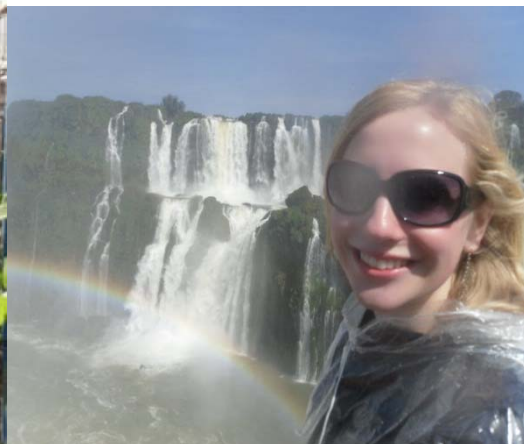


The Summer of Soybeans



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THE WORLD FOOD PRIZE



INTRODUCTION

My Story

Almost exactly a year ago, I had also just had an amazing summer adventure. August of 2009 I took my first trip to a Third-World country, Guatemala. It was while I was there that I first experienced hunger in a personal way. The experience was eye-opening, and I carried home with me a new passion to make a difference.



Guatemala, 2009

At the time, I was also working on a summer assignment for the start of a new year with the New Visions: Life Sciences program at Cornell University. The assignment was to write about a possible solution to food insecurity, but I had no idea at the time how this was connected to the World Food Prize. I ended up writing my essay about providing rural farmers in Third World countries a source of credit by securing their property rights, inspired by the stories and sights of landless farmers and families in Guatemala. My research for the essay had led me to books like The End of Poverty by Jeffrey Sachs and The Mystery of Capital by Hernando de Soto. The more I read about hunger and poverty in the developing world, the more interested and outraged I became.

My first few weeks in the New Visions program continued to reinforce my new interest in food security, as well as introduce me to the world of agriculture, which I had had little experience with. When I decided to attend the New York Youth Institute, I was not thinking about Iowa, the Global Youth Institute, and especially not the internship. In fact, it wasn't until they showed the Global Youth Institute video and called my name to say I was selected to go to Iowa that I realized I was about to have a life-changing experience.

The October 2009 Global Youth Institute was one of the greatest surprises of my life. I had not known what to expect when I arrived, and I ended up leaving a changed person. The three days I spent in Iowa altered my perspective dramatically and caused me to rethink many parts of my life, including what I wanted for a career. The most influential part for me was to see how much of a role science, a subject I had always loved, could play in food security and making the world a better place. Talking to real-life heroes, like the 2009 World Food Prize Winner Gebisa Ejeta and Kids Against Hunger founder Marcie Proudfoot, proved to me that one person can make a difference in this world. It was inspiring to be even just remotely connected to a community of people so dedicated to improving the lives of others around the world. And I knew it was a community I wanted to join.

When I got home, I didn't want my interest or excitement to fade away. I started reading everything I could find. I was especially inspired by the book Enough: Why the World's Poorest Starve in an Age of Plenty by Scott Kilman and Roger Thurow. The more I read, the more outraged and involved I became, and the more I realized I had to learn about agriculture. Once a week, I brought home a stack of books from my New Visions teacher about international agriculture and solutions to world hunger. I searched for knowledge about farming and food through movies, conferences, and the FFA, which helped me to gain a better picture of the role



With Dr. Gebisa Ejeta
at the Global Youth
Institute.

of agriculture in nearly everything we do. When I was presented with the opportunity to work with a researcher at Cornell, I knew I wanted to do something that could be related to food security and agriculture. My work with Arbuscular Mycorrhizal Fungi and its impact on food productivity was a great introduction into research and how it can relate to pressing world issues.

But through all this, I knew that a summer as a Borlaug-Ruan Intern would truly be the ultimate learning experience. I had still had so much to learn, and I saw the internship as a once-in-a-lifetime experience where I would be able to experience a new culture, broaden my worldview, gain knowledge of agricultural science, and explore my passions. The images of the interns had stayed with me from the Global Youth Institute and I knew I would regret it for the rest of my life if I didn't give it a shot and decide to apply.

And so, I found myself on a third plane this year. Not to Guatemala or Iowa, but to Brazil—a country whose economy is largely based on agriculture, a country with many problems and much poverty, but a lot of people working on solutions too. And I couldn't wait.

Brazil

Brazil is a big country. I learned this the hard way when I traveled to Southern Brazil for a seminar, and the climate changed so dramatically I nearly lost my toes to the cold. But whether I was near the gently rolling golden hills of wheat outside Londrina or the green mountainous forest of the southern regions, one thing remained constant. Agriculture was everywhere.

Brazil is an interesting and unique country because it is a Third World country that is on the rise, and it is doing so in an entirely unconventional way. Unlike the development trends of other countries, Brazil's progress is still largely agricultural based. And Brazil is a country that is looking to the future. Instead of investing in carbon based fuel systems, Brazil is looking towards solar, hydro, and crop based fuel technologies. Everywhere I looked I saw evidence of a developing green country. Whether it was the ethanol available at the gas pump, the recycling system, or EMBRAPA's research into sunflowers and soybeans as alternative sources of fuel, Brazil was an inspiring and thought-provoking place.

Of course, like most things, Brazil has a great deal of problems. My two months in Brazil opened my eyes to issues of unequal distribution of wealth, government corruption, class and social issues, and a magnitude of poverty I had never seen before. But Brazil is a country rich in natural resources, resourceful people, and innovative ideas. And I believe it is agricultural science that will serve as the factor that links those three things and make Brazil a place that could change food security around the world.



RESEARCH:

EMBRAPA: The Place, The People, The Purpose

In the last thirty years, Brazil has transformed into an incredible agricultural force. Brazil, which was once considered a food importer, is now in league with grain exporters like America, Canada, Australia, Argentina, and European Union. The rise in Brazil's farm production has been astounding. In ten years, from 1996 to 2006, the total value of Brazil's crop production increased 365%. Brazil is now the world's largest exporter in beef, poultry, sugar cane, and ethanol. The increase in Brazil's soybean production has been a large part of this success. Brazil is currently second in soybean exports, right behind the U.S. The amazing part of this is that Brazil has accomplished all this with little government subsidies and without deforesting the Amazon. How was this possible? Brazil is incredibly rich in natural resources; it has more free-farmland and more water than any other country. But the major reason Brazil has experienced such remarkable growth in agriculture is because of EMBRAPA.



The government-run EMBRAPA, or Brazilian Agricultural Research Corporation, is made up of over forty different research centers across Brazil, ranging from EMBRAPA swine to EMBRAPA tropical fruits. It was founded in 1973, when high oil prices were putting pressure on Brazil's agricultural subsidies program. Since that time, it has literally renovated Brazilian agriculture. One of the biggest projects that EMBRAPA was involved in was changing the Brazilian savannah, or cerrado, into farmable land. Norman Borlaug himself told the New York Times that "nobody thought the cerrado was going to be productive." Using methods like lime and rhizobium addition to the soil to reduce acidity and increase nutrient levels, the cerrado has been transformed and now accounts for 70% of Brazil's output. EMBRAPA also brought back the grass *brachiaria* from Africa, which serves as a grass feed on the cerrado, allowing for the increase in Brazil's beef production. EMBRAPA has also recently been at the forefront of no-till farming methods and agriculture and livestock integration. Yet, the most important thing EMBRAPA has done for Brazilian agriculture has to do with its work with the soybean. More specifically, this work has been done by EMBRAPA Soja (soybean), the research institute I was placed at, located in Londrina, Brazil.

Soybean is a temperate-climate crop, native to north-east Asia. All other big soybean producers, like Argentina and the United States, have temperate climates. But Brazil, excluding its southernmost states, has a tropical climate. It was EMBRAPA Soja's work with crossbreeding that turned soybeans into a tropical crop, allowing them to grow on the cerrado and other areas of Brazil. EMBRAPA Soja has also created varieties of soybean more tolerant to acid and with faster growing periods, allowing for increases in yields for farmers across Brazil. EMBRAPA Soja is becoming very involved in the development of genetically modified soybean seeds. Brazil itself is the second-largest user of GM soybean after the U.S. Just this year, EMBRAPA Soja was granted approval for the commercial development of its first GM variety, a line of soybeans resistant to a new class of herbicides.

