My Summer with Soybeans

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My family gave me their unconditional love and support.

Introduction

My journey to Brazil began in Mozambique. During the summer of 2010 I chose the south-eastern African country for my research subject and analyzed "Solutions for the World's Smallholders."

While participating in the 2010 Global Youth Institute and World Food Prize symposium, I was amazed at the knowledge flowing around me and the dedication that both students and professionals showed towards eradicating hunger. Ever since my Mozambique research, I had many burning questions that needed to be answered, such as "What can I do to help in addition to donating money?" I wrote about what the Mozambican government should do to improve its infrastructure, but what could I personally do? My questions were answered in Iowa as I learned about David Beckmann's leadership of Bread for the World and how I could become active in both my community and nationwide.

"Action against hunger walks on two legs. There's the leg of direct assistance, really helping people, and then there's the leg of advocacy, changing laws and structures. And you've got to walk on both legs," said 2010 laureate David Beckmann, quoting his organization's founder, Arthur Simon. I was not able to attend the "laureate's forum" section of the World Food Prize symposium, in which Mr. Beckmann said the quote above, but reading through the transcript later on made me realize the cooperative efforts of the anti-hunger movement. There are so many people in many different areas of work who are coming together to help fight world hunger. Even the name of the Borlaug-Ruan internship demonstrates that fact, linking an agronomist with a trucking company executive.

As I learned more about the Borlaug-Ruan internship, I became more prepared to understand world hunger that, in turn, gave me the skills to become personally involved. Everything about the internship's mission and objectives motivated me to be prepared for the greatest journey of my life.

EMBRAPA Soja and its Mission

The Brazilian Agricultural Research Corporation (EMBRAPA) is comprised of 45 research units located across Brazil. The soybean unit, EMBRAPA Soja, focuses research efforts on soybeans, sunflower, and wheat; other research units work with agricultural items such as coffee and farm animals. Its mission is to "facilitate, through research, development and innovation, solutions to the sustainability of production chains of soybeans and sunflowers, for the benefit of Brazilian society" (embrapa.br) Brazil is the second largest producer of soybeans in the world; since its agricultural sector is very important to the national economy (5% of GDP vs. USA's 1%) soybean is a valuable crop.

My internship supervisor was Dr. Neumaier. He was the liaison with the World Food Prize. Dr. Abdellnoor, one of the three scientists leading my lab (Plant Biotechnology), directed my research and advised me on my project. Many graduate students and employees worked in the lab. I worked closely with Danielle, an EMBRAPA employee, and Tatiana, a graduate student.

My Program

During the past several years, a devastating soybean disease has been ravaging crops across the world, most recently appearing in the United States. As the second-largest producer of soybeans in the world, Brazil was greatly impacted by the disease and researchers have been trying to find ways to combat this disease. Besides using pesticides, farmers are also able to use plants that are genetically resistant to the disease, Asian Soybean Rust (ASR), to reduce crop losses. I worked in the plant biotechnology lab, where many graduate students, post doctorates, technicians, and researchers have been isolating soybean lines with disease resistant genes. My research had two parts. First, I was involved with finding the best method of extracting DNA for experiment samples. Many methods of DNA extraction from plants exist, but most of these were created for different species such as Arabidopsis. Later on, my work focused on a process called Marker Assisted Selection. Markers (in this case, Simple Sequence Repeats were used) of known chromosomal regions were used to test whether or not a particular sample possessed the ASR-resistance gene.

One goal of the program was to increase the resistance of already-resistant plants by stacking gene loci. The other was to cross breed resistant plants from other climates and environments with cultivars that are native to Brazil.

My Research

Background of Asian Soybean Rust (ASR) and Review of Literature

Brazil is the second largest soybean producer in the world, second only to the US. Soybeans are an important part of its agricultural industry and to combating world hunger as a whole. In addition to being an excellent source of protein for humans, soybean also works well as animal feed, an energy source, and as material for soybean oil. Although Asian Soybean Rust (ASR) has been identified in the distant past, it has recently started to affect more and more soybean crop yields. Up to 80 percent of yields have been destroyed by ASR.

Asian Soy Rust disease is caused by *Phakopsora Pachyrizi* and spreads through urediniospores. In Brazil, ASR was first seen in 2001 and has spread throughout South America since then. Currently, the only effective method to fight ASR is the spraying of fungicide, which raise production costs and contaminates the environment. Scientists have identified 5 resistance genes, Rpp 1,2,3,4 and 5.

(Identification and Analyses of Candidate Genes for Rpp4-Mediated Resistance to Asian Soybean Rust in Soybean)

However, each resistance gene may not resist all strains of ASR. Therefore, since monogenic rust resistance may not be a stable solution, scientists have been trying to stack loci (pyramiding) by crossing resistant lines with a different Rpp gene each to make a more resistant plant. To test whether a parent is resistant, the plant is infected with a field isolate of P. Pachyrhizi and lesion type evaluated: plants with resistant genes have reddish-brown lesions (RB), while susceptible plants have tan lesions (TAN).

Once scientists mapped populations (the organism group used to make a particular genetic map) and screened them with SSR markers using Bulk Segregant analysis (BSA) to ID linked markers, they found several markers for Marker Assisted Selection. DNA markers can help ease the process of stacking different resistance loci into a single cultivar for a more sustainable resistance. SSRs, which are microsatellites used for mapping host resistance to soybean diseases. Researchers use SSR microsatellites because they have high polymorphism, co-dominance, reproducibility, good distribution throughout the soybean genome.

(Molecular mapping of two loci that confer resistance to Asian rust in soybean)

Since SSR markers are PCR based, they are easily assayed on gel electrophoresis systems. Soybean has over 1000 SSR markers across 20 linkage groups. Scientists use these markers to ID genome location of SBR resistant genes to help integrate these genes into modern breeding lines through marker assisted selection.

(Map Location of the Rpp1 Locus That Confers Resistance to Soybean Rust in Soybean)

The Experiment

The overall goal of the Asian Soybean Rust research at EMBRAPA is to increase the resistance of soybean plants to the disease. The researchers I worked with are trying to facilitate the breeding of plants and stacking of resistance genes by using Marker Assisted Selection.

Section 1

S1 Purpose

The purpose of this experiment was to test which method of DNA extraction from soybeans worked the best. Methods had to be low cost and fit the parameters of a high quality DNA sample. Danielle and I worked on this section of the experiment.

S1 Materials and Procedure

Several DNA extraction protocols were tested in this experiment.

TEST 1: von Post et al. 2003

Materials	Supplier
NaOH	Not specified
Tris-HCL	Not specified
EDTA	Not specified

A solution of 1M Tris-HCl (pH 8) was made. A buffer of 150µL Neutralizing solution was made from 30mM Tris-HCl (pH 8) and 1mM EDTA. An extraction buffer of 0.15M NaOH was created. Seed fragments were transferred to 1.5mL microtubes and ground with a pestle. 40µL of extraction buffer were added and the solution was mixed thoroughly. The solution was heated in a microwave oven at 10% power at 1400W for 30 seconds. 150µL of neutralizing solution was added to each tube. The solution was incubated at 4°C for 1 hour. Samples were diluted by 1:10 for the PCR reaction.

TEST 2: McDonald et al. 1994

Materials	Supplier
NaCL	Not specified
Tris-HCl	Not specified
EDTA	Not specified
Chloroform	Not specified
Isoamylic Alcohol	Not specified
Isopropanol	Not specified
CaCl2	Not specified
RNAse A	Not specified

A solution of 1mM CaCl2 was created. A solution of 40µg/mL RNAse A was created. A buffer was created from HCl, 300mM NaCl, 25mM EDTA, and 1% SDS. Another buffer (named TE) was created from 10mM Tris-HCl and 1mM EDTA. A final buffer was created using 24 parts Chloroform and 1 part isoamylic alcohol (24:1). 50mg seed powder was transferred to 1.5mL microtubes with 1000µL extraction buffer. The solution was mixed thoroughly and incubated for 20 minutes at 55°C. The solution was then centrifuged for 10 minutes at 10,000g. The supernatant was transferred into a new 1.5mL tube. 10µL Proteinase K and 10µL of 1mM CaCl2 was added and the resulting solution was incubated at 37°C for 1 hour. 900µL of chloroform isoamylic alcohol (24:1) was added and the solution was mixed by inversion for 5 min. The solution was centrifuged for 5 min. at 10,000 g. The supernatant was transferred to a new tube and 900µL cold isopropanol was added. After 2 minutes (for full precipitation), the solution was centrifuged for 5 minutes at 10,000g and air dried as a pellet for 10 minutes. The pellet was resuspended in a 300µL TE with 40µg/mL RNAse A and incubated for 30 minutes at 37°C. 900µL cold isopropanol was added. After waiting 2 minutes for precipitation, the sample was centrifuged for 5 minutes at 10,000g and air dried into a pellet for 10 minutes. The pellet was resuspended in 300µL TE.

