that was not just far away, but had a completely different culture and way of life. Would I be able to adapt?

I had learned to appreciate this trip more than I ever expected before I even left for it. When I applied for my Brazilian Visa, I was informed that I couldn't obtain one because I didn't have a U.S. passport. Since I wasn't a United States citizen, I didn't qualify for a passport, but I was a legal alien so I did have my green card. After a frustrating phone call with the Brazilian Visa Service agent, I hurriedly called Lisa Fleming, our Youth Programs manager, and explained the situation as devastation crept

over me. After a lengthy talk on the phone, Lisa and I worked out a plan. The next afternoon I went down to Lisa's office to fill out the rest of my visa application and also an application to obtain a Refugee Travel Document. We later found out that the travel document usually takes about a year to process. When I heard this news I thought that it was all over. The journey that I had worked so hard to achieve was slipping right through my fingers, right in front of my eyes. Although I was heartbroken and thought that this was the end of my journey, Lisa informed me that she might be able to expedite the process. The next day Lisa made a few phone calls and was able to find someone to help our case. She had spoken to someone who worked for the state legislator's office that would be able to help us out. After a couple weeks of anxious waiting, we were informed that my travel document was processed and on its way home to me. Although Lisa and I were ecstatic, we didn't waste any time. As soon as the document came into my possession, I went down to Lisa's office to make a copy of it and then send it off to the visa service along with my visa application. Now all we could do was wait and see if the Brazilian Consulate would accept my travel document in place of a U.S. passport. Lisa yet again had another trick up her sleeve. She spoke with Mr. Frank Fogarty, the Brasil Year Committee Chair for the Des Moines Area Community College, who just happened to personally know the Brazilian Chief Consulate. After a few phone calls and a week of anticipation, my travel document was on its way back, with my Brazilian Visa stamped inside, just in the nick of time, two days before my scheduled departure. I had to go through so much extra work to get my necessary travel documents and the fact that my entire trip hung from a string made me really appreciate the magnitude of the opportunity that I was being presented with.

As the plane got ready to land in São Paulo, I looked outside my window and just took in all of the landscape. The soil was unusually red and there was plenty of open green space. As we got closer and closer to the airport the landscape started changing and I saw cities with large buildings and even skyscrapers. I could see that the land had been influenced by American culture as I spotted a McDonald's sign in the distance. When we touched down, I became anxious, and I was ready to barge out of the plane door and go explore the airport's sights and sounds. As soon as I stepped out of the tunnel and into the

airport corridor I got an immediate adrenaline rush. There I was, on Brazilian ground, staring up at a sign that exclaimed “Bem-Vindo Ao Brasil”. The moment I looked around I realized that I was surrounded by all different cultures and ethnicities. I expected everyone to have a bronzed hue to their skin and dark hair and eyes, but what I saw instead surprised me. Mixed among the apparent “Brazilians” were Chinese, German, Japanese, Swedish, Indian, African American, and many more. This threw me aback for a second. Was I really in Brasil? I never realized that Brasil was such a melting pot of cultures, just like the United States. I was expecting to feel completely out of place, but instead, I felt as if I fit right in, that I belonged here.

The crowd hurried along, so without a second thought, I followed them, trying to blend in and look like I knew what I was doing. The crowd led me to the customs station where we went our separate ways into lines of “Brazilian Citizens” and “Foreigners”. When customs was done inspecting my travel document, I shuffled along trying to keep up with where everyone else was going and they eventually led me to the baggage claim. As I waited for my bag to circle around, I looked at the advertisements on the walls. They boasted famous coffees, cell phones, and duty-free shops. Excitement coursed through my body. I was really in Brasil. Once I got my bag, I started on my journey to find my check in gate. As I was wandering around, a woman walking near me turned to me and asked, “Onde é o banheiro mais proximo?” I looked at her with a bewildered look on my face. Had I known that she had just asked me where the nearest restroom was I would have replied with “Não sei”, “I don’t know”, instead I told her, “Eu sou Americana, não falo português”, “I am American, I don’t speak Portuguese”. After she let go a little chuckle, she gave me a warm smile and walked away. As she walked farther away, my face grew redder by the moment. She understood me! I was so excited by this little incident that a big smile appeared on my face as I continued on my way to find my check in gate.

After a long wait, I boarded my plane to Londrina, and I was on my way to my new home for the next two months. When we landed, instead of stepping out of the plane into a terminal, I set foot on the airport ground, instantly engulfed by the warm, humid

air. I looked around and saw the landscape blanketed by the rich darkness and brightly gleaming stars, a sight that I seldom get to see in the crowded brightness of Des Moines. I started walking towards the tiny airport, made my way through the sliding glass doors, and found myself standing right in front of the baggage claim. I anxiously waited for my bag and once I retrieved it, I turned to my left and walked out another set of sliding glass doors into the lobby of the little airport. As soon as I set foot into the lobby I spotted a familiar face. Standing a few feet in front of me was Ivania, my host mother, and her husband Sidney and daughter Carol. “Oi, tudo bem?” Ivania asked as she beamed at me. “Tudo”, I replied, and I kissed her on both cheeks, and I did the same with Sidney and Carol. Ivania pinched my cheek and chuckled. Carol explained to me, “In Brasil, one kiss, not two.” And so, having only stepped off the plane five minutes ago, I got my first culture lesson, and definitely not my last.