EMBRAPA Soja was established in 1975 with the mission to "provide competitive technological solutions for sustainable soybean development through generation, adaption, and transfer of knowledge and technologies, for the benefit of society", with objectives "to provide technological solutions that contribute to decrease social unbalances; to provide technological solutions that contribute to improve nutrition quality for human population." Its work with the soybean in the last few decades, as described, has been revolutionary. Brazil accounts for approximately one-third of the world's soybean exports. In twenty years, its soybean export has risen from 15m tons to over 60m. On just 6% of its arable land, Brazil supplies one quarter of the world's soybean trade. EMBRAPA Soja is continuing to work towards making improvement in soybean production. In the Biotechnology Lab of EMBRAPA Soja, where I worked, research is currently focusing on producing genetically modified soybean varieties that are more resistant to drought and flood. I worked on the development of drought-resistant soybeans under the guidance of Alexandre Nepomuceno, Renata Fuganti, and Silvana Rochenbach. Alexandre Nepomuceno received his PhD from the University of Alabama, and is currently the project coordinator of EMBRAPA's scientific cooperation project with Japan.

Drought Resistance Soybeans: Significance for Brazil and the World

Though Brazil has had incredible success with the soybean, it is still facing some major issues. Drought is one of the largest contributors to soybean production loss in Brazil, and losses are only expected to climb with continuing climatic change. In the 2004/2005 seasons, the southern states of Brazil, which are responsible for 40% of national soybean production, lost more than 25% of their crop yield to drought. In Rio Grande do Sul, losses were over 70%. In 2008/2009, losses had a direct cost of 2.7 billion U.S dollars. It is small soybean farmers, especially from the southern states of Rio Grande de Sul and Parana, who suffer the most from these losses. Financial difficulties often force them to move north, contributing to issues of deforestation. And losses are only predicted to increase. UNICAMP projections suggest that agricultural losses could surpass US \$4 billion annually by 2020 because of increasing temperatures, with half of the losses in soybean. The development of GM drought-resistant soybeans would help to combat these harmful losses and provide financial and economic benefits to both small and large producers. Such technology could spread to other developing countries, like Africa, who has similar land to Brazil and also suffers serious losses due to drought.



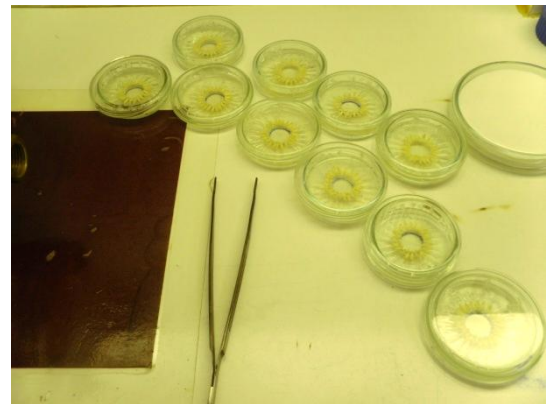
What is DREB? The Japan-Brazil Project

DREB, which stands for dehydration-responsive element binding factor, is a family of transcription factors, or DNA-binding proteins that is part of the AP2/ethylene-responsive element binding protein family of DNA binding proteins (AP2/EREBP). DREB proteins, which act as *trans*-acting elements, bind to DRE, a *cis*-acting dehydration responsive DNA regulatory element. This signaling process results in the induction of downstream genes involved in drought resistance and initial stress response in the plant, primarily through protecting cellular structures during dehydration. Responses can include stomata closure, repression of cell growth and photosynthesis, and activation of respiration. It is the DREB1A and 2A genes which encode for these DREB transcription factors, which in turn activate other genes. Overexpression of DREB results in the introduction of stress-tolerance genes under a non-stressed environment, improving the plants tolerance to drought.

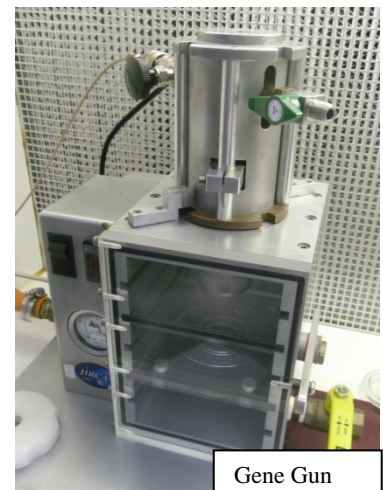
In 2007, EMBRAPA joined with JIRCAS, the Japan International Research Center for Agricultural Science, in the effort to develop a drought-resistant line of soybeans. Japan creates plasmids with the DREB construct and ships them to EMBRAPA. EMBRAPA and JIRCAS also share researchers. There were several people at EMBRAPA from Japan, and several of my friends in the lab were preparing to travel to Japan to work.

Overview of Process: Plasmids from Japan to a Final Cultivar

When plasmids arrive at EMBRAPA Soja from Japan, the research laboratory performs a plasmid digest to check that the construct is present (the plasmid is broken up into pieces using restriction endonucleases and a gel is run to check that the number of base pairs is correct). Then, *E. coli* are made electro competent, an electric shock disables the bacterial membrane and the plasmids are incorporated into the bacteria. The bacteria multiply, and the plasmids are replicated. The plasmids are then purified from the bacteria using a process known as Mini-Prep. One of two methods, either the bioballistics or agrobacterium method, is then used to transform a soybean embryo. My research mainly focused on the bioballistics method. Here, soybeans are sterilized and the embryos are extracted. The embryos are then set up and placed inside a gene gun, where plasmid DNA-coated microparticles are shot at the embryos. The gene gun creates a vacuum and the embryos are bombarded with the microparticles at a speed of about 1500km/h. Shot embryos are then placed in the phytohormone BAP (benzylaminopurine) overnight with the presence of the growth inducer cytosine. Embryos are then transferred to a MS agar medium and placed in the growth chamber. After 45 days, the developed embryos are transferred to a sand and Vermiculite mixture (1:1) and left in the growth chamber, where they are watered with a nutrient solution. The soybean plants are then transferred to the greenhouse, where they grow to full maturity. Here PCR is used to test which



Extracted soybean embryos arranged in preparation for the gene gun.



Gene Gun

plants are positive for the gene. This process involves performing DNA extractions for each of the plants and running a PCR gel. The resulting gel is then compared to the known DREB bands, and positive plants can be determined. Positive plants are then autocrossed many times, creating several generations. Each generation is tested to see that the plants are positive for the gene. Greenhouse drought experiments are also performed. When the line is developed enough, experiments are moved to the EMBRAPA fields, where data is collected on the plant's reaction to drought-stressed conditions. EMBRAPA's breeding program then works with these things to create a final cultivar which can be sold commercially.

Though I was mainly involved in the DNA extraction and PCR analysis portion of this process, I was also able to work with the bioballistics method of transformation, as well as medium preparation and soybean maintenance.



History of the P58 Line

It started with a mistake. One weekend, three years ago, a worker had been in the greenhouse and accidentally turned off the watering system. When Alexandre Nepomuceno came in that Monday and saw all his newly transformed soybean plants, after a weekend without water, wilted and shriveled, he was furious. The worker had most likely ruined months and months of work! After writing a letter of complaint and fuming in his office, Alexandre went back to assess the damage. This time when he visited the greenhouse, he noticed something. There was one plant that stood out among the others. As Alexandre said, it was “smiling up at him”, healthy, green, and tall among a crowd of shriveled up soybeans. The repairman's mistake had inadvertently saved months and months of work by inducing a drought circumstance and highlighting a soybean plant that had been successfully transformed with the DREB1 gene!

The plants in the greenhouse were of the BR16 variety (a common non-transgenic commercial variety in Brazil) that had undergone the bioballistics method of transformation in an attempt to introduce the DREB1A gene. As it turns out, that one surviving plant would be the starting point of the P58 line of DREB1A soybeans, one of the first and most important lines to be identified as positive. This line of soybeans with the DREB1 gene has now been tested physiologically, agronomically, and molecularly. Plants were tested first in greenhouse conditions and then under field conditions. An unlucky season with large amounts of rain altered the field results, but in the greenhouse the plants showed improved drought tolerance. Though there is still an intimidating amount of research left to do and problems to solve, it is the P58 line that shows the most potential of becoming a cultivar that can be sold commercially.

My Project: Work with Crosses

Introduction:

One of the biggest problems with the P58 line has to do with the number of inserted genes. When the bioballistics method of transformation is performed, there is no way to control how many copies of the gene are inserted into the genome of the soybean, or where in the genome they will be inserted. Unfortunately, most of the P58 plants have between two to three copies of the gene, and sometimes in unstable areas of the chromosome. This can cause problems for breeders later. When the plants have those extra copies, it can lead to genetic silencing and deletion of the gene later on when the plants are multiplied or crossed with other varieties.



