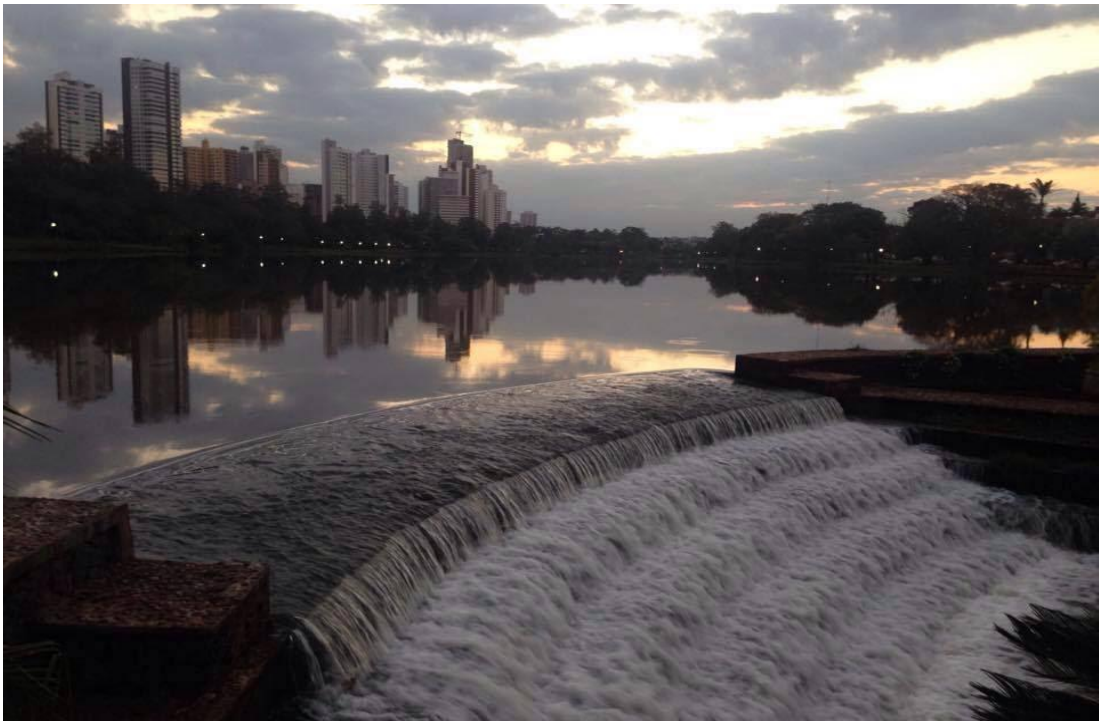
LONDRINA "THE LITTLE LONDON OF BRAZIL"



Galactinol Synthase (GolS) Gene Expression in Soybean under Drought Conditions

Nawadir (Nosa) Ali
Des Moines, Iowa
The World Food Prize Foundation
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The Brazilian Agricultural Research Center-Embraba Soja
Londrina, Brazil

THE WORLD FOOD PRIZE

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Oi!

My name is Nosa Ali and I am a senior at Des Moines Roosevelt High School and Central Academy. I was selected to represent my high school and the State of Iowa as in intern for the prestigious Borlaug-Ruan international internship this past summer. During my sophomore year I decided to participate in the Iowa Youth Institute as a part of my extended learning course at my high school. For the IYI I researched and wrote about how human right violations are impacting food security in Burma. I, along with 250 other students, presented our research papers to college professors and researchers at Iowa State University. I was then selected to represent my high school at the Global Youth Institute the following October where 150 students and myself once again presented our research papers. This time our panel consisted of international leaders and researchers. The Global Youth Institute was an extraordinary experience and a great opportunity to gain knowledge about the international perspective on food security and ending hunger.

At GYI I also had the opportunity to interact with interns from previous years and learn about the different intern opportunities available after the Global Youth institute. The Borlaug-Ruan internship stuck out the most to me because I have always wanted to do research in another country while experiencing the culture. After submitting my paper and completing my interview, I was fortunate to have been selected as a Borlaug-Ruan intern in Londrina, Brazil with the Brazilian Agricultural Research Corporation (EMBRAPA). My internship would start June 19, 2016 to August 18 2016. I was extremely grateful to have been selected for such a prestigious internship and a chance to make an impact on the world while still in high school.