As I climbed into the cab of their Chevy Blazer, I noticed all the cars around us were a little odd looking. I soon realized that they were all European models, and most of them had manual transmissions. We lumbered along with the quiet hum of the Blazer in the background as I took in all the sights and sounds of this strange new place that I was in. I noticed lavish restaurants and shops intermingled with salvage yards and run down buildings. “Is Londrina a rich city?” I asked my family. They looked at me. “I don’t understand,” replied Carol. I tried again. “Londrina, has many money?” I asked as I made some hand motions for “money”. They seemed to get this question. Sidney replied this time. “In Londrina, many money. Many people live Londrina has many money, many people has little money, no money.” Over the next couple of months, I would come to realize the magnitude of this statement.

Londrina is a city that is considered by many in the country as well off, but there is a vastly uneven distribution of income. This became apparent to me when we were stopped at the next stop light. A man in his mid-forties came up to each of the cars stopped and tried to sell some fruit. His features were weathered and his eyes looked tired. I wondered to myself whether or not this was a common occurrence. My curiosity must have given me away because Ivania said, “Many people say for you to buy.” I knew

instantly what she was trying to tell me. While many people in the city were plagued by hunger and famine, others were living comfortably in their homes. This kind of uneven distribution of income created an impoverished community seemingly unable to recover from the plight of hunger, but as the Brazilians say, “Eu sou brasileira, e não desisto nunca”, “I am Brazilian and I never quit”.

The next morning I began my work at Embrapa. My supervisor, Dr. Ricardo Abdelnoor showed me around the center and the labs that I would be working in for the next two months. I had been assigned to the Laboratories of Genetic Expression and Molecular Markers. Dr. Abdelnoor informed me that for the first week or so I would just be observing and learning techniques, and then after that I could jump in and help out with a project. During the next week I basically followed around the different people in the lab and observed what they did, what kinds of techniques were done, and just the basic schedule of the day. It wasn't until a couple weeks later that I was told that I could help Magda Beneventi with her research. So it had been settled. I would be helping Magda with her research on soybean stress and drought tolerance.

While I was in Brasil I had the opportunity to visit Foz do Iguaçu and the Cataratas do Iguaçu, or Iguaçu Falls, which is on the border of Brasil, Argentina, and Paraguay. Iguaçu Falls was a completely different experience from Londrina. The city, Foz do Iguaçu, was mainly a tourist town, but it still had the quaint charm of Londrina. The waterfalls themselves were beautiful and breathtaking. The word Iguaçu means “great water”, and rightfully so. The waterfall system consists of almost 300 individual waterfalls. Although most of the waterfalls are actually on Argentina's side, a more spectacular panoramic view can be seen from Brasil's side.



My family and I walked the trail down to a bridge that sat right at the edge of one side of the falls. We were standing in the Garganta del Diablo, The Devil's Throat, and we were actually feet away from the plunging edge of the falls and right up next to a thundering wall of water. I felt like I was standing at the gates of heaven as I cautiously



peered over the edge of the bridge and into the depths of the water below. The trip to Iguazu Falls was amazing and breathtaking. I never knew that a place with such beauty and power existed. The falls

really opened my eyes to my world around me, made me see the big picture, and gave me a greater appreciation for nature.

During my stay in Londrina I found myself not missing the things that I thought I needed. Instead of wanting my cell phone, computer and MP3 player, I missed my family, my friends, and my boyfriend. I realized that life doesn't revolve around the possessions you own, the kind of house you live in, the car you drive, the food you eat, or who you know. What is important is the kind of character you possess, the kind of person you are, and how you maintain the relationships in your life. I went to Brasil thinking that I already had a good sense of self. I thought knew who I was, and this trip wouldn't change who I was inside, but I was wrong. Brasil wasn't just a summer trip for me. It's a trip that will stay in my heart until the day I die. The things I saw in Brasil will stay with me forever and remind me everyday that I am incredibly lucky to have everything that I do. I found that I came back from Brasil with a great appreciation of nature and simple living. I was also more aware of the people around me and my surroundings. Brasil truly retaught me the meaning of the old elementary school slogan, "Treat other people the way that you want to be treated."

Background

The soybean was introduced to Brasil in 1882 in the northeastern state of Bahia. Starting in 1900 the soybean was a minor crop of Brasil, only grown on a small-scale, being used solely for hay production to feed dairy cattle and bean production to feed pigs. Brasil didn't realize the potential benefits of large commercial production of soybeans until the 1960s. In the mid-1950s a government decision was made to increase wheat production. A summer crop was needed to follow the wheat-growing season. Since they return nutrients into the soil and allowed farmers to use the same farm machinery for production, soybeans were chosen to follow the wheat season.