Recently, a cross was performed in an attempt to create segregation lines that will result in a plant with only one copy of the DREB1 gene. P58 plants have been crossed with BR16 soybeans, the original non-transgenic variety. This cross is very strategic, because, except for the one gene difference, BR16 and P58 have the same genetic background, leading to fewer complications in the crossing process. The goal of this cross is to create at least one plant with only one copy of the DREB gene in one stable location. This one plant can then be autocrossed to create a cultivar where the plants have only one copy of the DREB1 gene in a stable region of the chromosome.

The P58 line and its drought resistance capabilities are a huge accomplishment, but this project, to create a line with only one copy of the gene, represents the crucial next step in order for drought resistant transgenic soybeans to make it to the commercial stage.

My Project:

My work at EMBRAPA involved the third generation of the P58XBR16 crosses. The second generation was grown in the greenhouse, and underwent drought conditions for thirty days. The ones that died were thrown away. When I arrived at EMBRAPA, it was time to first test the remaining 111 plants of the third generation to make sure they had the DREB1 gene. From there, it will be time to test which of the plants positive for the DREB gene have only one copy.

During my internship, after a few weeks of working with the bioballistics method and plant transformation processes, I was put in charge of testing which of the crossed plants were positive for the gene. This was a very important part of the experiment, and I was honored and excited to be in charge. Renata, my project coordinator, also had some other DREB1 lines that she wanted me to test along with the crossed plants. This included P3069 (1-10), P1378 (1-10), P59 (1-10), and P1142 (1-10).

Hypothesis:

We hypothesized that 75% of the plants would be positive for the gene. We concluded this based on our knowledge of genetics and the lab's past experiences with soybean crosses. Though we were expecting 75%, 100% positive plants was the goal.

Materials and Methods:

Collecting Samples

Each plant was grown in an individual pot in a vermiculite and soil combination (with a ratio of 1:1). To collect a sample, I used scissors to cut a small piece of a leaf off the plant. I tried to collect samples from young leaves because they have fewer proteins that can potentially get in the way during extractions and PCR. The sample was put in the corresponding labeled eppendorf tube. We had tubes numbers 1 through 112 for the crosses (with 111 as a skip because it was dead), P3069 numbers 1 through 10, P1378 numbers 1 through 10, P1142 numbers 1 through 10, and P59 numbers 1 through 10. The tubes were kept on ice while we collected in the greenhouse. The scissors were wiped clean between each collected sample. Once the samples were collected, the racks with the tubes were wrapped in plastic and kept in the -80° Celsius freezer until we were ready for DNA extraction.

DNA Extraction

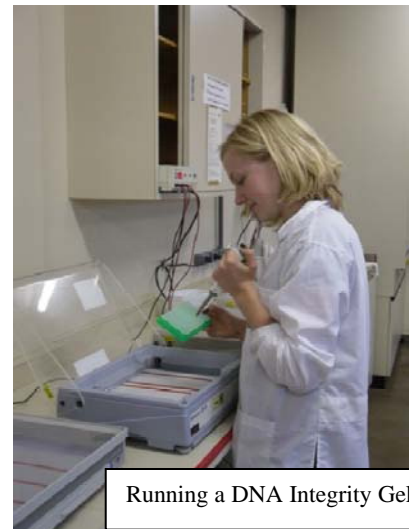
Because we had so many samples to process, we used a slightly revised version of EMBRAPA's regular DNA extraction protocol. Before starting the process, we labeled a second set of tubes with the same numbers (described above) and added two Teflon white grinding balls to each sample tube.

Prepare a Gel: See Appendix A

DNA Integrity and Applying a Gel: See Appendix B

PCR: See Appendix C

Gel Electrophoresis and Viewing Results: See Appendix D



Running a DNA Integrity Gel



Viewing Results of Gel



Performing PCR

Experience:

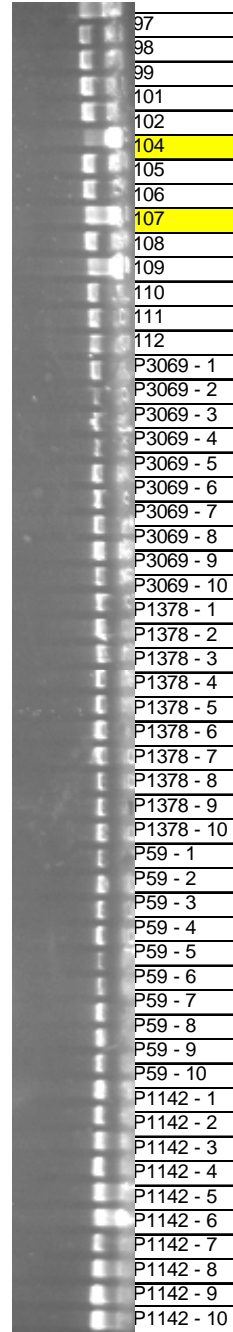
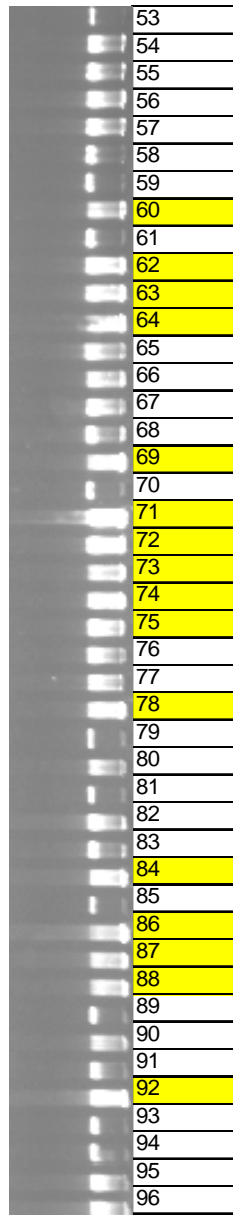
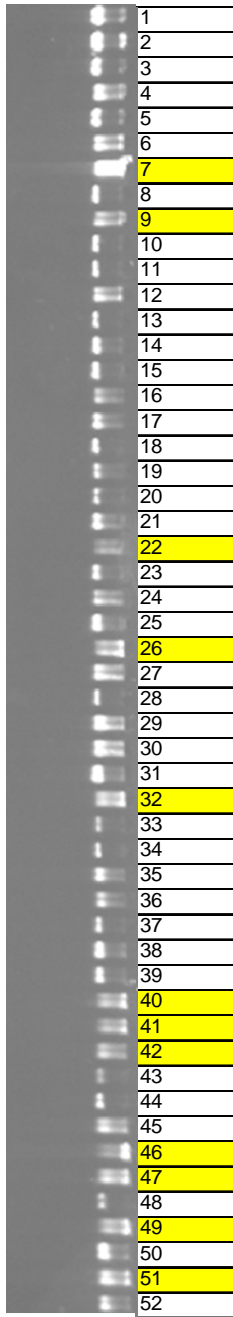


Figure 1: Gel Electrophoresis of DNA Integrity. Samples were collected from all of the crossed plants and the DREB1 lines. DNA extraction was performed. A 2% w/v agarose gel was run and the results were analyzed to determine for which samples the DNA had degraded and the extraction needed to be performed again. The highlighted samples were determined to be degraded. (Note: Sample #100 was lost during the extraction process and needed to be repeated.)

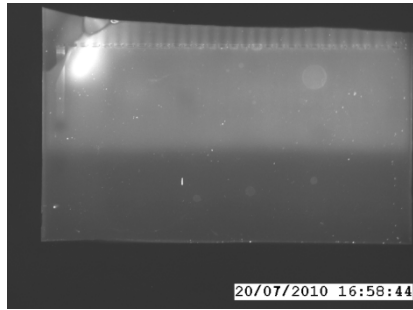


Figure 2: Gel Electrophoresis of DNA Integrity for Samples where Extraction Failed. DNA collection and extraction was repeated for the highlighted samples. A 2% w/v agarose gel was run to view the results.

The gel was blank, meaning the extraction had failed.

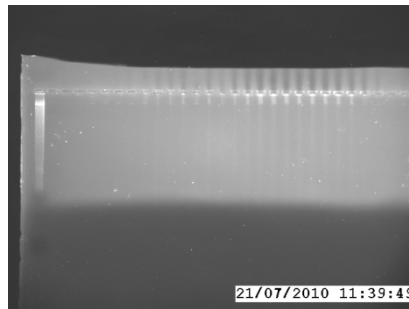
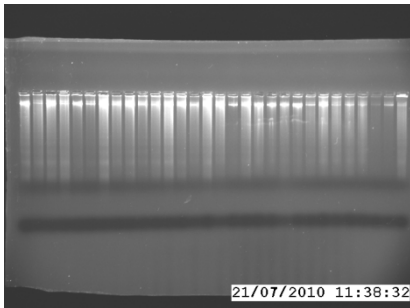


Figure 3: Gel Electrophoresis #2 of DNA Integrity for Samples where Extraction had Failed. Collection and extraction were repeated and a gel was run.