FIRST IMPRESSIONS

I did not realize that stepping out of the plane and into the Sau Paulo airport would be the last time I would hear the English I was most familiar with and the culture I was used to. The airport was my first real experience with what Brazilian culture would be like. Everyone around me was speaking Portuguese loudly while many stood in crowded lines for food that looked very much American. One thing I learned about Brazilians is that they love the American culture and are willing to wait hours in long crowded lines to experience anything American. Like any airport, there was a lot of chaos, which ironically made me feel comfortable because that was something I familiar with.

When I landed in my host city Londrina, the first things that caught my attention were the extremely red soil, colorful houses, and graffiti art covering almost every street wall. Despite the immediate cultural shocks, I was positive I was going to grow to love this city, country, and culture as soon as I received a warm welcoming hug from my host family.

I received a great welcome come at the Embrapa lab. The first day I meet all of the important people at Embrapa who made my internship possible including my supervisor Liliane M. Mertz Henning who is a lead researcher in the plant biotechnology lab, Alisson Dias who is a university student and intern at Embrapa, and all of the other grad/post grad interns.

Everyone wanted to get to know me and was very curious to hear my English. They were also very excited to practice their English with me and also teach me a little Portuguese. During my visits to the greenhouse and lunch hour I had the opportunity to interact with many Embrapa employees who all had interesting stories about visiting Iowa or touristic states like California, New York, and Florida. There was one intern in my lab that had even lived in Iowa for a year while attending Iowa State University. It was definitely nice to share stories with her about the Iowa State Fair and cool places she visited in Des Moines.

Figure 2 Londrina, Brazil Red Soil

Figure 3 Brazil Street Graffiti

ABSTRACT

Brazil is facing one of the biggest threats in agriculture due to forecasted changes in climate. An increase of 1 degree Celsius would lead to devastating losses in Brazil. This threat makes it extremely important for Brazil to make changes to its soybean production before significant damage is done. The GOLS gene is an important tool that could be used to prevent such devastation from happening. In order to test out this gene and produce transgenic plants, agrobacterium transformation was used as a way of inserting the gene into soybean seeds. After the seeds have been transformed and germinated, it is expected transgenic T1 soybean plants with resistance to drought and cold-stress will be harvested. Then the DNA will be extracted from the T1 plants, Polymerase Chain Reaction (PCR) will performed with specific primers to GOLS gene in order to determine which plants were positive (transformed), gene insertions and segregation will be evaluated, and then finally the lines for the next generation of plants will be selected. Eventually by the T3 generation, we should be able to remove the plants from the green house and into the field of experiments to test out whether the plants are efficient in being resistant to drought conditions. The soybean samples were successfully transformed with 92% transformation efficiency. The transformed soybean plants demonstrated higher tolerance to drought stress than the conventional BRS184 genotype that it was tested against. This efficient tool can not only be implemented in Brazil, but in drought regions around the world.

Figure 4 Soybeans in Drought

EMBRAPA SOJA

Embrapa Soja is the Brazilian Agricultural Research Corporation responsible for developing sustainable soybean production practices in Brazil. Embrapa was founded on April 16, 1975 and has since expanded to become Brazil's largest and most important public research facility. Embrapa has become a model for many tropical regions and countries when it comes to development of agricultural practices and technologies. It has 47 research units stretching from the Amazon in the North to Rio Grande do Sul in the south. In total, Embrapa employs more than 9,000 people; 2,000 of which are researchers. Three-fourths

Figure 5 EMBRAPA Entrance

of the employees at Embrapa have a doctoral degree, which indicates how competitive this organization is. Embrapa has played a key role in transforming Brazil's agricultural practices. This includes making Brazil one of the world's top exporters of soybean and corn. Besides focusing on soybean and corn research, Embrapa's facilities also specialize in animal and environmental research. Embrapa has many international partnerships including Japan, The United States, Germany and 78 bilateral agreements with 56 countries and 89 institutions. Embrapa is an important public institution for Brazil because for every R$1 invested in Embrapa, R$13.20 are generated.