TEST 3: Kotchoni and Gachomo 2009

Materials	Supplier
SDS	Not specified
NaCL	Not specified
Ethanol	Not specified
Isopropanol	Not specified
ddH2O	Not specified

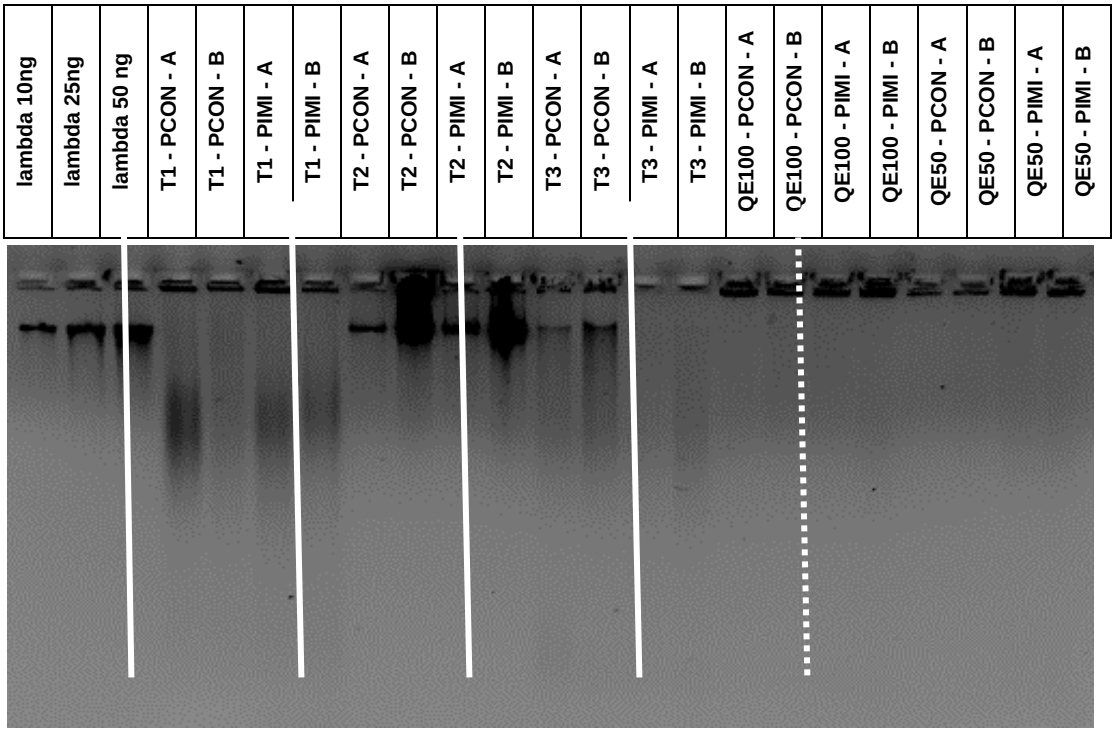
Solutions of 70% ethanol and cold isopropanol were created. An extraction buffer with 1% SDS and 0.5M NaCl was created. The seed powder was transferred to 1.5mL microtubes. 400µL extraction buffer was added. Solution was macerated and vortexed. The solution was centrifuged for 1 minute at 13,000 rpm. The supernatant was transferred into a new tube and 400µL isopropanol was added. The solution was mixed by inversion and centrifuged for 1 minute at 13,000 rpm. The supernatant was discarded and the DNA pellet was washed with 500µL 70% ethanol. The solution was centrifuged at 13,000rpm for 1 minute. The ethanol was discarded and the pellet was air dried. The DNA was dissolved in 50µL ddH2O and the extract was stored at 4°C for immediate use. (stored at -20°C for long term storage.)

TEST 4: QuickExtract Seed DNA Extraction Solution (from EPICENTRE Biotechnologies) Seed samples were crushed into small pieces. 10mg of sample was weighed out. The seed fragments were placed into a 500µL tube and 100µL of QuickExtract Seed DNA Extraction Solution was added. The solution was mixed by vortexing. The sample was heated at 65°C for 6 minutes and then heated at 98°C for 2 minutes. The sample was placed on ice. 1µL of sample was used as the template for PCR in 25-50µL reaction volumes.

For comparing techniques:
Nanodrop analysis was used to find the volume of DNA in the extracted fluid. PCR and then gel electrophoresis of the extracted fluid (both not diluted and diluted) used to test DNA integrity. Qubit assay used to produce absorbance spectrum to test how much of each substance is in the extraction (peaks mean different things)

S1 Findings
Spectra peaks. 230 nm: carbohydrates. 260 nm: DNA (what we want) 280 nm: proteins. Because we have peaks of substances other than DNA, the samples are contaminated. Best case scenario would be only a peak at 260 and nothing else. That would show that only DNA was extracted and there are no contaminants. Test 3 was the best during the PCR and Gel Electrophoresis test, which is most important. Test 3 was also good on the spectra. For the electrophoresis, only the control test 2 (already established method) and test 3 had visible bands in the results.