The results still seemed to suggest the DNA had degraded (notice the streaked quality of the gel). I was unsure how to proceed, but Silvana advised me to move on and perform PCR for all the samples, with the repeated extractions at the end.

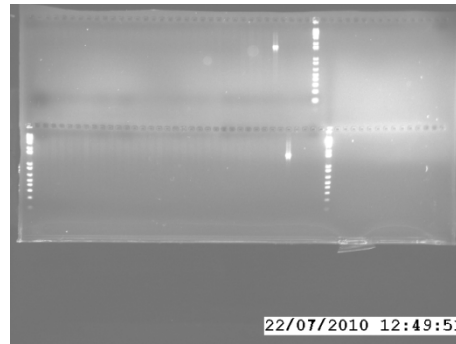
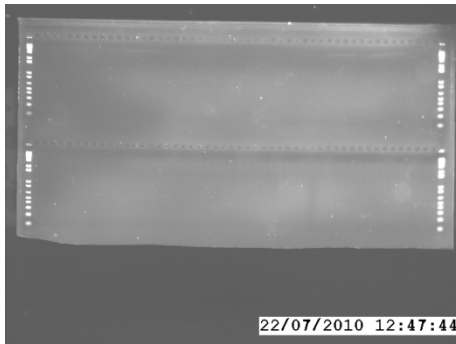


Figure 4: Gel Electrophoresis for first PCR. The first PCR was performed using forward primer 29AP5H (GGGAAAGCTTGCCATAGATGCAATTCAATTCAATCAAAC) and reverse primer NostProx (GTTTGAACGATCGGGGAAAT). We used five controls: rd29DREB1A (a positive plasmid control), P58 (positive plant control), BR16 (negative plant control), and two water blanks. The 29AP5H-NOSTP thermocycler program was used. I ran a 3% w/v agarose gel and viewed the results of the PCR under a UV light.

The PCR was unsuccessful. The gel came out blank for all the samples and even the positive controls did not show up, indicating something went wrong with the PCR reaction.

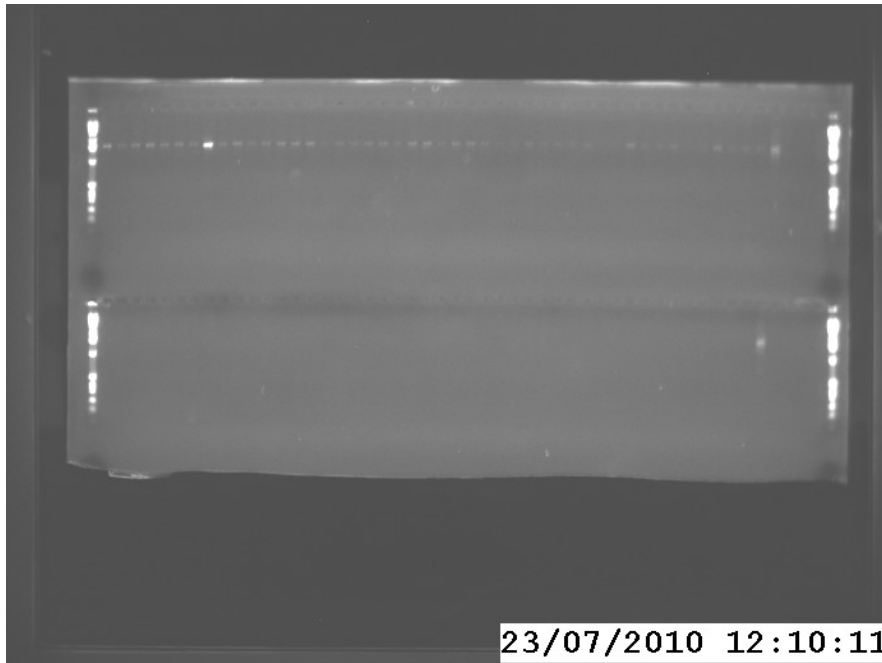


Figure 5: Gel Electrophoresis of PCR for first plate. PCR was repeated for the first plate (all samples with good DNA) using the same primers and controls as the previous PCR. I ran a 3% w/v agarose gel and viewed the results.

The results of this second PCR were very strange. The top row of the gel showed many plants positive for the DREB1 gene, indicated by the white band at the appropriate location. The bands were faint, but it was clear that the gene was present. But, nothing showed up in the bottom half of the gel. As shown above, the second row of the gel had no white bands indicating the presence of the DREB1 gene. It is very unlikely that the DREB1 gene would coincidentally be in the first half of the samples and not the second. I had to assume that there was an issue with the PCR protocol, but the lab members and I could not determine whether it was faulty primers or an issue with the thermocycler that had caused the results. So, we decided to repeat the PCR again with the same primers but using a different thermocycler (in case that had been the cause of our erroneous results). This way, if this PCR also failed, we could be certain the issue was with the primers we were using. Silvana also decided to perform the PCR herself to make sure the error was not in something I was doing during the procedure. (Note: for this PCR the samples were rearranged so that the repeated samples at the end were now integrated into the order. The new order of the samples was #s 1-112, skipping #64 (no sample) and #103 (plant dead), P3069 #s1-10, P1378 #s1-10, P59 #s 1-10, and P1142 #s 1-10). The DREB1 lines were given their own row when running the gel).