HISTORY OF BRAZILIAN AGRICULTURE

Agribusiness has been and continues to be the greatest and most important industry in Brazil. Brazil's agriculture industry has seen major transformations in less than the last 50 years—specifically in the 1970's. Before 1973, agricultural practices were predominant in southern Brazil where the weather is semi-temperate and the soil is fertile and well irrigated. The northeast and the midwest regions of Brazil were not as attractive to farmers due to the weak quality of the soil. The northeast is much drier with less nutrient-rich soil, not tolerant to droughts, and lacks infrastructure. Midwest Brazil was underdeveloped, very poor, had low cultivation, and its land was viewed as unsuitable for farming. Other factors that prevented farmers from migrating to the Midwest were the lack of roads, atrocious infrastructure, and high transportation costs due to its isolation from the rest of the country. During this time Brazil, was also a major importer. However, significant change for Brazil came in the early 1970's. The discovery of sustainable soybean growth in the region once believed to be impossible for cultivation and low land prices in the midwest began the agricultural boom for Brazil. The value increase of soybean in the international market also contributed to the unexpected interest in soybean. Before the agricultural boom, coffee was the plant of interest and soybean was one of the least cultivated crops. Currently, soybean is the most important crop in Brazil followed by corn and beans. Soybean had limited uses, but now has expanded in both consumption and non-consumption products. In a span of 50 years, soybean production increased from 160% to 328% with small increases in land area usage but a major increase in productivity. Soybean and corn continue to dominate crop production in Brazil with 80% of land area dedicated to its growth.

In Brazil, soybeans are planted in the spring and are considered the second summer crop. Corn is planted either in September and cultivated in October or planted in February and cultivated in April. Everyday counts when it comes to planting and harvesting. Brazil follows a no-till policy and plants its soybean immediately after the harvested corn. The fact that agribusiness contributes nearly 20-25% of Brazil's GDP explains the urgency for planting immediately. Brazil's economy is greatly dependent on agribusiness and most importantly soybean production. Technological developments were the greatest investments in strengthening Brazil's agribusiness. A few implemented techniques include adaptation of soybean in low latitude regions, advances in fertilization, improved machinery, integrated crop with livestock, zoning, use of transgenic crops, property management, and usage of the no till system which increases the organic matter and keeps moister in the soil. Causes of rapid growth can also be credited to entrepreneurship, producer's access to quality information, termination of subsides which eliminates unproductive farmers from the market, and favorable global markets for soybean.

The agricultural boom has transformed Brazil from one of largest importers to the second largest agricultural exporter in the world. Since the 1970's, Brazil has shown exponential growth in production and continues to demonstrate its potential for becoming the largest producer after surpassing the United States. Brazil has an abundance of arable land suitable for agriculture, a favorable climate year-round, plenty of water to irrigate, technology to adjust to various conditions, and deep soil that is easy to manage. The greatest advantage forecasted for Brazil is the increasing world population, which is leading to the growth of the global economy, increasing and ever changing consumption habits; less grain and more meat, increasing per capita income, greater demand for alternative oil for biodiesel, and the expectation that Brazil will account for 40% of agricultural production increases in the next decade.

Although Brazil is experiencing excellent growing conditions, there are factors that can possibly affect the growing environment of soybean. These include global warming, which is leading to temperature increases and drought conditions. A possible increase of just 1 Celsius could greatly alter the climate of both the south and the north of Brazil.

INTRODUCTION

Plants have unique ways of responding to water deficit and drought conditions. They can either experience physiological or biochemical changes such as shift in gene expression. These genes may work as osomprotectancts by enabling the plant to survive extreme osmotic stress. Raffinose family oligosaccharides (RFOs) play a key role in desiccation tolerance of seeds. (cite) Without its presence desiccation tolerance of seeds may not occur. Galactional Synthase (GolS) is considered a key-catalyzing enzyme in the biosynthesis of oligosaccharides of the raffinose family (RFO). The GolS gene can be used as a vital instrument to control the desired levels of RFOs under certain stress conditions. In Arabidopsis plants, galactional synthase was observed to provide cold-stress tolerance. In this study a specific GolS gene was used to catalyze RFO biosynthesis in order to generate drought tolerant conditions in soybean. Gene segration of the GolS gene will first be observed then tested under ideal drought conditions in the greenhouse.