In the 1970s, soybeans became Brasil's leading primary crop. Being able to capitalize on the similarities in climate and methods of production, Brazilian soybean producers were greatly aided by American agricultural expertise. Brasil'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 Brasil.

In 1975 Embrapa Soja, (Embrapa Soybean) was established. 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."

At Embrapa I worked on soybean drought tolerance with Magda Beneventi. Magda had earned her undergraduate degree in biology at Londrina State University and she was currently working on obtaining her Masters degree. Since soybeans are such a valuable crop in Brasil, research in this area is ongoing. Because of its high protein content, there is virtually no area in the world that would not benefit from soybeans, but

areas of the world that are arid or have unstable climatic conditions are unsuitable for growing today's varieties.

Although my previous experience with biotechnology was limited, I found that it greatly prepared me for my work at Embrapa. As a junior in high school I was able to take a biotechnology class that taught me the basic building blocks to genetics and genetic engineering. The summer of my junior year I worked at Pioneer Hi-Bred International as an intern in the Seed and Crop Technology department. At Pioneer I learned a lot about corn and corn hybrids, obtaining research, and doing field work. I thoroughly enjoyed both of these experiences and I believe that they helped me tremendously with my work at Embrapa.

The soybean plant is sensitive to environmental stresses. When a situation such as drought strikes, yield and economic losses can be devastating to growers. My work with Magda used genetic engineering to try to solve the problem of drought tolerance. 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 fairly quickly, speeding up the entire experiment.

Methods

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 that were used were of the Conquista variety. First, the seeds were sterilized with a 70% alcohol solution and then placed in a 1:1 solution of H₂O and sodium hypochlorate. After the sterilization, the seeds were placed in a container of water and allowed to sit overnight. 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. The next day embryo extraction was performed. The embryos were extracted from the seeds using sterilized tweezers and a scalpel. The tweezers were used to keep the seed in place while the scalpel cut the seed in half, thus exposing the embryo. These extractions are performed in petri dishes in the presence of water. It is a slow process, but it is vital that the embryos are not damaged during transformation. Once the embryos are removed from the seed, they were dried on filtration paper in an autoclave machine, and placed in small glass plates containing phytigel, an agar substitute used as a growth medium for plant tissue. The embryos were positioned in the phytigel 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, DNA of interest must be prepared.

The focus of the project was attempting to develop transgenic plants containing the drought-tolerance gene DREB. Tungsten microparticles are used by the gene gun to transport the DNA into the embryos. The tungsten microparticles, which tend to stick to each other, are prepared by placing them for 15 minutes in a sonicator to spread and separate them. After 15 minutes, 50uL of DNA, 50 uL of CaCl₂ and 20uL of Spermidem are placed on the microparticles. The CaCl₂ and Spermidem help bind the DNA to the microparticles. The mixture is centrifuged for 10 seconds to eliminate the supernatant. Afterwards, the microparticles are washed with 150uL of 100% alcohol solution. Then the centrifuge process is repeated with 24uL of alcohol. Now the prepared mixture

containing the DNA microparticles is spread on sterilized membranes. 3.2uL of mixture is spread on each membrane. 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.

Soybean embryos are then placed in the gene gun in the direct path of the accelerating microparticles. 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 and enter the embryos at the nucleus and are introduced into the genome.

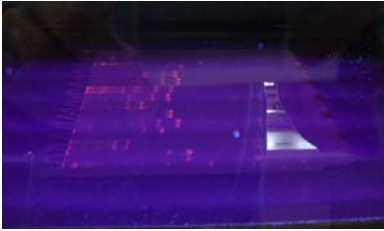


After being shot, the embryos are placed in a phytohormone called BAP (benzene aminopurine) in the presence of cytokine, a growth inducer. The embryos are kept within the phytohormone overnight while the cytokine induces growth of the meristematic section of the embryo. The following day the embryos 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 for 45 days. After 45 days, 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 for 15 days. Throughout the duration, plants are watered with a nutritive medium. After another 15 days in the growth chamber, plants are transferred to the greenhouse where they remain for another 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 cover



the plants for 7 more days then the bags are completely removed and the plants are left to grow in the greenhouse for a month.

After the month of greenhouse growth is up, Polymerase Chain Reaction, PCR, is done 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 was 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 DREB and the gene's presence within the samples is 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 and a stack of paper towels is placed on top of 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 later used to get an accurate quantification of PCR products.

Results

As I was nearing the end of my internship, Magda and I found that there were two plants that had been successfully transformed. Although we had indeed found good results, I was at the end of my stay. The results were found just two days before I was to return home. Magda and I spoke about the potential next steps that were to be taken. Magda and her advisor, Dr. Alexandre Nepomuceno discussed using Real Time PCR to determine an accurate amount of gene expression in the plants. The last time I was in contact with Magda, she informed me that they had started using Real Time PCR analysis with the samples but there was some sort of contamination in the samples or the solution. They continue to work to find the source of the contamination.

Conclusion

Drought can cause tremendous amounts of devastation to farmers and severely damage their yields. 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 Brasil'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 the potential of biotechnology in soybeans is reached, it will dramatically increase yields and help feed millions of people around the world.