S1 Data, Results and Conclusions



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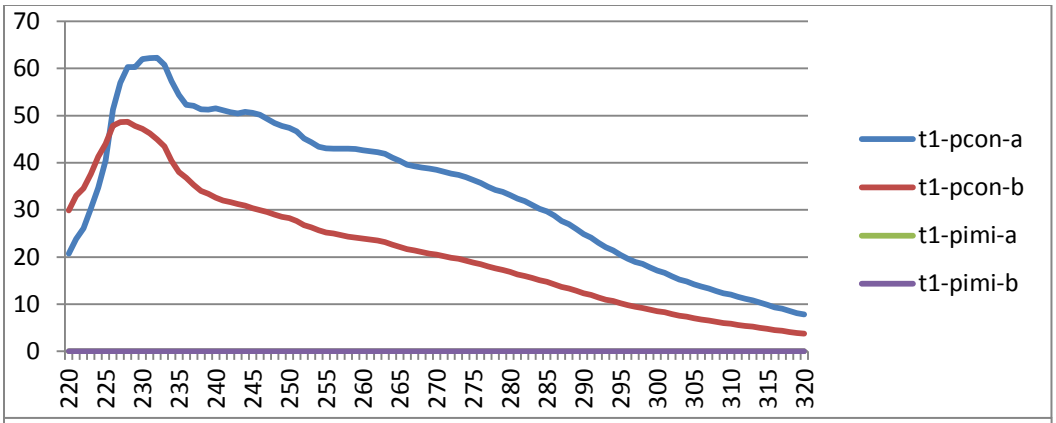
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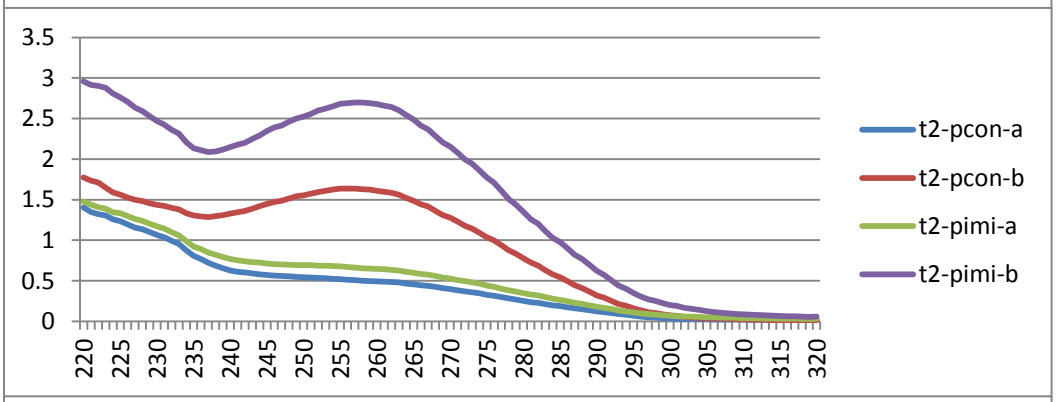
t1-pimi-a
t1-pimi-b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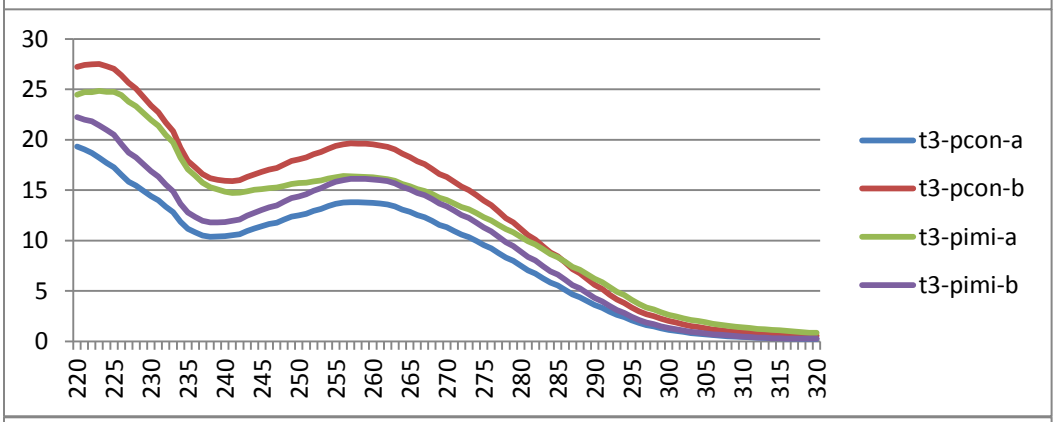
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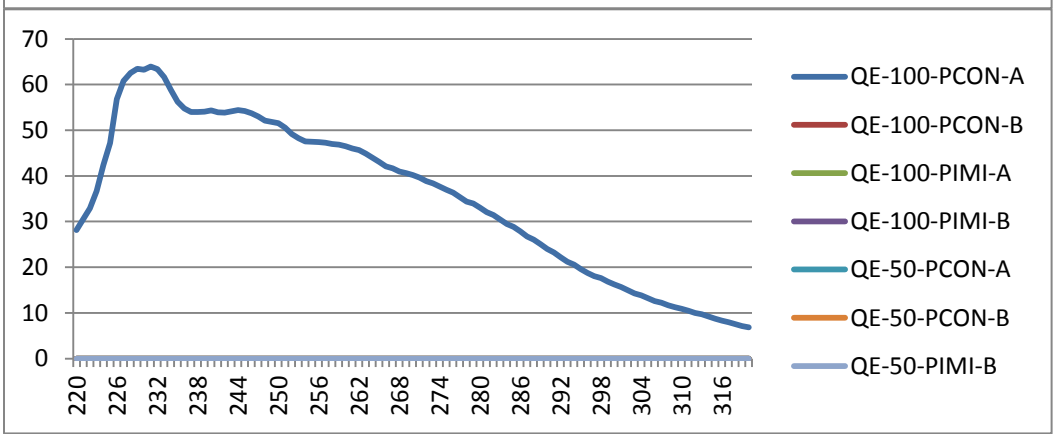
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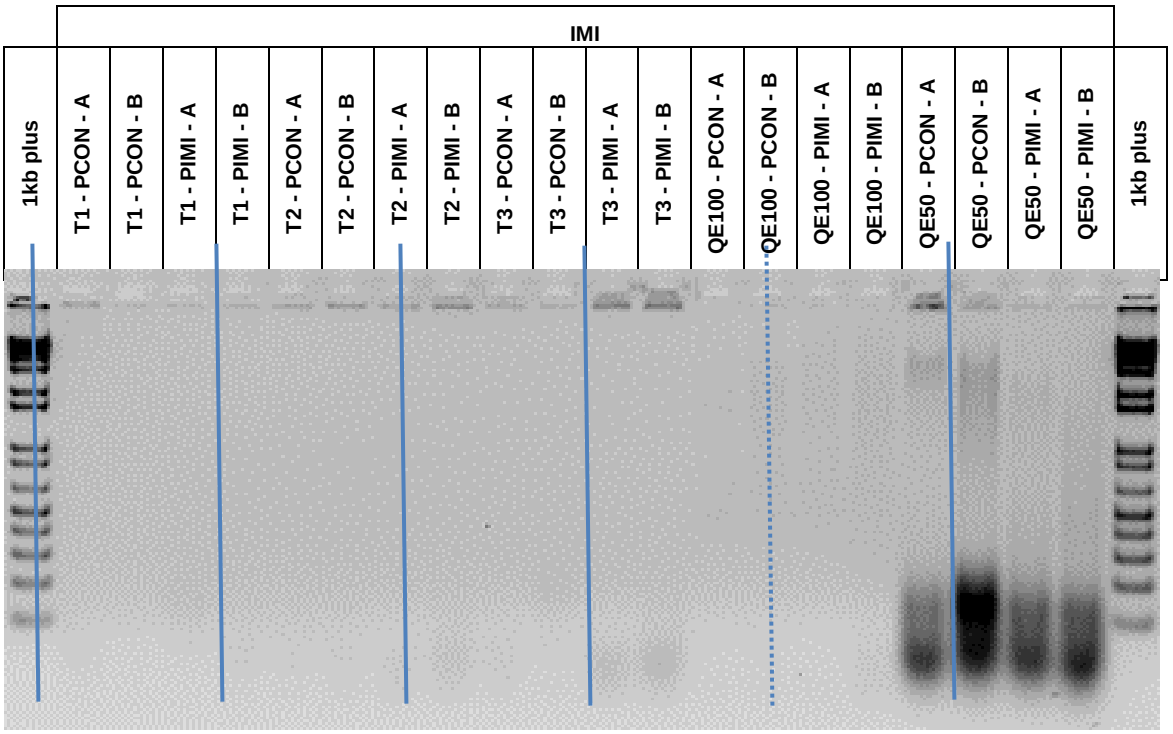
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T2 - PIMI - A
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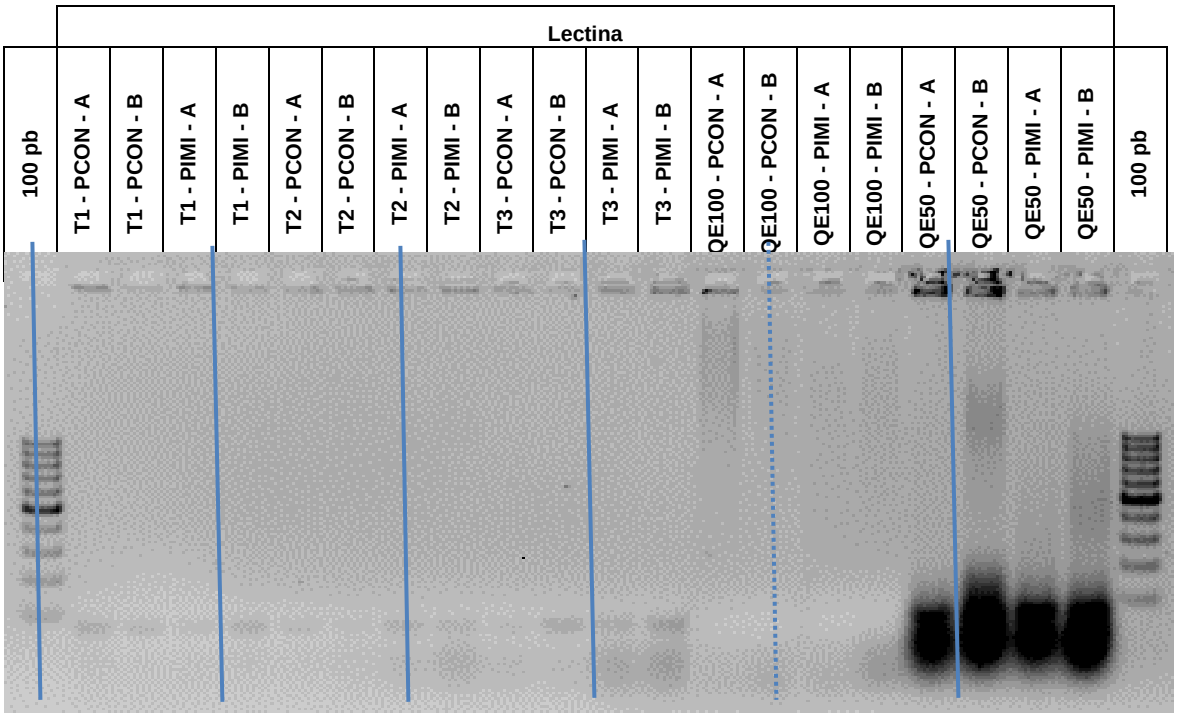
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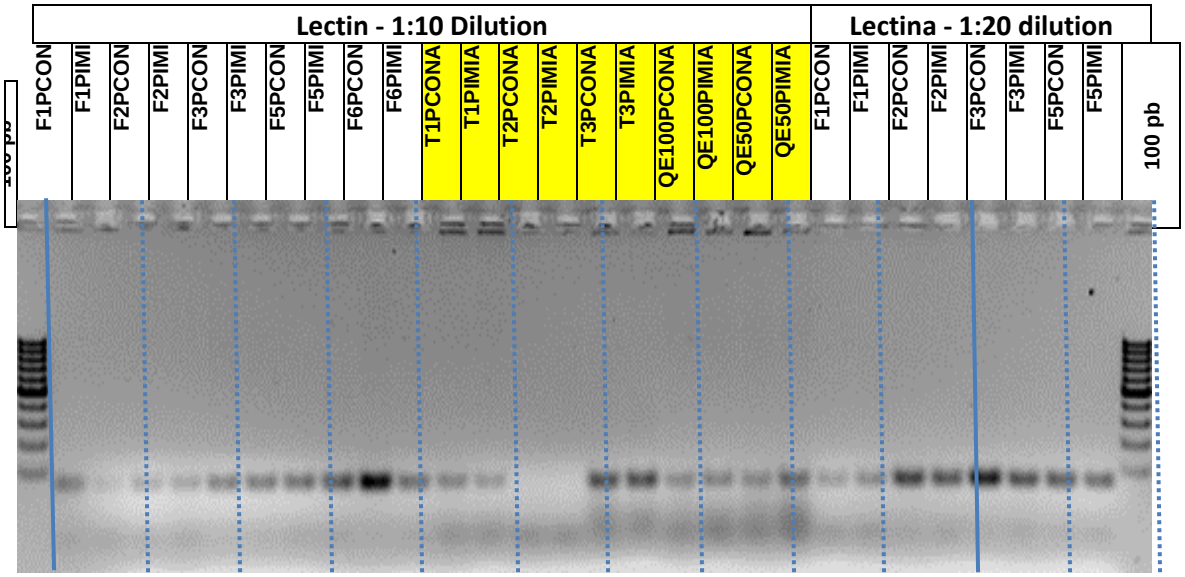
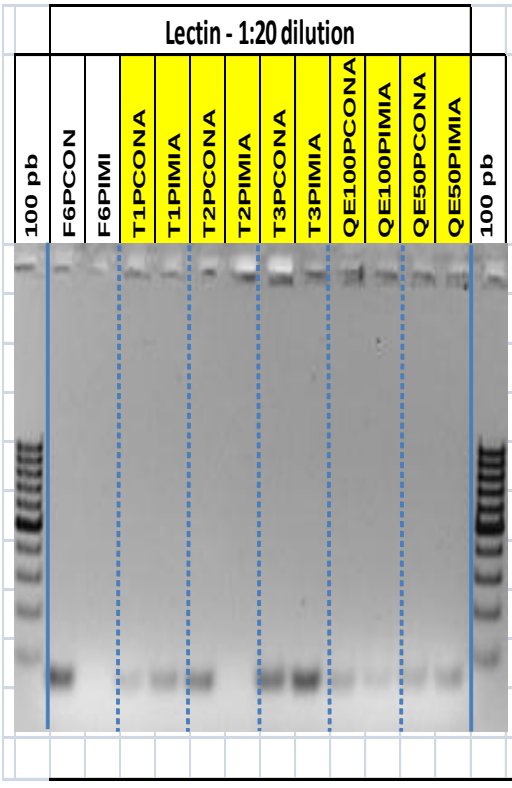
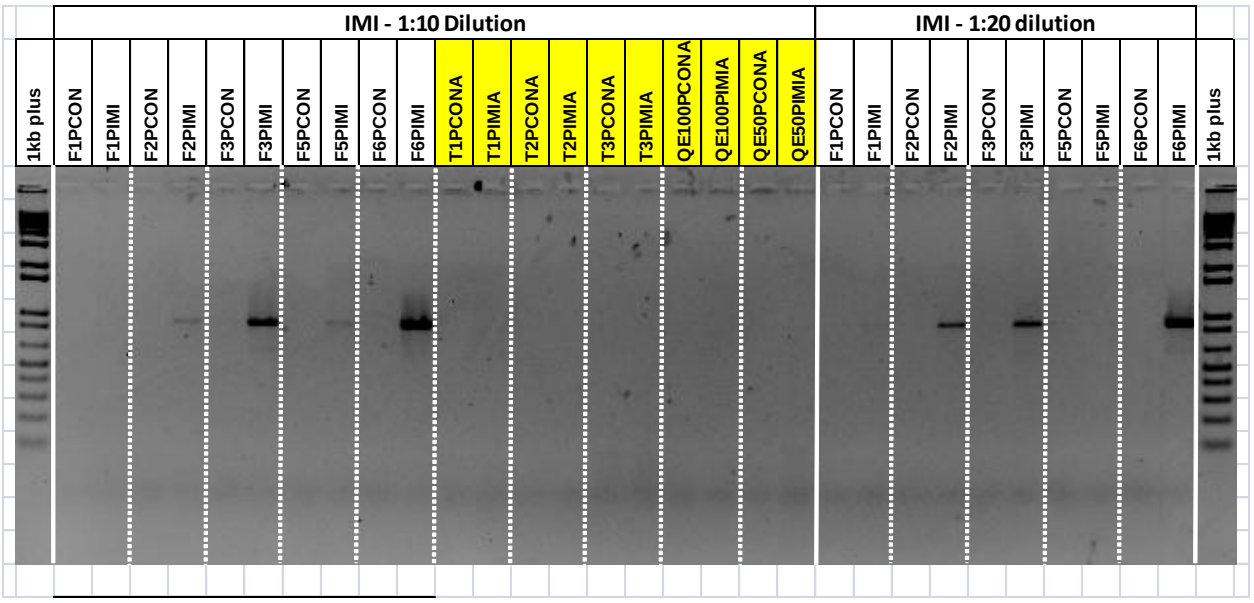
100 pb