MATERIALS AND METHODS

The experiment was based in the developing and characterization of transgenic soybean plants transformed for drought tolerance. The GOLS gene was used in the soybean transformation process. This gene is involved in the synthesis of oligosaccharides belonging to the raffinose family (RFOs). For *Experiment 1* seeds were transformed in the Embrapa lab using agrobacterium and transformation protocol *Agrobacterium tumefaciens (*ISU). Mature seeds were harvested from the transgenic plants. (Figure 8) The soybean seeds where then germinated using germination test paper in the seeds lab under ideal conditions. After 4-5 days the germinated seeds were transferred into soiled pots and transferred to the greenhouse. (Figure 9) After the plants grew to V2 stage, leaves were collected for DNA extraction. The DNA extraction was performed following the *Vegetal DNA Extraction (Doyle & Doyle)* After the DNA extraction PCR reaction was performed using specific primers to amplify the gene. *Experiment 2* involved checking the performance of the transgenic plants under drought stress greenhouse conditions. Transgenic line soybean seeds and conventional seeds were

Figure 8 Transgenic Soybeans seeds

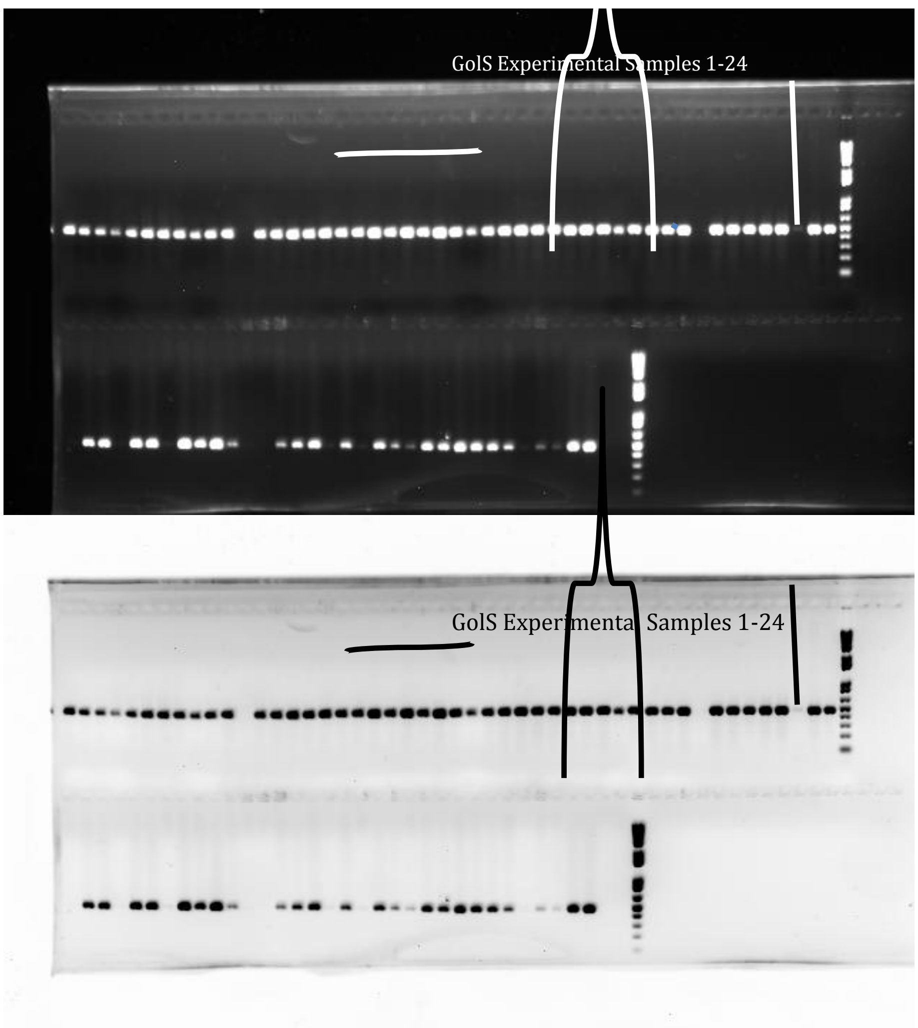
Figure 8 Planting the Seeds

germinated using the same procedure, dipped into nitrogen fixation solution in, and potted. Pots were then randomized within their respective blocks to ensure that no one plant receives a certain condition over another. Both conventional and transgenic plants were allowed to reach V2 stage then covered with plastic cover to initiate drought conditions. Only the control plants continued to receive water. Plants physical appearances were analyzed, water consumption was monitored, and Photosynthesis was measured after drought conditions were obtained.