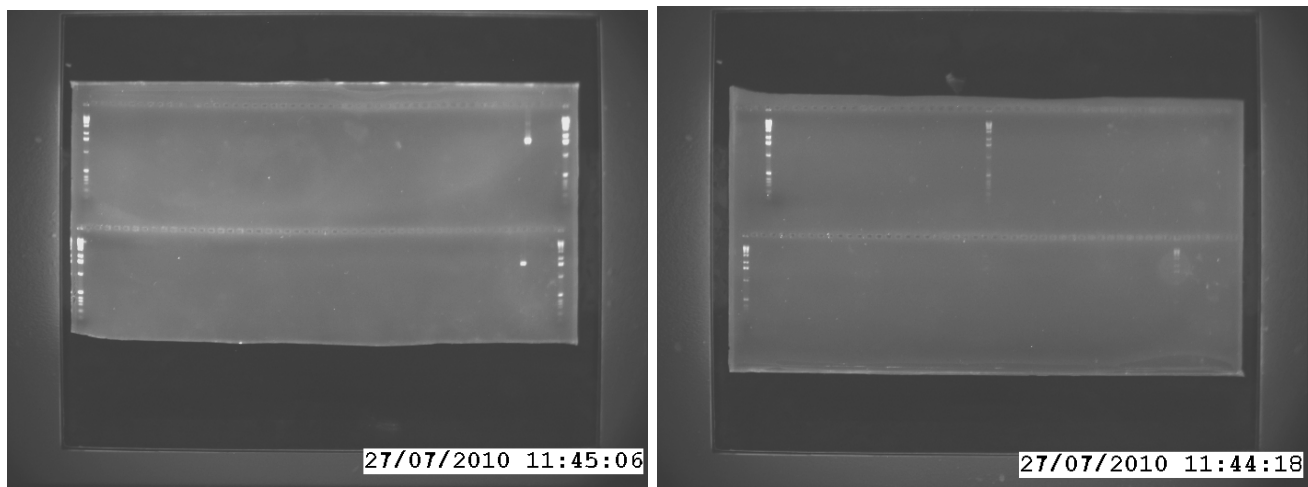
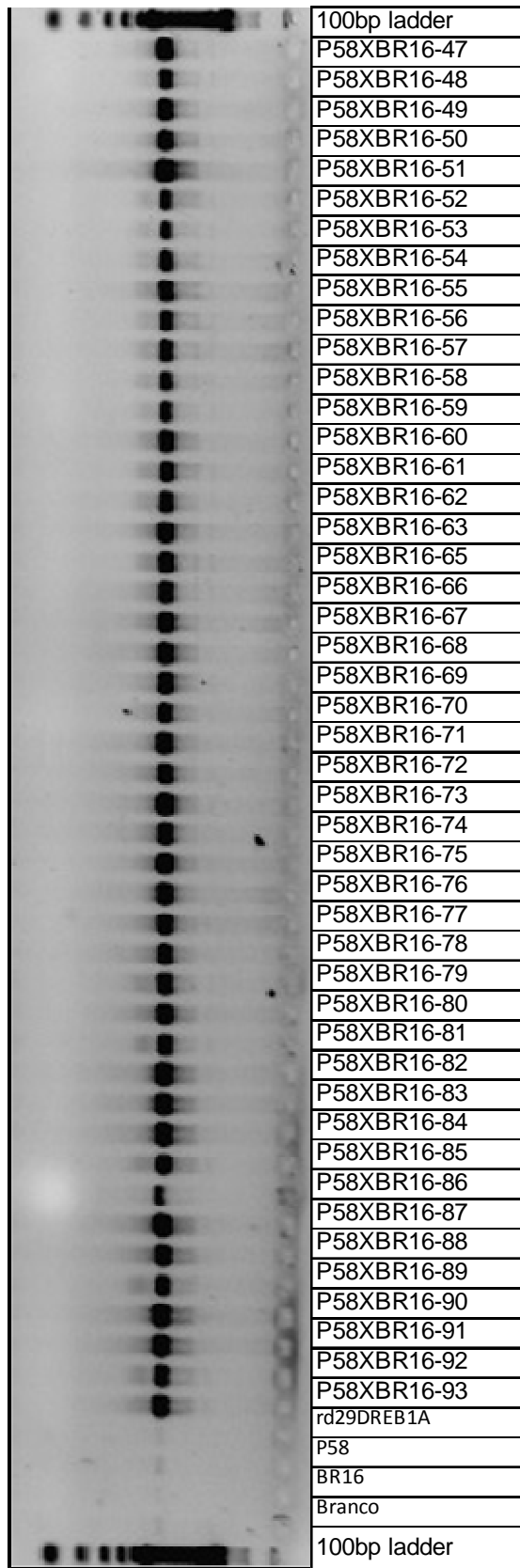
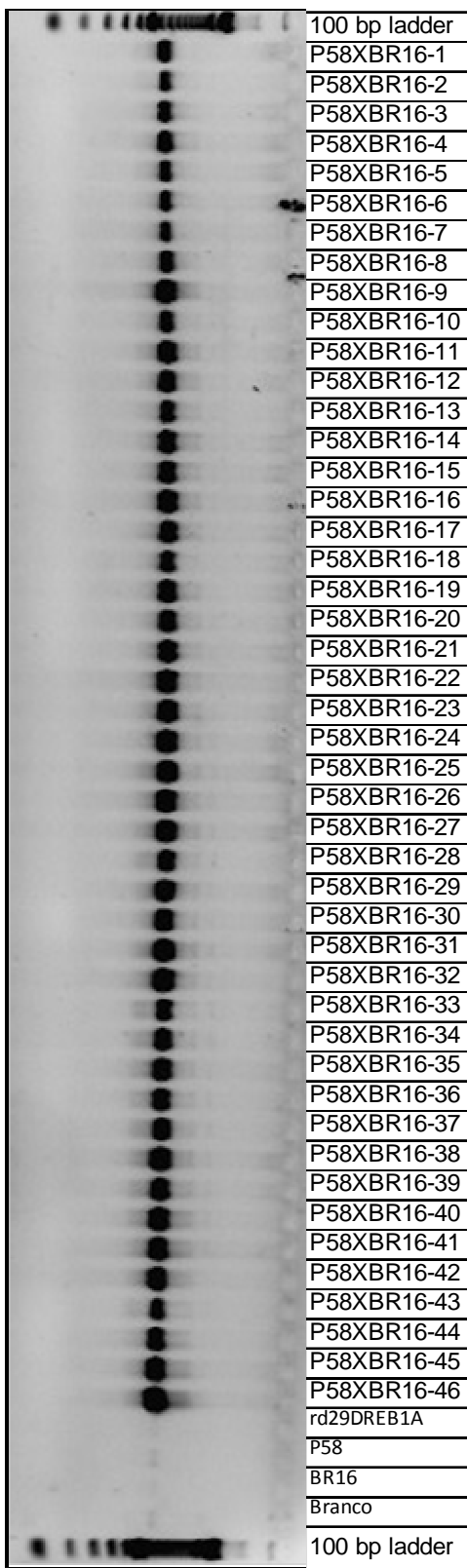


Figure 6: Gel Electrophoresis of Third PCR performed. Same primers and controls used.

The results show another failed PCR. Even the newly replaced positive plant control (P58) did not show up as positive for the gene. This confirmed my suspicion that the problem was with the primers I was using.



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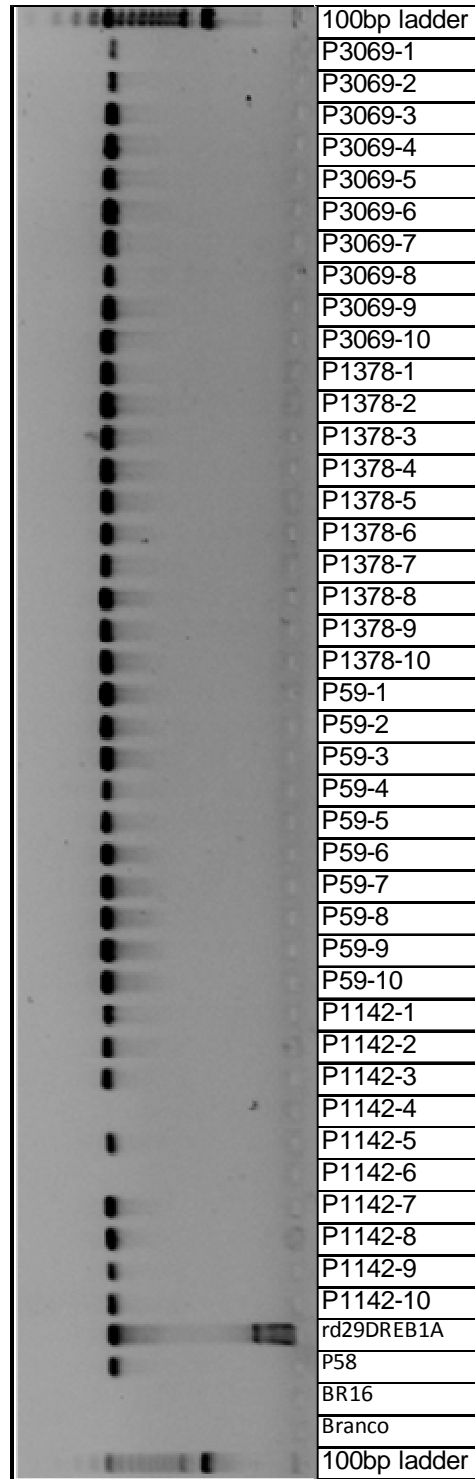
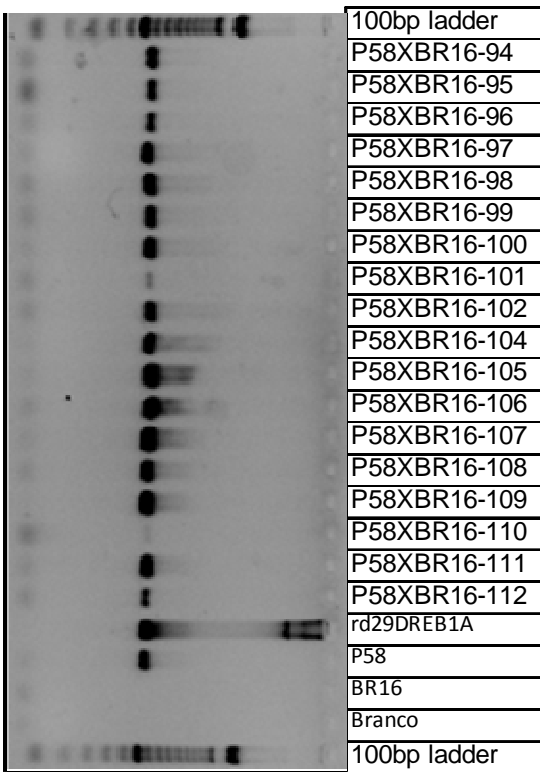
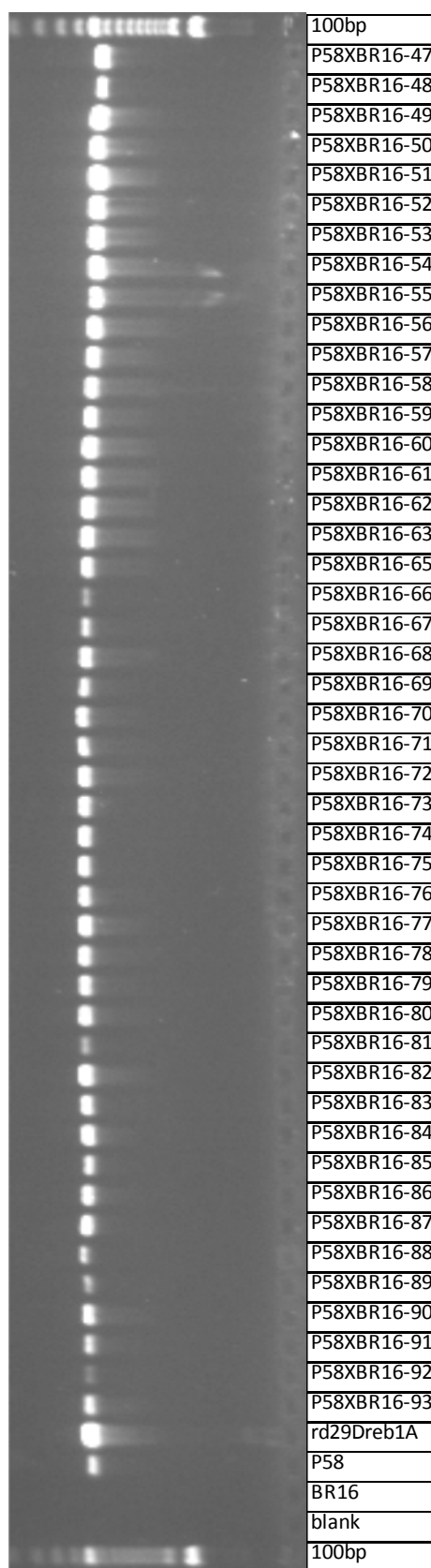
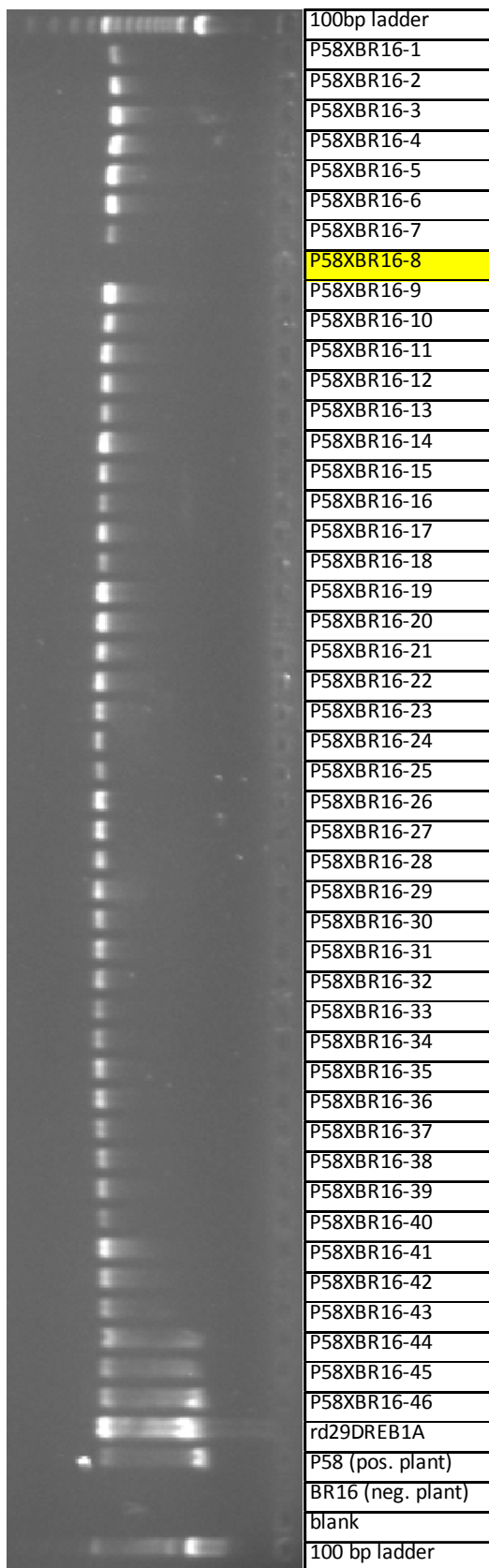