The highlighted ones are the results of the extraction tests on seed samples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IMI - 1:20 dilution
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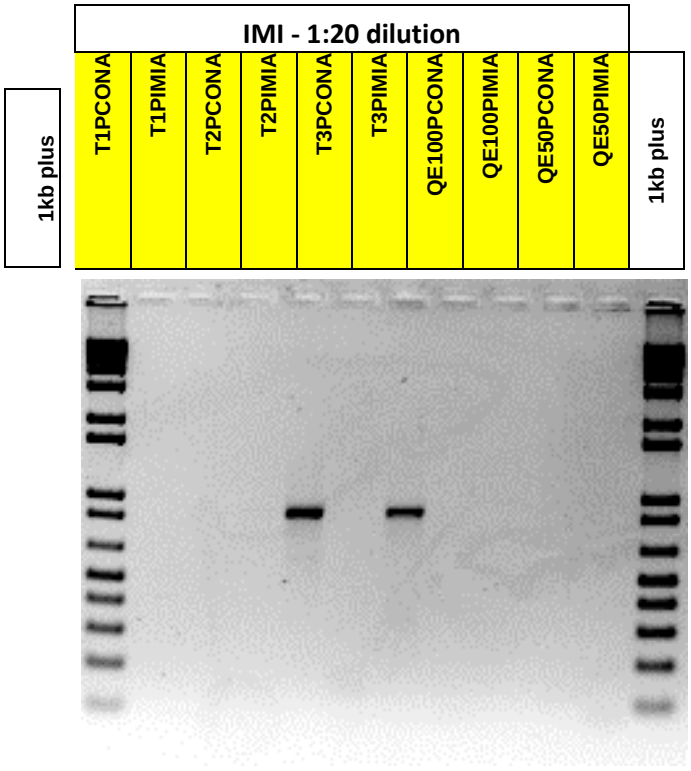
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QE50PCONA
QE50PIMIA
1kb plus

Sample ID	ng/ul	A260	A280	260/280	260/230
ti-pcon-a	2132.98	42.66	33.17	1.29	0.69
ti-pcon-b	1196.83	23.94	16.82	1.42	0.51
t1-pimi-a	-	-	-	-	-
t1-pimi-b	-	-	-	-	-
media	**1664.91**	**33.30**	**25.00**	**1.36**	**0.60**
t2-pcon-a	24.64	0.49	0.25	1.94	0.46
t2-pcon-b	80.52	1.61	0.78	2.07	1.12
t2-pimi-a	32.34	0.65	0.35	1.86	0.55
t2-pimi-b	133.89	2.68	1.35	1.99	1.09
media	**67.85**	**1.36**	**0.68**	**1.97**	**0.81**
t3-pcon-a	687.26	13.75	7.51	1.83	0.96
t3-pcon-b	976.65	19.53	11.17	1.75	0.84
t3-pimi-a	813.31	16.27	10.37	1.57	0.74
t3-pimi-b	803.07	16.06	8.92	1.80	0.95
media	**820.07**	**16.40**	**9.49**	**1.74**	**0.87**
QE-100-PCON-A	2324.19	46.48	33.02	1.41	0.74
QE-100-PCON-B	-	-	-	-	-
QE-100-PCON-B	-	-	-	-	-
QE-100-PIMI-A	-	-	-	-	-
QE-100-PIMI-B	-	-	-	-	-
QE-50-PCON-A	-	-	-	-	-
QE-50-PCON-B	-	-	-	-	-
QE-50-PIMI-B	-	-	-	-	-
media	**2324.19**	**46.48**	**33.02**	**1.41**	**0.74**