[GolS Primer] B-Actina-Intron F   CCCCTAACCCAAAGGTCAACAG

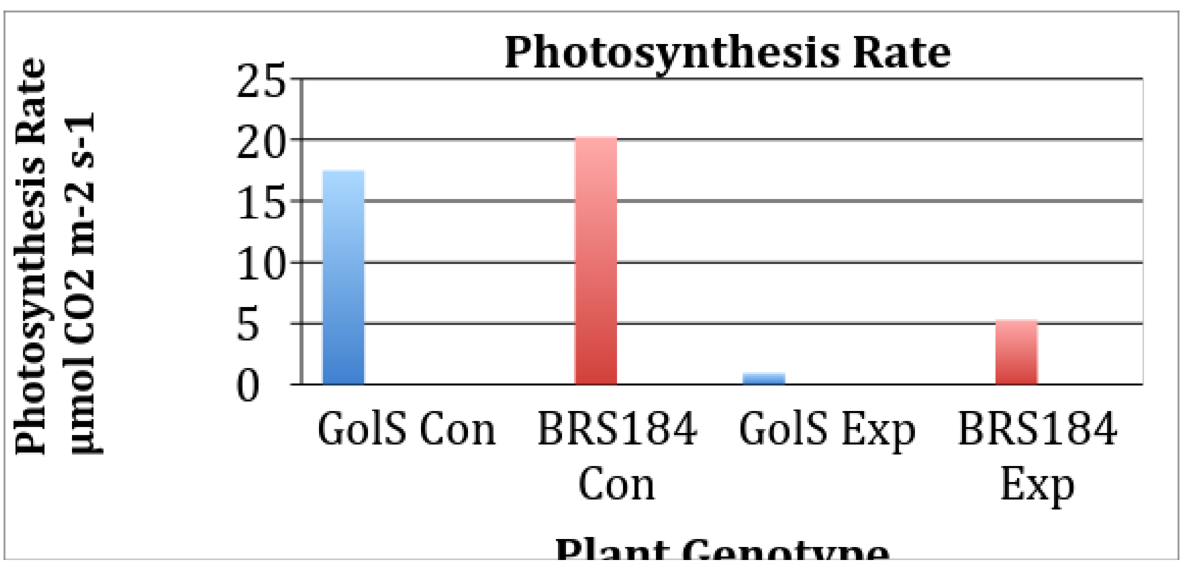
[GolS Primer] B-Actina-Intron R GGAATCTCTCTGCCCCAATTGTG