Figure 7: Gel Electrophoresis of 4th PCR using different primers. PCR was repeated again but used a different set of primers. This time the forward primer DREB1A-N (ATGAACYCAYYYYCYGCTTTTTCTG) and reverse primer DREB1A-N (TTAATAACTCCATAACGATACGTCG) were used with the same controls as the previous PCR and a new program on the thermocycler (AgroDREB2). I ran the results and viewed them under UV light. They looked promising, so I aligned them on Excel to their corresponding sample name.

Finally, this gel showed promising results. As shown above, most of the plants showed positive results for the DREB1 gene. Unfortunately, the results of Plate 1 (the first two rows) show that the negative controls (BR16 plant and water) amplified slightly. We assumed that it was just caused by contamination when applying the gel and would not disprove our results. Still, it was necessary to repeat the PCR to confirm that our results are valid by making sure that we came out with blank negative controls. We also decided to use a different set of primers as another way to confirm our results.



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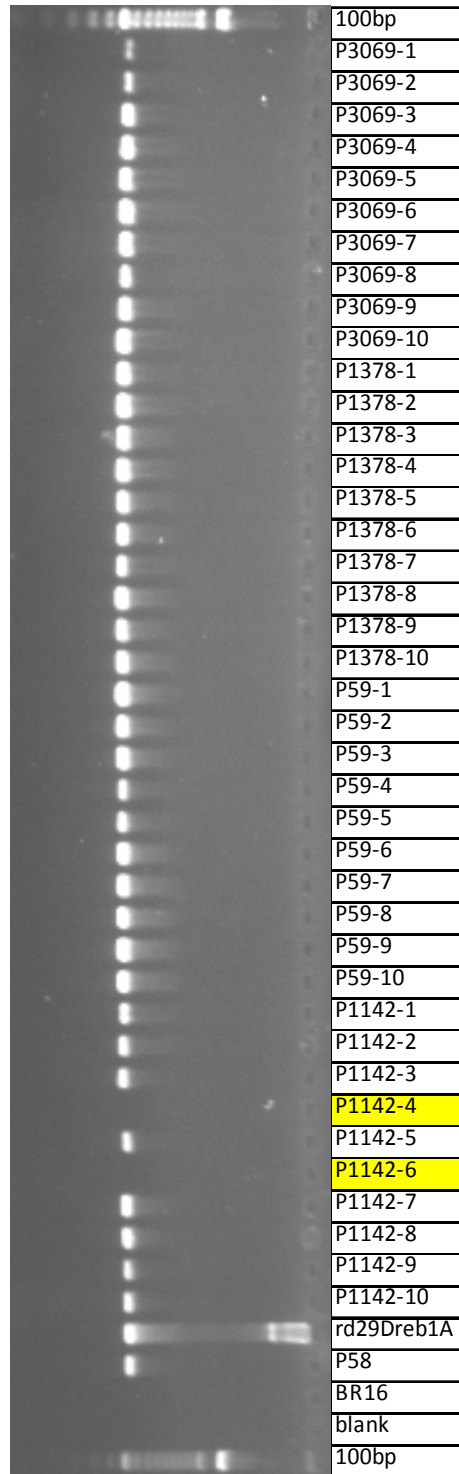
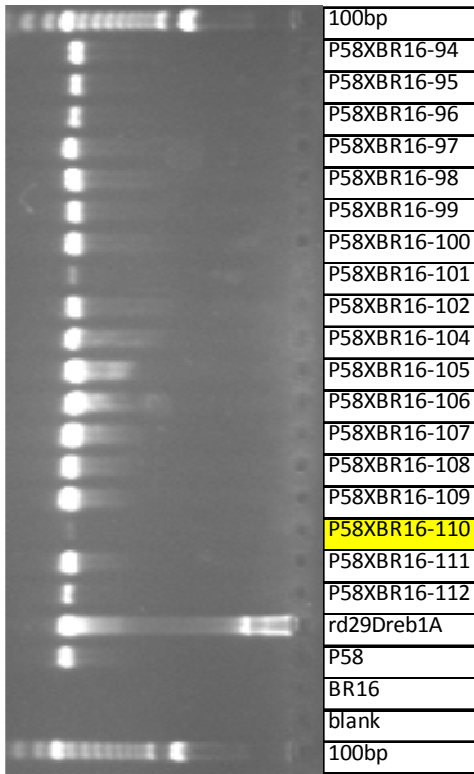


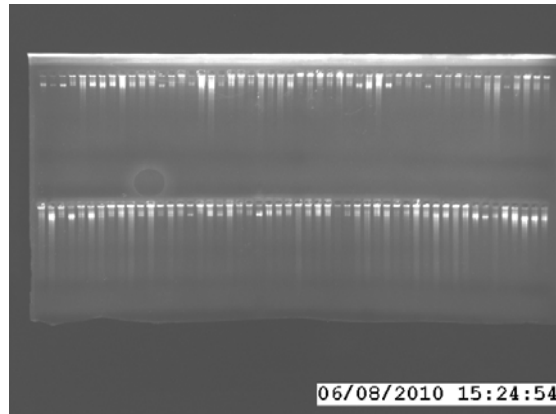
Figure 8: Gel Electrophoresis of 5th PCR using different primers. For our final PCR, we used the forward primer AtRd29DREB (CCAATAGACATGGACCGACTACT) and reverse primer AtRd29DREB (GTTCTCTAACCTCACAAACCCACT) with the same controls and thermocycler program as the previous PCR. Yellow highlighting represents samples that are not positive for the DREB1 gene.