Starting material	Protocols	Samples	Nano Drop [ ] ng/uL	Media	Standard Deviation	Variation Coeficient (%)	Qubit [ ] ng/uL	Media	Standard Deviation	Variation Coeficient (%)
Leaves	Protocol 1	c1-pcon	431.19	400.16	22.79	5.7	4.88	4.95	0.54	11.0
		c1-pimi	367.94				4.92			
		c1-f1	386.75				4.32			
		f1-pcon	405.69				4.92			
		f1-pimi	417.79				5.96			
		f1-f1	391.57				4.72			
	Protocol 2	c2-pcon	202.62	159.39	37.48	23.5	2.26	4.98	6.16	123.7
		c2-pimi	196.33				17.40			
		c2-f1	132.67				4.32			
		f2-pcon	156.54				2.16			
		f2-pimi	164.01				2.26			
		f2-f1	104.18				1.48			
	Protocol 3	c3-pcon	82.61	41.50	24.74	59.6	6.52	5.49	2.33	42.4
		c3-pimi	41.94				5.88			
		c3-f1	17.44				1.99			
		f3-pcon	56.56				9.00			
		f3-pimi	29.33				5.16			
		f3-f1	21.11				4.40			
	Protocol 5	c5-pcon	42.18	35.23	8.30	23.6	-	1.17	0.37	31.7
		c5-pimi	33.12				1.22			
		c5-f1	21.44				0.79			
		f5-pcon	42.24				1.20			
		f5-pimi	41.21				1.75			
		f5-f1	31.18				0.91			
	Protocol 6 (control)	c6-pcon	69.07	45.78	17.68	38.6	6.40	4.81	1.65	34.2
		c6-pimi	35.23				1.95			
		c6-f1	36.4				5.20			
		f6-pcon	67.85				6.32			
		f6-pimi	35.41				4.16			
		f6-f1	30.71				4.84			
Seeds	Protocol 1	ti-pcon-a	2132.98	1664.91	661.96	39.8	102.80	46.54	37.62	80.8
		ti-pcon-b	1196.83				27.68			
		t1-pimi-a	-				31.40			
		t1-pimi-b	-				24.28			
	Protocol 2	t2-pcon-a	24.64	67.85	50.50	74.4	3.36	5.94	2.23	37.6
		t2-pcon-b	80.52				7.96			
		t2-pimi-a	32.34				4.80			
		t2-pimi-b	133.89				7.64			
	Protocol 3	t3-pcon-a	687.26	820.07	119.01	14.5	18.64	27.47	18.97	69.1
		t3-pcon-b	976.65				55.60			
		t3-pimi-a	813.31				14.36			
		t3-pimi-b	803.07				21.28			
	Quick Extract - 100 uL	QE-100-PCON-A	2324.19	2324.19	-	-	7.72	8.54	1.32	15.5
		QE-100-PCON-B	-				10.00			
		QE-100-PCON-B	-				7.16			
		QE-100-PIMI-A	-				9.28			
	Quick Extract - 50 uL	QE-100-PIMI-B	-	-	-	-	11.84	9.85	2.53	25.7
		QE-50-PCON-A	-				6.16			
		QE-50-PCON-B	-				10.96			
		QE-50-PIMI-B	-				10.44			

Section 2

S2 Purpose

The purpose of this section was to identify Simple Sequence Repeats (SSR) for use in Marker Assisted Selection of genes that confer resistance to Asian Soybean Rust (ASR). Tatiana and I worked on this section of the experiment.

S2 Materials and Procedure

PCR: Samples of soybean DNA were obtained using an extraction protocol and placed in a 96-well plate. A DNA mix was created:

	Volume	Final
Water (Milli-Q 3X distilled)	3.0 µL	---
Buffer 10X	1.0µL	1X
MgCl2 (50mM)	0.3µL	50mM/µL
dNTP (2.5mM)	0.52µL	2.5mM
Primer F (forward) (2µM)	1.0µL	2µM
Primer R (reverse) (2µM)	1.0µL	2µM
Taq Polymerase (5 U/µL)	0.2µL	1U
DNA (10mg/µL) (SAMPLE)	3.0µL	30mg

*The formula above is for one sample of DNA. For 10 samples, multiply everything by 10. Mix was added to the samples using a pipette. In experiments with many primers, mixes are sometimes made without primers. Primers are added later separately. Samples were placed into the thermal cycler (Veriti 86 well Thermal Cycler' from Applied Biosystems) on a SSR Fast program:

1. 94°C for 1 min.
2. 94°C for 40 sec.
3. 55°C for 40 sec.
4. 72°C for 40 sec.
5. 72°C for 1 min.
6. 4°C forever

Polyacrylamide Gel Electrophoresis (PAGE):

Alcohol was used to clean glass plates. Two glass plates were put together and sealed using a rubber frame seal. Plates were clipped together using binder clips. Mixes were prepared. Mix A was the running gel, which is applied first in between the plates.

Mix A: Running Gel:

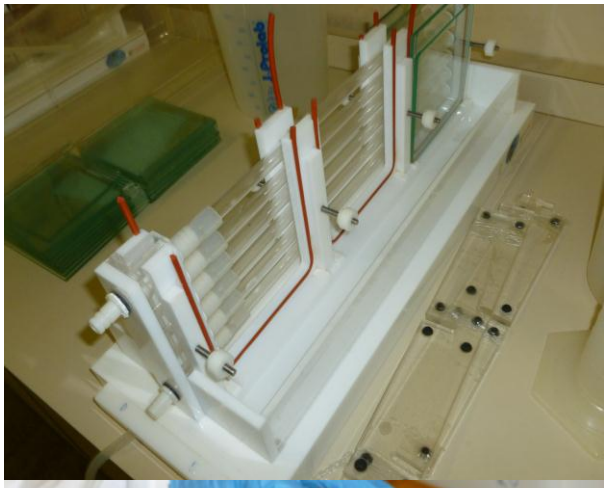
	Volume
Water (Milli-Q)	3.3mL
Polyacrylamide	8.9mL
Tris HCl (1.5mL)	4.2mL
Ammonium Persulfate 10%	63.8µL
TEMED	15.8µL

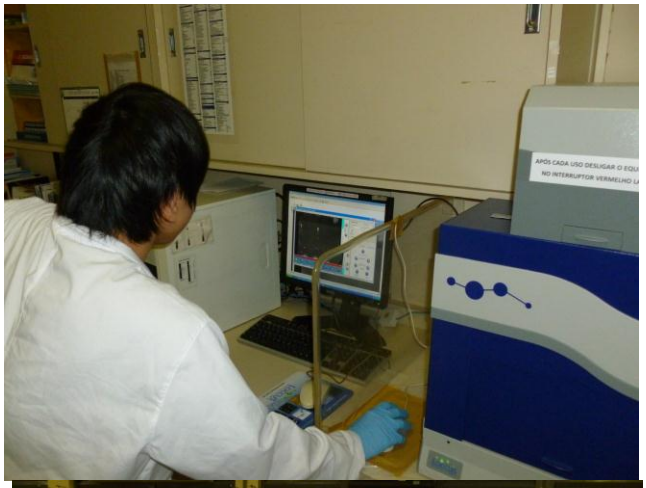
Running gel was mixed with a syringe. The running gel was applied in between the plates using a syringe. A line of alcohol was placed in between the plates as well. After 20-25 minutes, the alcohol was poured out. Running gel had already solidified. Stacking gel, Mix B was applied.

Mix B: Stacking Gel:

	Volume
Water (Milli-Q)	2.04mL
Polyacrylamide	0.6mL
Tris HCl (0.5M)	0.96mL
Amonnium Persulfate	30µL
TEMED	4µL

Comb was placed between the plates immediately after applying stacking gel with syringe. Plate was let rest for 30 minutes, then covered with Saran wrap and placed in refrigerator. When ready for electrophoresis: Plates were placed in position in the electrophoresis apparatus. The middle portion was filled with Tris-Glycine 10X buffer until full to the edges. Sides were filled with buffer as well. Electric cords were plugged in and the electrophoresis procedure was run at 250 volts for around 6 hours. After run time, plates were taken out. Rubber seals were removed. Plates were placed in boxes filled with ethidium bromide solution (250mL H20; 60µL ethidium bromide). Boxes were placed inside shaker for 10-15 minutes. Plates were removed. Loccus Biotech L Pix molecular imaging machine was used for gel electrophoresis images (high setting on Transiluminator function).