DATA AND ANALYSIS

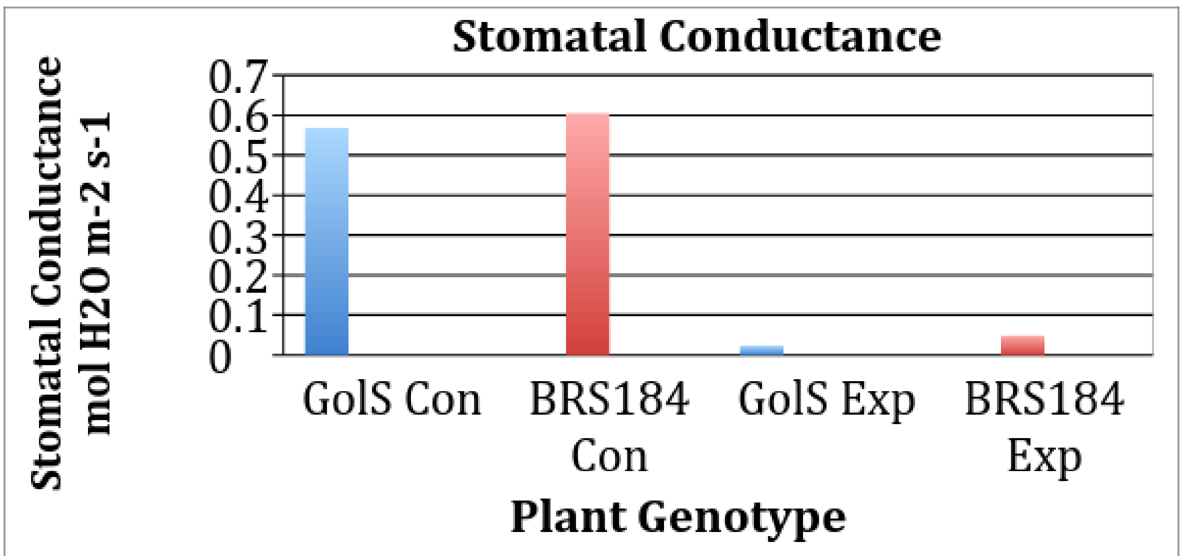


To run the PCR, forward primer B-Actina-Intron F and reverse primer B-Actina-Intron R were used. The first PCR that was run did not produce sufficient results due to wrong calculations of the forward and reverse primers. Figures 3 illustrates the 2nd PCR results of Experiment 1 GolS plants. 22 of the 24 plants successfully exhibited the gene and were considered healthy to continue to grow and produce T3 seedlings. Figure 4 shows one of the 22 successfully transformed soybean plants that will be harvested soon for T3 seedlings and grown once again.

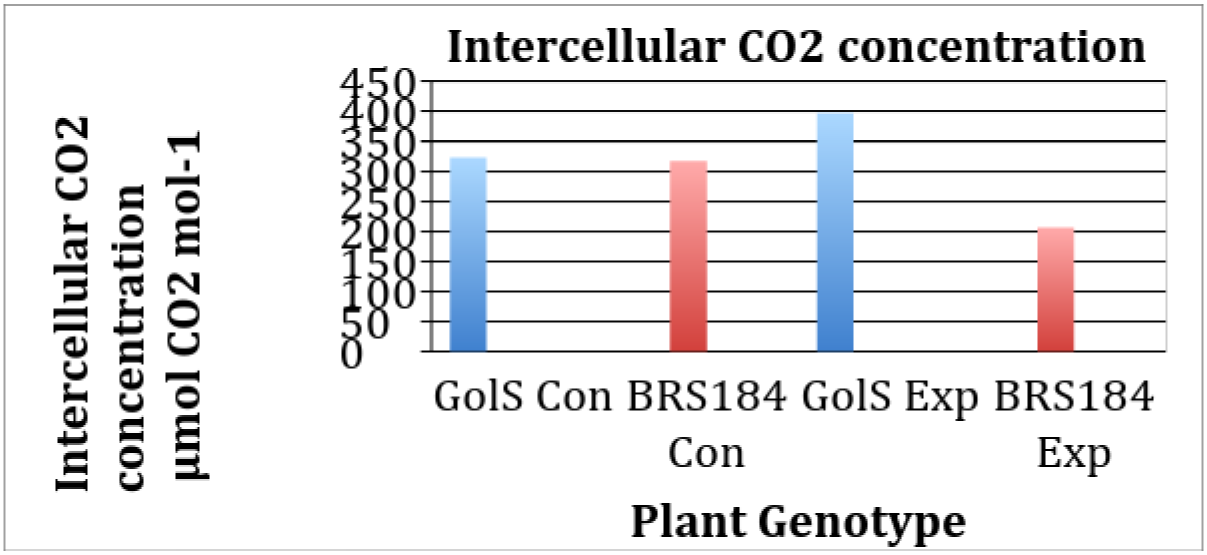
Plant Genotype	**Intercellular CO2 Concentration *u*mol CO2 mol-1 co2**					
GolS Control	**13.3**	**22.1**	**13.7**	**22.3**	**13.3**	**20.4**
BRS184 Control	**20.8**	**18.4**	**24.6**	**20.5**	**16.3**	**20.8**
GolS Experimental	**1.87**	**0.16**	**-0.79**	**-2.64**	**-0.98**	**6.41**
BRS184 Experimental	**4.97**	**3.94**	**6.64**	**4.78**	**3.45**	**8.10**



Plant Genotype	**Stomatal Conductance to H20 mol H20 m-2 s-1**					
GolS Control	**0.523**	**0.629**	**0.553**	**0.628**	**0.504**	**0.577**
BRS184 Control	**0.571**	**0.473**	**0.713**	**0.685**	**0.636**	**0.553**
GolS Experimental	**0.026**	**0.019**	**0.013**	**0.012**	**0.023**	**0.044**
BRS184 Experimental	**0.039**	**0.046**	**0.066**	**0.046**	**0.036**	**0.063**