The PCR results came out looking good, with nearly all positive plants and with blank negative controls and positive positive controls (confirmed that our results were valid). The yellow highlighting represents samples that are not positive for the DREB1 gene. Except for one plant (#8), all of the crosses are positive for the gene and, except for two plants (P1142 #s 4 and 6) all of the DREB1 lines prove to be positive as well.

Conclusion of Experience: It is important to note the reasons behind why only the last two PCR's worked. The method of DNA extraction we used left a large amount of protein behind in the sample. This protein can get in the way of the PCR reaction, especially near the promoter regions of the gene, where the binding first takes place. The last two sets of primers I used were different in that they only worked around the gene and not around the promoter regions. We believe that this meant less protein in the way of the PCR reaction, and the reason behind the successful last two PCR's.

These results were very good, but it was still necessary to repeat the entire procedure again, starting from a new sample collection. Our results were valid, but it is important to confirm the results using a sample from a different part of the plant.

My last two weeks at EMBRAPA were spent trying to replicate the results. Unfortunately, errors occurred with DNA extraction. Samples were collected and extractions were performed three times and each time the extraction failed. For nearly all of the samples the gel showed that the DNA was degraded (a streaky white bar instead of a solid band) and the results also showed an excess of protein in the samples (indicated by a white band near the top of the gel column). See below for an example of one of the integrity gels of a failed extraction.



We tried revising the DNA extraction protocol many times in order to discover a solution. For the second extraction, instead of using liquid nitrogen to break up the plant sample, we decided to use a mashing tip as part of the original method. This way, the sample was broken up while being suspended in TE buffer, and therefore had less of a chance of degrading (with the dry ice method the buffer is added after the sample is mashed). When this possible solution failed, we made new reagents for the TE buffer and new TE buffer. I had to leave Brazil before I was able to test whether the extractions would work with the new buffer. Renata updated me when I was back in the United States, and said the extractions had still failed. The lab is now looking into a new DNA extraction protocol that will use phenol and hopefully be more effective at removing excess proteins.

Discussion of Results:

The experiment proved to be a difficult challenge of problem-solving. In the end, my results turned out better than we expected. All of the crossed plants and nearly all of the different lines of DREB1 plants turned out positive (all but two). Our hypothesis was actually incorrect because, instead of 75%, 98.7% of the crossed plants turned out positive. We did run into some difficult problems with the DNA extraction when attempting to repeat the results, but once the issues with the protocol are resolved and the results confirmed, the lab will be able to move on to the next step (see future research). Overall, the experiment has proven to be a success. Silvana, the lab manager, and Renata, my project manager, were extremely excited to see how many of the plants were positive. The repetition of the experiment is causing some problems, but members of the lab are already looking forward to testing which of the positive plants only have one copy of the gene.

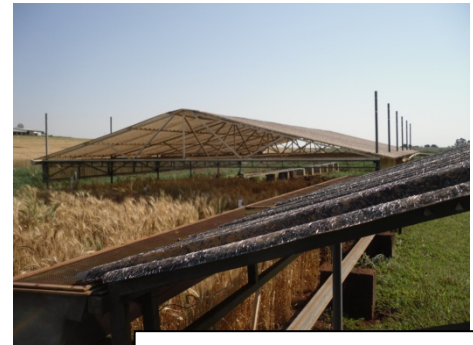
Future Research:

The next step for this experiment is to determine, of the plants positive for the gene, which ones have only one copy. Since so many plants are found to be positive for the DREB1 gene, real time PCR will be performed to give more accurate information on the amount of gene expression. This will allow the lab to quickly get rid of the plants that obviously have more than one copy. After that, Southern Blot Analysis will be performed to give a more accurate measure the relative amount of the DREB1A gene in the samples. Once it is determined which plants are not only positive for the DREB1A gene, but also have only one copy, EMBRAPA researchers will autocross these plants and continue to generate more seeds. When a large enough store of seeds has been produced, it will be time to plant the seeds in the EMBRAPA fields for further experimentation and data collection. In the fields, further drought studies will be conducted and data will be taken on a multitude of things, including photosynthesis and growth rates.

Conclusion and Significance:

Despite being the second highest producer worldwide, Brazil still suffers major losses in its soybean production due to drought. Biotechnology, and the development of transgenic soybean plants that are more tolerant to drought, could provide an important mitigation strategy for this issue. Such a development would improve agribusiness in Brazil and food security around the world.

Though the project I was involved in, and in charge of, represents only a small part of the overall process, it was still a very important part. In order for the P58 transgenic soybeans to make it to the commercial stage, it is necessary that lines be created with only one copy of the gene. Having GM soybean lines with only one copy of the DREB1A construct is also key to introducing this drought tolerance trait to other commercial varieties adapted to different Brazilian soybean producing regions, and regions around the world. EMBRAPA's breeding program can use this one copy line in their crosses with other genotypes of interest.



EMBRAPA's rain shelter system that is used to simulate drought circumstances.



Field Study equipment

If EMBRAPA finds successful crosses with only one copy of the gene, this will also open many doors for further experimentation and serve as a lead on for other important questions. Using the one copy gene line I helped to identify, EMBRAPA's breeding program will be able to create more cultivars with drought resistant qualities that can be suited to different regions of the world. The lab will continue to research how these crosses react to drought conditions in the field and different crosses. Overall, this project will serve to further the state of knowledge about transgenic soybeans and, more specifically, how to deal with multiple copies of an inserted gene. Our techniques and experimental design can serve other scientists working to create successful transgenic crop lines.

EXPERIENCES & REFLECTION

I was sitting in the plane in Ithaca, waiting for lift-off, and I was already getting choked up thinking about my parents and friends. I remember thinking, "I haven't even left the ground, and I'm already homesick." But then, firmly, I said to myself, "Zoe, this is what adventure is all about."

My experience as a Borlaug-Ruan Intern was one of the most enriching experiences of my life, and definitely a great adventure. I came home with a little bit of everything: some knowledge in the Portuguese language, a new-found confidence and sense of independence, skills in laboratory and genetics work, a passion for making a difference, and many, many memories.

Brazilians love to work hard and play hard. Working at EMBRAPA for nine hours a day was often exhausting, but always invigorating. I was amazed at how much independence I was given at the lab. Having a project that I had so much control over was an incredible experience. I knew the data was very important to the lab, so I pushed myself to get results. It was frustrating to get so many failed PCR's and DNA extractions but it taught me a very valuable lesson; science does not always go as planned. Every time the results came out badly, it was up to me to work through the possible causes and manipulate the protocol to find a solution. When I needed help, there was always someone there, but it was great to have the option to work through things myself.

It was also in lab that I made some of my closest friends. The language barrier was an issue at first because many of the graduate students only knew a little bit of English. But as time went on, things got easier and I found myself getting better and better at Portuguese! Some of my fondest moments from Brazil were of going out with the girls from the lab to movies and dinner.

I became especially close with one graduate student, Juliane Marinho. We worked together on extractions for many days and this gave us a lot of time to talk, joke, and work on our English and Portuguese. Juliane even invited me to stay at her family's farm for a weekend. We rode horses, played soccer, and made pamonha and doce de leite. It was one of my favorite experiences from Brazil. Being on a farm also reminded me of what we were working for in the lab. Everyone's efforts at EMBRAPA were geared towards helping the Brazilian farmer. When I said good bye to Juliane my last day, we both cried. It still amazes me that we were able to become such good friends when a lot of the time we barely knew what the other was saying! It just goes to show that, no matter how cliché it sounds, friendship can cross cultural barriers.