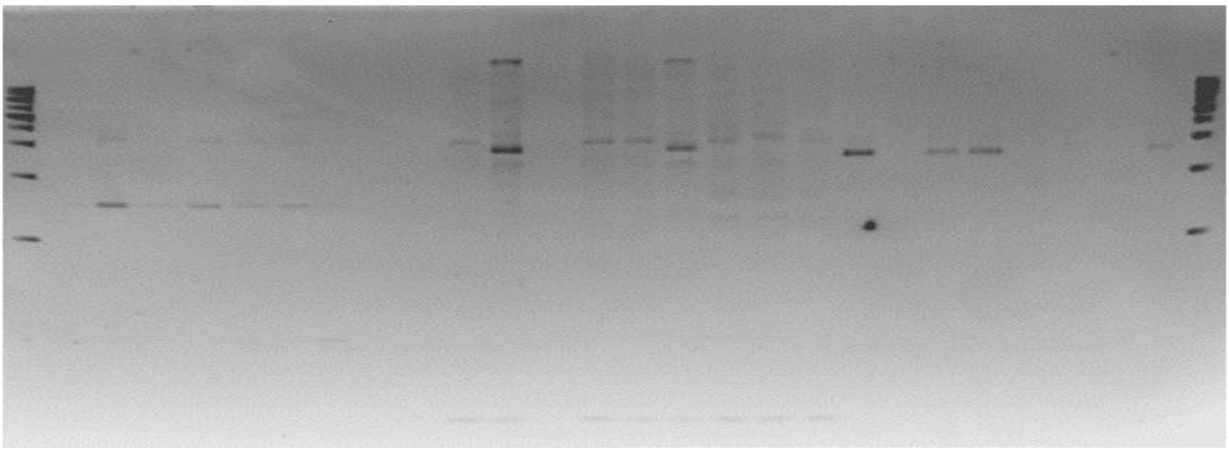




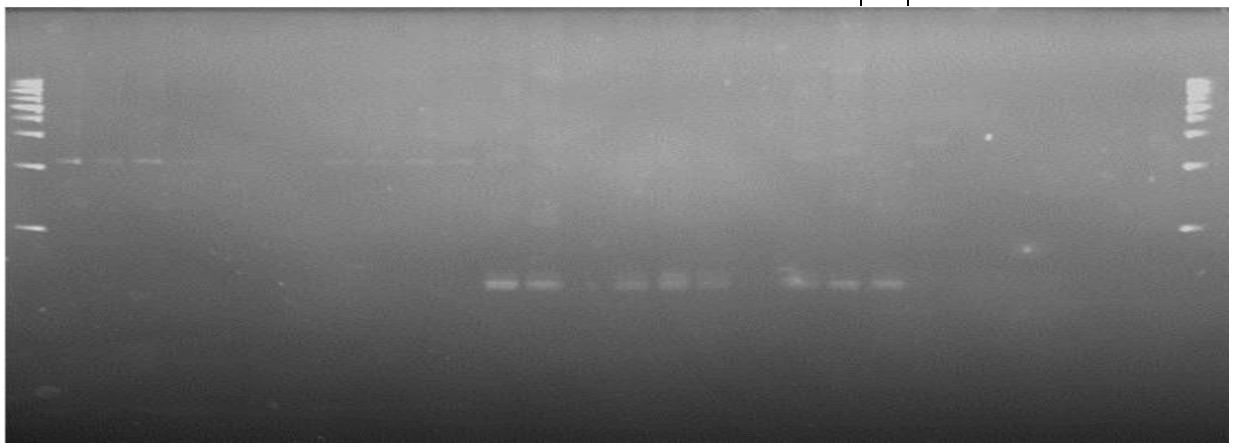
S2 Data

The following are gel electrophoresis photographs resulting from the process above:

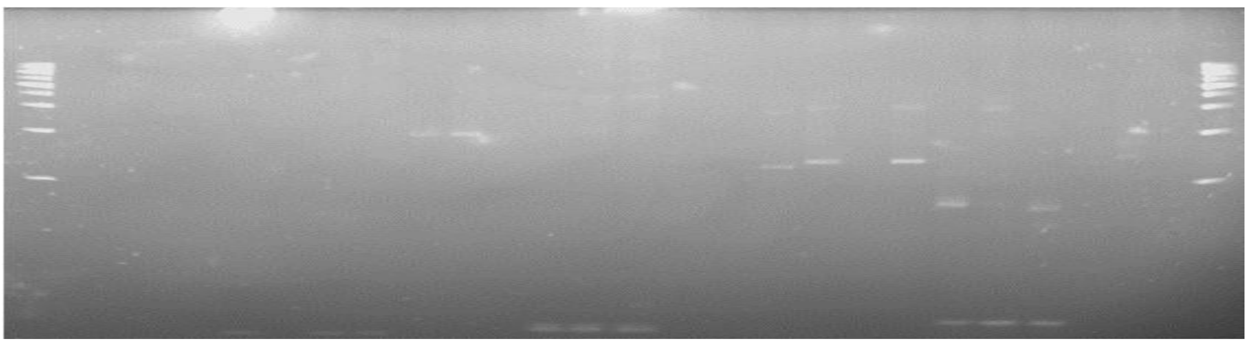
PCR																														
Rpp1																														
	Sct_187								Sat_372										Sat_117										
Ladder 100pb	**PI561356**	**Sambaíba**	**E10-572**	**PI587880A**	**E10-596**	**PI594538A**	**BRSMG 751SSR**	**E10-1643**	0	PI561356	Sambaíba	0	E10-572	PI587880A	E10-596	PI594538A	BRSMG 751SSR	E10-1643	**PI561356**	**Sambaíba**	**E10-572**	**PI587880A**	**E10-596**	**PI594538A**	**BRSMG 751SSR**	**E10-1643**	Ladder 100pb



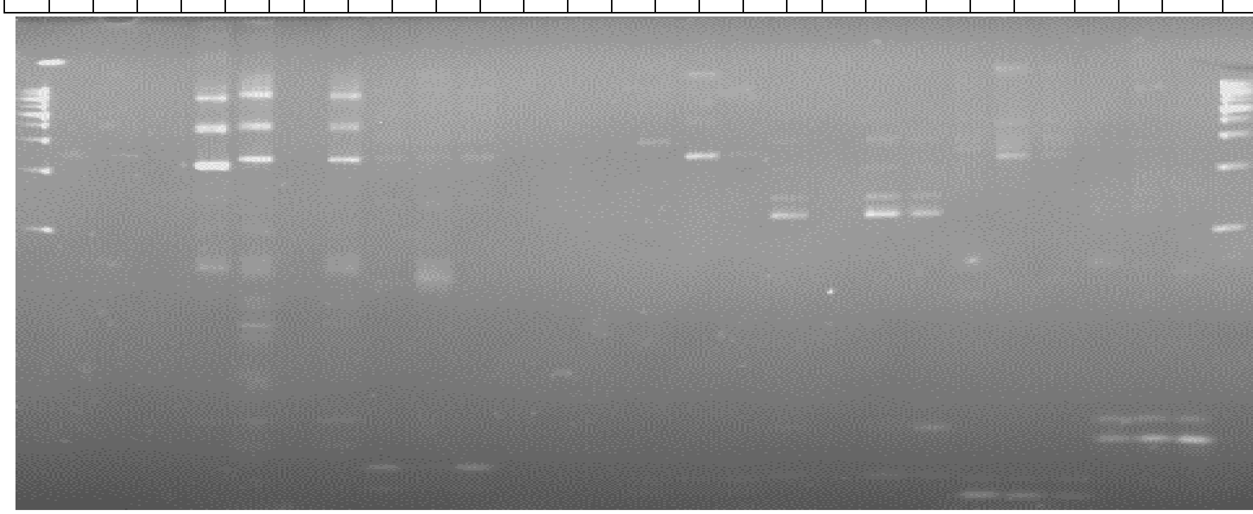
PCR																														
Rpp1																														
	Satt472								Sct_199								Sat_064													
Ladder 100pb	PI561356	Sambaíba	E10-572	PI587880A	0	0	E10-596	PI594538A	BRSMG 751SSR	E10-1643	**PI561356**	**Sambaíba**	**E10-572**	**PI587880A**	**E10-596**	**PI594538A**	**BRSMG 751SSR**	**E10-1643**	PI561356	Sambaíba	E10-572	PI587880A	0	0	0	0	Ladder 100pb			