Plant Genotype	**Intercellular CO2 Concentration *u*mol CO2 mol-1 co2**					
GolS Control	**335.3**	**314.0**	**335.6**	**311.6**	**331.2**	**311.9**
BRS184 Control	**312.5**	**309.8**	**314.1**	**324.0**	**333.7**	**311.3**
GolS Experimental	**262.2**	**316.9**	**469.5**	**701.3**	**441.7**	**142.0**
BRS184 Experimental	**178.3**	**240.3**	**214.4**	**210.0**	**228.7**	**170.4**



Data table 1 and graphs 1,2, and 3 show the results of experiment 2 which compared the photosynthetic rate, stomatal conductance, and intercellular CO2 concentration of the GolS and BRS184 plants. The experimental GolS plants experienced less water loss than the conventional plants as shown by the stomatal conductance data table and graph. However, the GolS plants had lower photosynthesis rates, which were expected considering they were not experiencing drought conditions at the same high rate as the conventional plants. The GolS plants also had higher intercellular concentration levels, which mean they are able to hold in CO2 much more than the conventional BRS184 plants. The results are great considering these are only second-generation plants.

CONCLUSION & FUTURE DIRECTIONS

In experiment 1, transformation efficiency was tested on soybean plants transformed with the GolS gene using PCR. The PCR results showed 92% transformation efficiency, which is quite good considering the seeds transformed, were T2 seeds. In experiment 2 the Transgenic GOLS plants proved to have high tolerance to drought stress when compared to the conventional as hypothesized. These two experiments are only the initial steps in identifying GolS as a prime gene for drought resistance. Many more trials will need to be carried out in the greenhouse before the transgenic soybean plants can officially be put on the market. It is recommended that trials up to generation 4 be administered because the first three generations lack stability and usually produce seeds that may not be positive to the desired gene. This method can be vital to many regions around the world where drought is common.

CULTURAL EMERSION

The Brazilian culture is definitely one of the most diverse cultures I have ever experienced. The culture within itself is composed of many other cultures such as African, Arab, Lebnonese, German, Italian, Spanish, Portuguese, and Japanese. Brazil is interestingly home to the largest Japanese population outside of Japan. I lived in a gated community called "Condominium Sun Lake" which was not diverse at all but my host moms Divania and Ivani made sure to expose me to different places where I was able to experience the cultures. Some of the fondest memories I have include being able to go to a Lebanese restaurant where I was able to speak my native language, Arabic, with the head chief and taking road trips to larger cities such as Curitiba and Guratuba, where I was able to visit famous Brazilian architecture, museums, beaches, markets, and restaurants.

My host mom, Divania, is neighbors with a few professors at the local university EUL. Although I have never done anything of this sort, I agreed to give three different lectures about Iowa and American culture, the American health system, and religions in America. My hos family tagged along with me to every lecture and my host brother did the translation. A few of the students now basic English and could follow quite a bit but for the majority English was not one of their languages. I thought the language barrier would make the lecture awkward but the delayed laughters allowed me to laugh at my jokes with them. Overall, the students were extremely pleasant and interested in how a 17-year-old girl could have the opportunity to travel around the world. They were equally as excited to learn that the World Food Prize Foundation and Norman Borlaug were responsible for my internship sponsorship. The lab experiences were with no doubt great learning experiences but I don't think I would have learned about Brazil and the impression of America on the Brazilian people than rom a group of enthusiastic college student.

Lectures at EUL

Students asking questions throughout the presentation

AWKNOWLEDGMENTS

Before I arrived in Brazil I had expected to see the Brazil I had always saw on T.V; futbul, carnival, and poverty. However, that was not quite what I saw in Londrina. Instead I saw great diversity, very welcoming and humble people, and a country full of culture. I cannot explain in words how thankful I am to have had this opportunity to experience Brazil, make lifelong friendships along the way, and, most importantly, work to solve global hunger. There are so many individuals who have made a great impact on my life and I want to thank them for that. Thank you for making me feel comfortable when at times I was dealing with cultural shock and homesickness. Thank you to those who have ever made me smile or laugh. Thank you to those who have helped make me a better person in any way.

I would most importantly like to thank Norman Borlaug, Ambassador Kenneth M. Quinn, Lisa Fleming, and the World Food Prize Foundation. Thank you Norman Borlaug for being an inspiration to young students like myself and for creating a program that has/will continue to impact many students. I hope that my experience carried your legacy and impacted the lives of many, as you would have wished. Thank you Ambassador Kenneth M. Quinn for ensuring the World Food Prize and its programs run efficiently. Thank you Lisa Fleming for being so dedicated to the youth programs. I have spent several days in your office watching you work long hours to ensure every student/BR intern has a safe and unforgettable experience whether that be IYI, GYI, or the BR International Internship. Thank you to the entire World Food Prize Foundation for continuing the conversation about global hunger.