A lot of my time that wasn't spent at the lab was spent on a bus. Bruna, my host sister, was competing in a series of beauty pageants while I was there. For several weekends in a row, about twenty of Bruna's friends and I would travel by bus to the pageant to cheer her on. Even though this meant a lot of late nights with crazy Brazilian teenagers, the experience allowed me to see a

huge amount of Brazil. One of the most shocking memories for me was when we traveled to Sao Paulo for Bruna's final contest. I had been sleeping on the bus when someone shook me awake and told me to look out the window. We were passing through the favelas on the outskirts of the city. All I could see for miles were hundreds of tiny concrete buildings mashed together. The poverty was stark and overwhelming. But the worst part was that it never seemed to end. We kept driving and driving, and the sheer magnitude of the favelas was what got to me the most. It was hard for me to imagine how many people this meant were living in such poverty.



When I wasn't on a bus or in the lab, I was watching soccer. I was incredibly lucky to be in Brazil during the World Cup. Every single game was a huge event in the lives of every Brazilian, and it was an amazing thing to witness. Work at EMBRAPA was canceled on game days, and everyone would crowd around a TV in a downtown bar to watch the game. It was like the whole country was holding its breath. After one game, which Brazil won, I went down to the main street of Londrina with my host sister. The place was in a state of utter chaos. People were everywhere dancing, music was playing, and the street had become one big party. Brazilians really do know how to celebrate.

The things that amazed me the most was the people. Everyone I met was so warm and friendly, I felt like I had been living in Brazil for years. Whether it was Renata Fuganti, my project coordinator, taking me shopping and out for pizza, or the graduate students going to a movie with me, I was always accepted with open arms. I felt very at home with my host family too, it was almost like I had two real sisters!

One concept that I carry with me to this day has to do with "The Brazilian Way". Whenever I ran into a problem in the lab that seemed unsolvable, someone would always say, "Be Brazilian, do it the Brazilian Way." Brazilians are very proud of their ability to find unique solutions to complex issues. I like to think I took a bit of this unique sense of perseverance and creativity home with me.

It was inspiring to see everyone at EMBRAPA working towards creating a product that would increase food security in Brazil and around the world. The experience showed me to an even greater degree the role that science can play in international agriculture and in combating world hunger. Science has always been an interesting subject to me. But what I found this summer is that working towards making a difference, even in a miniscule way, makes science much more fulfilling and empowering. As I look to a future in science, I will always remember my experience in Brazil, and how it felt to be part of a bigger cause.

I was sad to leave Brazil. The experience was so enriching in a multitude of ways that I just didn't want the adventure to end. The internship sparked my interest in a lot of things, including the country of Brazil itself. I am currently taking a History of Brazil class and am considering taking Portuguese next fall now that I know some basics. I know I want to return to Brazil, and perhaps even EMBRAPA so I can see some of my friends again.

World hunger is a pressing issue affecting billions of people. The outlook often looks grim and I am sometimes overwhelmed by the issue at hand. But then I think of all the hard working PhD's, graduate students, and technicians at EMBRAPA and everyone at the World Food Prize, and I am reminded of how many people are working towards a difference, and that change is possible.

ACKNOWLEDGEMENTS

I would sincerely like to thank my host family, the Nepomuceno's, for opening up their home to me and providing me with so much support and hospitality. Thank you especially to Alexandre, who filled the roles of both host father and mentor incredibly. Your guidance and direction helped make my internship an extremely fulfilling experience.



Thank you as well to Renata Fuganti, who served as my project coordinator, and Silvana Rothenbach, who were always there to help me along. I wish you, Silvana, the best of luck in Japan; I know our English lessons together will serve you well.

Deepest thanks to my many friends at the laboratory: Larisa Giroto, Cibelle Engles, Juliana Leite, and especially Juliane Marinho. I never would have thought I could have made such a great friend in such a short amount of time, and with a language barrier as well. I will miss you dearly, and our friendship was one of the most valuable parts of the experience.



I would also like to thank my former teacher, Michelle Sutton. If not for her, I never would have known of this incredible opportunity. I would also like to thank my parents, for supporting me throughout the entire process.

Thank you to EMBRAPA Soja, for allowing me to be an intern and providing me with such a valuable experience.

My deepest gratitude goes to the World Food Prize Foundation. I would especially like to thank Ambassador Quinn, Lisa Fleming, and Keegan Kautzky who made this internship experience possible. I am eternally grateful. And thank you to Norman Borlaug, for though I never had the opportunity to meet him, I know his legacy lives on through this internship and his life will always serve me as a source of inspiration and passion.



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Appendix A: DNA Extraction

1. Alternate between putting samples (two at a time) in dry ice and vortexing. This process, with the Teflon balls in the tube, works to break up the plant sample. Vortex and dip the sample in dry ice until the leaf sample is sufficiently broken up.
2. Once the samples have been broken up, add 300 uL of Doyle Doyle (Extraction Buffer) to each sample. The Doyle Doyler must have been in the banho-mario (water heater) for at least 15 minutes before adding.
3. Vortex each sample for 5 seconds
4. Put samples in Styrofoam holder in banho-mario for 30 minutes at 60° Celsius
5. Under the hood, add 300 uL of chloroform to each sample.
6. Wrap racks with tubes in plastic wrap, and mix manually for 5 minutes.
7. Centrifuge samples at 14,000 rpm for 5 minutes.
8. After centrifugation, the sample will have separated between inorganic and organic matter. Under the hood, pipette 200 uL of the top layer (sobrenadante) of liquid into the second set of labeled tubes, using a new tip each time. Make sure the sample numbers correspond.
9. Add 200 uL of chilled isopropanol to each sample.
10. Wrap sample racks in plastic and mix samples manually for 5 minutes.
11. Incubate samples in freezer at -20° Celsius for 30-40 minutes.
12. Centrifuge samples at 14,000 rpm for 5 minutes.
13. Drain liquid of each tube into a waste beaker, leaving DNA pellet (not visible at this point).
14. Add 200 uL of chilled 70% ethanol solution to each sample.
15. Centrifuge samples again at 14,000 rpm for 5 minutes.
16. Drain ethanol of each tube again into a waste beaker.
17. Set up tubes in sequential order with their mouths against a paper towel. Do this for 20 minutes, or until the tubes are dry.
18. Meanwhile, prepare a TE Buffer and RNase solution. The solution should be 99.2% TE Buffer and .8% RNase. When preparing solution, account for pipetting error.
19. After mixing solution, add 100 uL to each tube.
20. Wrap sample racks in plastic and incubate samples at 37° Celsius for 30 minutes.
21. Store samples in -4° Celsius fridge.

Appendix B: Preparing a Gel

1. Measure 3 grams of agarose in weighing room
2. Put on gloves and add agarose to 500 mL beaker (exclusively for making gels)
3. Measure out 300 uL of TE buffer using graduated cylinder and add to beaker.
4. Cover beaker with plastic and poke holes.
5. Microwave for approximately 3 minutes, or until the agarose has completely dissolved and the liquid is clear. Stop occasionally during heating to mix.
6. Cool beaker by swirling in a bucket of cold water.
7. Once beaker is cool to touch, add ethidium bromide at .1% of total concentration of solution (in this case, add 30 uL of ethidium bromide). Use the pipette that is exclusively for ethidium bromide use.
8. Mix sample and pour into a balanced gel electrophoresis tray. Spray with alcohol solution to get rid of bubbles.
9. Place combs (use the larger well side) and let gel sit for 10 minutes.
10. When ready, place gel in electrophoresis machine, sitting in buffer solution.

Appendix C: DNA Integrity and Applying Gel

1. Pipette 5 uL of each DNA sample (from extraction) to a clean plate. Go in sequential order and make sure to keep track of what sample you are on. Change tip each time.
2. Add 2 uL of buffer dye to each sample using dye applicator
3. Apply 7 uL of each sample to individual wells of gel. Between each sample, rinse tip in buffer in gel box. Change tip occasionally (like every ten samples). Keep careful track of where you are in the sequence- do not skip or repeat a sample!
4. Run gel for about two hours at 120 volts.
5. View gel under UV light and save image to computer.

Appendix D: PCR

1. Transfer 3 uL of each DNA sample to a clean plate.
2. Prepare PCR reaction master mix.
3. Pipette 22 uL of master mix to each DNA sample.
4. Seal plate tightly, and put in thermocycler.

Appendix E: Gel Electrophoresis and Viewing Results

1. Prepare a gel as described above, except at 1.2% agarose concentration for 300 mL.
2. On ice, add 2 uL of buffer dye to each sample in PCR product rack.
3. Apply 10 uL of each sample to each well of gel (working from the bottom up).
4. Leave a well empty in beginning and end for the ladder and apply four controls (positive plasmid, positive plant, negative plant, water) to the end of each row.
5. Run gel at 120 volts for approximately two hours.
6. View gel under UV light and save image to computer.