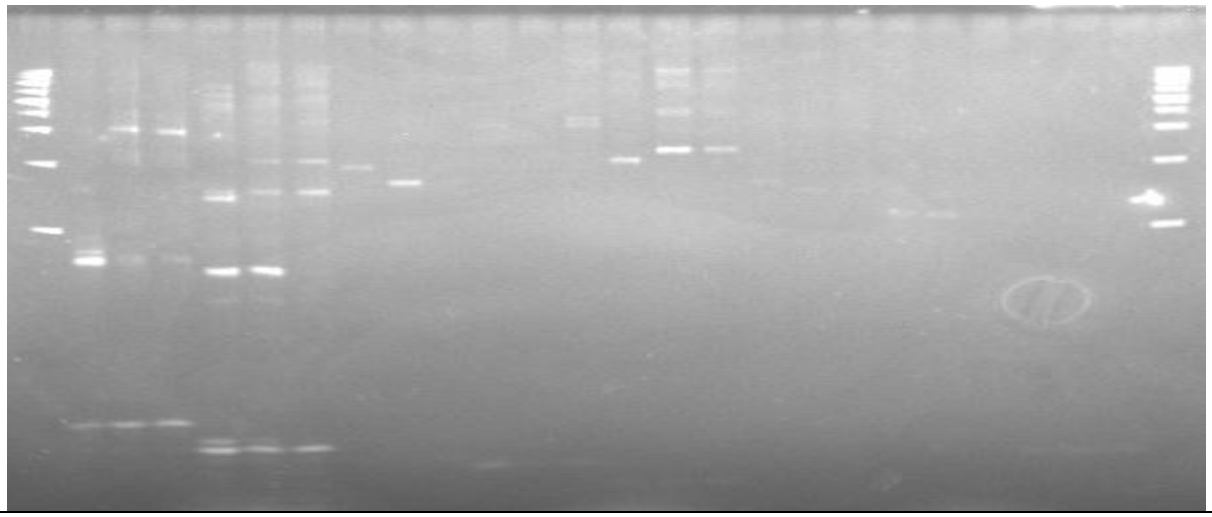
PCR																											
Rpp1					Rpp2																						
	Sat_064				Sat_93				Sct_001			Sat_361					Satt215				Sat350			Sat380			
Ladder 100pb	E10-596	PI594538A	BRSMG 751SSR	E10-1643	PI197182	0	BRS262	E10-520	PI197182	BRS262	E10-520	PI197182	BRS262	E10-520	0	0	PI197182	BRS262	0	E10-520	PI197182	BRS262	E10-520	PI197182	BRS262	E10-520	Ladder 100pb



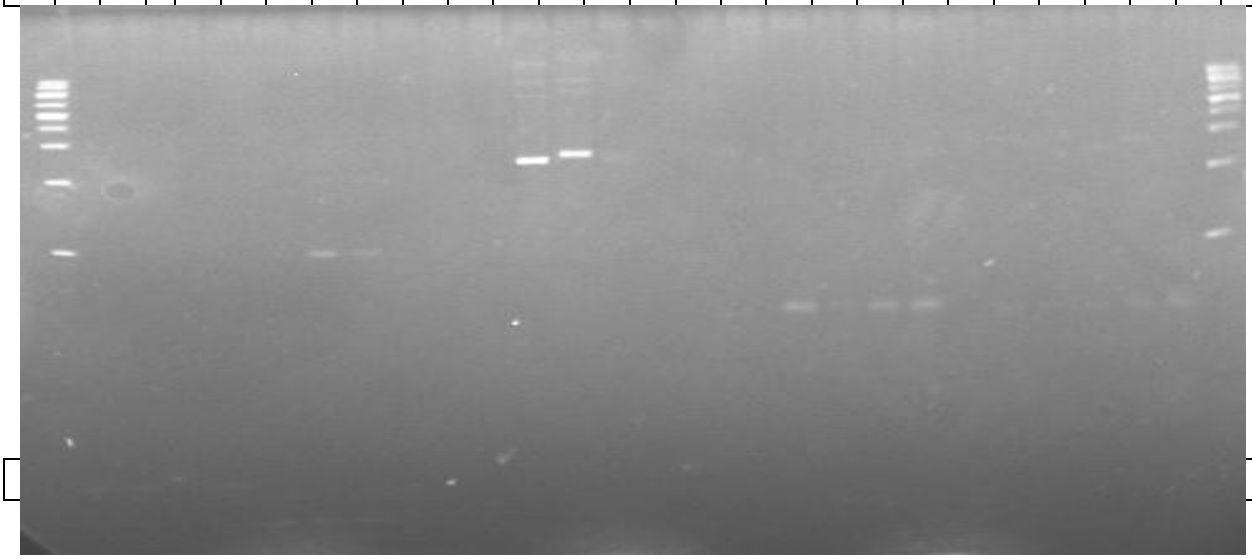
PCR																											
Rpp2																											
	Satt183			Sat622				Sat_165			Satt620			Satt621			Sct_193				Sat_151			Sat_412			
Ladder 100pb	PI197182	BRS262	E10-520	PI197182	BRS262	0	E10-520	PI197182	BRS262	E10-520	PI197182	BRS262	E10-520	PI197182	BRS262	E10-520	PI197182	0	BRS262	E10-520	PI197182	BRS262	E10-520	PI197182	BRS262	E10-520	Ladder 100pb



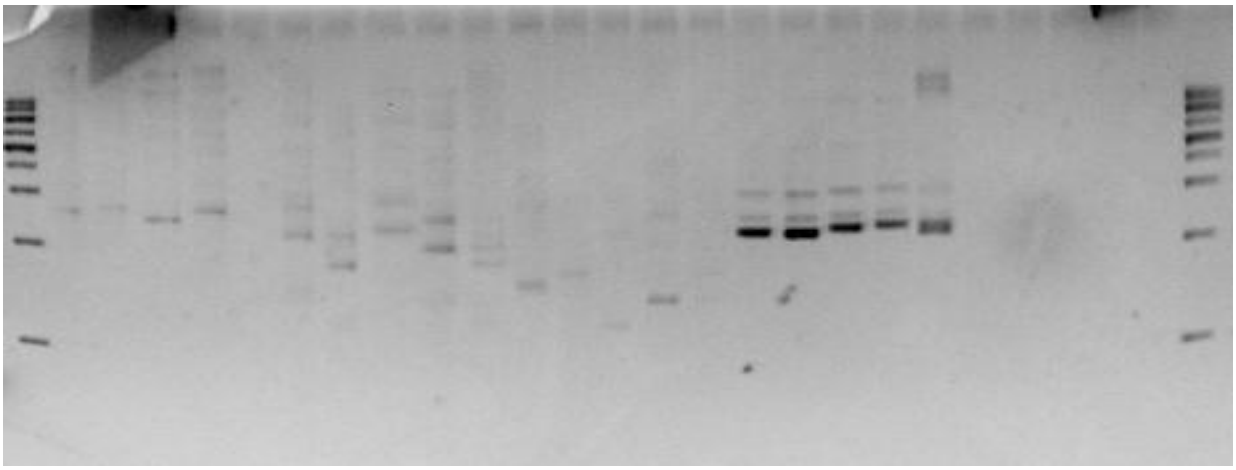
PCR																										
Rpp 2						Rpp3																				
	Sat_350			Sat244			Satt307			Satt202			Sat_263			Satt079			Satt460			Sat_238			
Ladder 100 pb	PI197182	BRS262	E10-520	**PI197182**	**BRS262**	**E10-520**	Hyuuga	Invernada	E10-518	**Hyuuga**	**Invernada**	**E10-518**	Hyuuga	Invernada	E10-518	**Hyuuga**	**Invernada**	**E10-518**	Hyuuga	Invernada	E10-518	**Hyuuga**	**Invernada**	**E10-518**	**Ladder 100 pb**



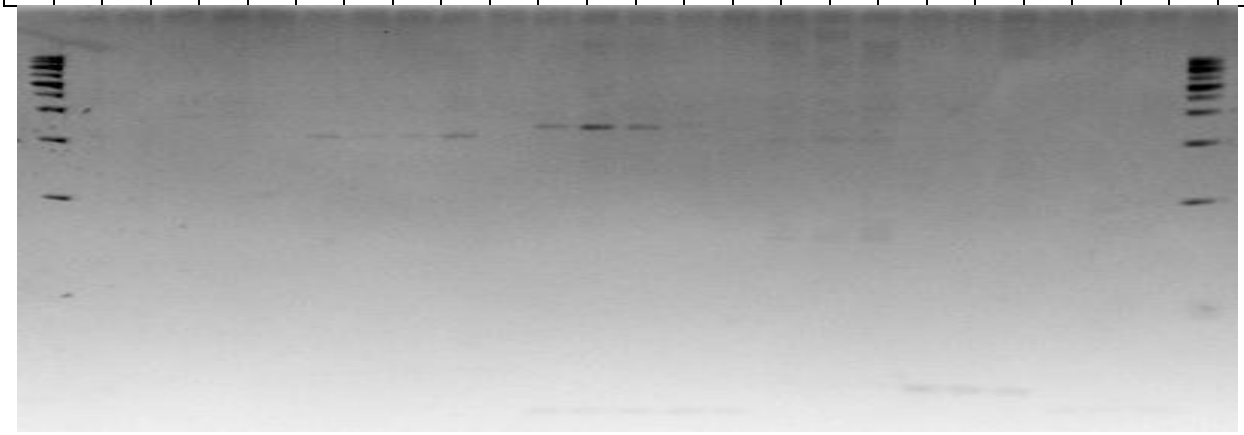
PCR																										
Rpp 3																Rpp4										
	Satt708				Sat_142			Satt100			Staga001			Sct_028			Satt288					Satt517					
Ladder 100 pb	Hyuuga	Invernada		E10-518	**Hyuuga**	**Invernada**	**E10-518**	Hyuuga	Invernada	E10-518	**Hyuuga**	**Invernada**	**E10-518**	Hyuuga	Invernada	E10-518	Sambaíba	E10-504	PI459025A	BRS284	E10-563	**Sambaíba**	**E10-504**	**PI459025A**	**BRS284**	**E10-563**	**Ladder 100 pb**