Thank you to my teachers and specifically Mrs. Dunley for encouraging me to participate in the World Food Prize Programs and for the endless support along the way. Thank you, Scott Schonberg, for building my interest in scientific research for the past four years. Thank you to my principal Kevin Biggs and Science Bound director Dr. Hargrave for taking the time to write me great recommendation letters. Thank you to my friends and especially my family for the endless support! Skype calls and messages from you definitely made homesickness gradually disappear.

I would like to extend great thanks to Embrapa Soja for accepting me as an intern for these past two months. The knowledge, skills, and resources I have gained in the short time I have been at Embrapa will definitely further my education and help begin the next journey of my life. Thank you to my supervisor Dr. Liliane for the friendship, support, and guidance you have provided. Thank you to my grad partners, Alison and Andre, for being there to help me along my entire journey. Thank you to all the graduate and post-graduate interns in my lab for the conversations, laughter, short Portuguese lessons, and adventures outside the lab. A special thanks to two girls I will never ever forget, friends I never thought I would build such a strong friendship with, and labs buddies I will miss joking around with. Thank you, Elizandra and Martina.

Last but not least, I would like to thank my amazing host families that have allowed me to stay in their homes. Although I made many friendships and memories in the lab, my most memorable ones will be the days I spent in your homes. Thank you, Divania and Pedro, for treating me like your daughter and always making sure I felt comfortable and was enjoying my time. I really enjoyed our daily conversations and weekly travels. Thank you, Pedro Jr., for being the best host brother. Thank you, Ivani, for allowing me to pronounce your name wrong and for always taking me to the store so I could buy chocolate for my chocolate chip cookies. Thank you for the warm welcome into your family. I really enjoyed every meal we prepared together and the many conversations we had. Thank you, Eglaida and Amelio, for a fun weekend together!

Thank you, Brazil, for an unforgettable summer.

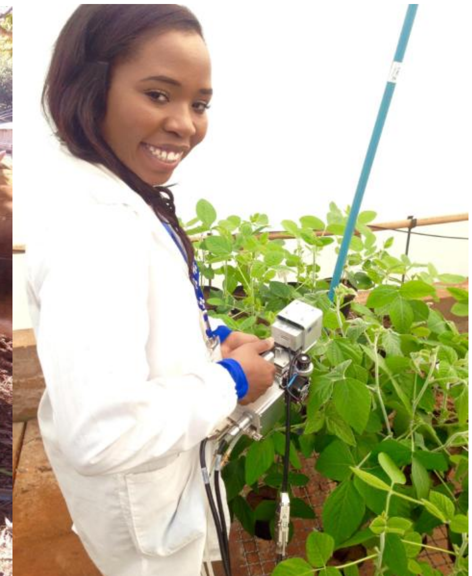
PICTURES


Embrapa
Soja
Empresa Brasileira de Pesquisa Agropecuária
Ministério da Agricultura, Pecuária e

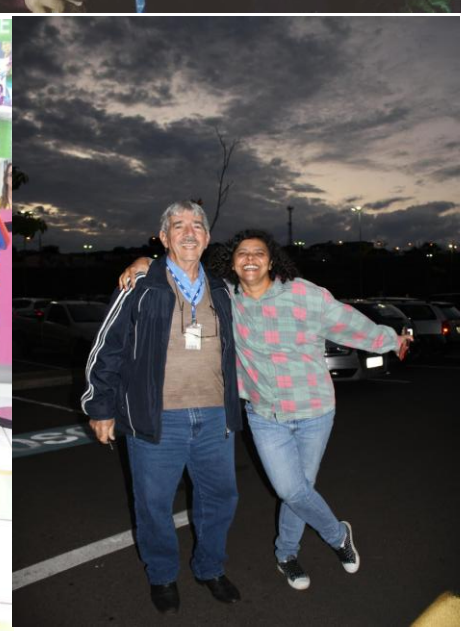

Bradesco
TRANSITO DE BICICLETAS


LAB. BIOTECNOLOGIA VEGETAL
LAB. BIOINFORMÁTICA







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