Rpp4																							
	Satt503					Sat_164					Satt012					AF162283					Sct_199					
Ladder 100 pb	Sambaíba	E10-504	PI459025A	BRS284	E10-563	**Sambaíba**	**E10-504**	**PI459025A**	**BRS284**	**E10-563**	Sambaíba	E10-504	PI459025A	BRS284	E10-563	**Sambaíba**	**E10-504**	**PI459025A**	**BRS284**	**E10-563**	Sambaíba	E10-504	PI459025A	BRS284	E10-563	**Ladder 100 pb**



PCR																									
	Rpp4															Rpp5									
	Sat_117					Satt472					Satt612					Sat_275			Sat_208			Sat_166			
Ladder 100 pb	**Sambaíba**	**E10-504**	**PI459025A**	**BRS284**	**E10-563**	Sambaíba	E10-504	PI459025A	BRS284	E10-563	**Sambaíba**	**E10-504**	**PI459025A**	**BRS284**	**E10-563**	Shiranui	BRS232	E10-683	**Shiranui**	**BRS232**	**E10-683**	Shiranui	BRS232	E10-683	**Ladder 100 pb**



S2 Analysis and Conclusions

The samples in this experiment came from a parent generation of soybeans. In the future, the researchers at EMBRAPA will extract DNA samples from the filial generation and run this experiment again. That generation will become a parent generation as the plants are crossed. This cycle will continue until a stacked Rpp resistance is conferred upon the soybean line. Below is an analysis chart. The SSR primers were chosen for their proximity to the gene region of Rpp X. The experiment tested whether or not these SSR primers actually worked to identify the presence of Rpp X.

Locus/GL/ Chromosome	SSR according to Soybase	Parent Analysis
Rpp1/G/18	Sct_187	No polymorphism
	Sat_372	Polymorphism between PI561356 and Sambaíba and E10-572 has the resistant band
		Polymorphism between PI587880A and Sambaíba and E10-596 has the resistant band
		Polymorphism between PI594538A and BRSMG 751SSR and E10-1643 has the resistant band
	Sat_117	No amplification
	Satt191	Have not tested yet
	Satt472	No Polymorphism
	Sct_199	No Polymorphism
	Sat_064	No amplification
Rpp2/J/16	Sat_093	No amplification
	Sct_001	No Polymorphism
	Sat_361	No Polymorphism
	Satt215	Polymorphism between PI 197182 and BRS262 and E10-520 does not have the resistant band
	Sat_255	Have not tested yet
	Satt 380	No amplification
	Satt183	No Polymorphism
	Satt622	Polymorphism between PI197182 and BRS262 and E10-520 does not have the resistant band
	Sat_165	No amplification
	Satt529	Have not tested yet
	Satt621	Have not tested yet
	Sct_193	No Polymorphism
	Sat_151	Polymorphism between PI197182 and BRS262 and it appears that E10-520 does not have the resistant band
	Sat_412	No Polymorphism

	Satt280	Appears that the Polymorphism between PI197182 and BRS262 e E10-520 has the resistant band
	Sat_366	Polymorphism between PI 197182 and BRS262 and E10-520 does not have the resistant band
	Satt 620	No amplification
	Sat_350	No Polymorphism
	Sctt011	Have not tested yet
	Satt244	No Polymorphism
Rpp3/C2/6	Sat_263	Polymorphism between Hyuuga and Invernada and E10-518 is heterozygous
	Satt079	No amplification
	Satt460	No amplification
	Sat_238	No amplification
	Satt708	No amplification
	Sat_142	Have not tested yet
	Sat_251	Have not tested yet
	Satt100	No amplification
	Staga001	Polymorphism between Hyuuga and Invernada and E10-518 does not have the resistant band
	Sct_028	No amplification
	Satt202	No Polymorphism
	Sat_252	Have not tested yet
	Satt307	No amplification
Rpp4/G/18	Sat288	No Polymorphism
	Sat_143	Have not tested yet
	Satt517	No Polymorphism
	Satt503	E10-504. This band shows the same susceptible pattern as its parent Sambaiba
		Polymorphism between PI459025A and BRS 284
	Sat_164	E10-504 não apresenta mesmo padrão de banda suscetível do pai suscetível Sambaíba
		Polymorphism between PI459025A and BRS 284 and E10-563 does not have the resistant band
	Satt012	No amplification
	AF162283	No Polymorphism
	Sct_199	No Polymorphism
	Sat_117	No amplification
	Satt191	Have not tested yet
	Satt472	No Polymorphism
	Satt612	No Polymorphism
Rpp5/N/3	Sat_275	Polymorphism between Shiranui and BRS 232 and E10-683 has the resistant

		band
	Sat_208	No Polymorphism
	Sat_166	No Polymorphism
	Satt485	No amplification
	Satt584	Polymorphism between Shiranui and BRS 232 and E10-683 Is heterozygous
	Sat_084	Polymorphism between Shinarui and BRS232, E10-683 was not amplified
	Sat_280	Polymorphism between Shiranui and BRS 232 and E10-683 has the resistant band
	Satt080	No Polymorphism
	Sat_266	Polymorphism between Shiranui and BRS232 and E10-683 has the resistant band
	Satt387	Have not tested yet
	Sat_236	Have not tested yet
	Sat_033	No amplification
	Passed (Good primers)	
	Discarded	
	No amplification	

The point of this entire project (of which I was only present for a small part of) at EMBRAPA is to adapt resistant cultivars to the Brazilian agricultural environment. Scientists crossed a resistant cultivar (which is not adapted to Brazil) and a Brazilian cultivar (which is not resistant to ASR) in hopes of producing a plant with native adaptations but is also resistant to ASR. However, there is no way to physically see whether the children have inherited the "correct" gene region. It's impossible phenotypically, so researchers use genotypes: Simple Sequence Repeats to test if the filial generation has the resistance gene. So, the experiment I conducted was used to find whether or not a specific SSR can actually indicate if a child plant has the resistance gene. I was testing if a SSR was useful in identifying resistance genes.

Food Security

Soybeans are a vital crop for improving food security in the world. As described by EMBRAPA Deputy Head of Technology Transfer, Amelio Dall'Agnol, new agricultural techniques and processes that were first tested on soybeans in Brazil spread to other crops because of soybeans' use as a model organism. (FAO "The Role…")

Soybean, a major crop, is still expanding throughout the world. Both an excellent source of calories and protein, soybeans are widely used in human food, vegetable oil and animal feed. Saving the production of soybean from being devastated by ASR is a very important task that researchers at EMBRAPA have undertaken. Keeping costs down by reducing the need for expensive fungicides is also a goal.

(FAO Commodities)

According to the Illinois Soybean Association, soybean is a healthy and nutritious food, present in three of the USDA's MyPlate food groups. It has been shown to fight heart disease. Increased production of soybeans, lowered costs, and thus greater availability will help benefit food-insecure people throughout the world. (Illinois Soybean Association)

Brazil

My surface immersion in Brazilian food (meat!) and culture was amazing. But after a while, what I really liked the most about Brazil is the warmth of its people, the sense of development, and the diversity. Everyone I talked to in Brazil was very courteous and opened up to conversation quickly. I also loved how Brazil as a country is rapidly growing and developing into a South American power. I felt that spirit of advancement especially keenly through my involvement with science at EMBRAPA. Finally, Brazil also made me feel welcome as it is an amazing melting pot of cultures (gauchos, European, Asian, indigenous, Caribbean) and ethnicities just like the U.S.

Personal Reflection

I've always been interest in science. Growing up, I leafed through encyclopedias and other reference books to learn more about our natural world. In high school, I took many science courses, competed in events such as science fair and USA Biology Olympiad, and interned in university research labs. Until my World Food Prize experience that culminated with my BR internship, however, science was extremely individualistic. The Borlaug-Ruan internship changed my perception of science and proved to me that science is not just a researcher sitting alone at his workbench; it's also people cooperating while arranged around a room, a lab, multiple labs, and the world.

I observed this collaboration immediately in my first couple of days working at EMBRAPA. I saw graduate students, technicians, and researchers all helping each other with an assortment of tasks. Many people took the time to help their lab-mates who weren't even working on the same project or research area. A graduate student named Noelle had worked in Japan for 8 months. She taught other students and me some extraction techniques she had learned there. Another student named Valeria gave me tips and helped me save time on PCR reactions. I later passed on these skills to lab-newcomers. While these two examples occurred within a lab, I also witnessed cooperation between laboratories and EMBRAPA units as well as internationally between EMBRAPA and the Japanese company JIRCAS.

In Brazil, I finally understood that science is really about cooperation between people to learn more about nature. This internship spurred my passion for science by revealing its aspect of collaboration.

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Pictures