AN INVESTIGATION OF VIRUS INFECTIONS IN SWEET POTATO CROPS

THE WORLD FOOD PRIZE

Fabian Leon

Borlaug-Ruan International Intern

World Food Prize Foundation

International Potato Center

Lima, Peru

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ONLINE RESOURCES

International Potato Center	http://cipotato.org/
World Food Prize	http://worldfoodprize.org/

INTRODUCTION

Hunger can come in many shapes and sizes, it can be found in every part of the planet, and it can be caused by a plethora of factors. I've seen hunger and poverty in my neighborhood in Kentucky and I have witnessed the hungry pleas of kids on the streets of Mexico. I have grown up with a perspective of being grateful for my food security and have understood, and appreciated, how hard my parents had to work as agricultural laborers so that I was raised with a hunger for knowledge, instead of food.

I have been instilled with a sense of responsibility to take every opportunity I can in order to further agriculture and do my part in securing sustainable agriculture methods to help feed the world. My appreciation for agriculture and hard work has molded me into the student I am today. It is what brought me to the International Potato Center in Peru and it is what keeps me working towards furthering my involvement in the most important scientific field of research: Agriculture.

CENTRO INTERNACIONAL DE LA PAPA

The International Potato Center, commonly referred to as CIP for its Spanish acronym, was founded in Lima, Peru in 1971 with the purpose of delivering sustainable solutions to the world problems of hunger, poverty, and degradation of natural resources by furthering research and development in the areas of roots and tubers. CIP still has its headquarters in Lima, Peru but has also built up a global presence with offices in 20 developing countries across Asia, Africa, and Latin America.

VIROLOGY

The study of viruses and virus-like agents was introduced to me entirely by the staff of the laboratory at CIP. Segundo Fuentes, especially, is such a spectacular teacher that had me completely debriefed on any experiment I ever had to undertake. My lab mentors, namely Ana Perez and Marco Galvez, made sure I understood concepts of virus interactions and lab equipment logistics and Heidy Gamarra and Carlos Chuquillanqui trained me for work in the field and greenhouse.

THE WORLD FOOD PRIZE

AN INVESTIGATION OF VIRUS INFECTIONS IN SWEET POTATO CROPS AROUND THE WORLD

Fabian Leon

World Food Prize

Borlaug-Ruan Intern 2015

International Potato Center (CIP)

Lima, Peru

ABSTRACT

The Sweet Potato (Ipomoea Batatas) is a root vegetable native to regions in America. In Peru, varieties of potatoes serve as a staple foods found in almost every meal from the Andes Mountains down to the coast in Lima. Sweet Potatoes, especially, have a plethora of health benefits and large amounts of Beta Carotene and are thus important for Vitamin A intake in people's diets all around the world.

It is thus vital to protect Sweet Potato crops from viral infections that occur all over the world. A few examples of Sweet Potato viruses are Sweet Potato Feathery Mottle Virus (SPFMV), Sweet Potato Chlorotic Stunt Virus (SPCSV), and Sweet Potato Vein Clearing Virus (SPVCV). It is also possible for a plant to be co-infected by two viruses making for synergistic, more problematic, diseases as with the case of Sweet Potato Virus Disease (SPVD). These viruses especially impact impoverished families and those living far from cities, demographics that rely more heavily on the sweet potato for food.

Viruses can have many effects and to analyze just how they impact production yields, the virology lab, including myself, at the International Potato Center has taken to examine samples from Sweet Potato crops in Peru to further study the impacts that virus infections have and don't have. This study will be serve to assist in a project running samples that

were retrieved from plants from Peruvian crops through extraction, Polymerase Chain Reaction, and Gel Electrophoresis methods to further investigate virus infections.

INTRODUCTION

Malnutrition, poverty, and food insecurity have always been problems that agriculturalists have tried to combat. Protecting harvests from diseases and pests have also been constant struggles that have been associated to ensuring food security. A branch of Agricultural science that has been vital for understanding crop diseases has been the focus of Virology within the larger frame of Plant Pathology.

Peru, as a country whose staple foods include potatoes and tubers, is a prime location for research into the interactions of diseases amongst potato and sweet potato generations and varieties. Once understood, the viral infections can be strategically combatted with efficient methods throughout Peru and the rest of the world.

MATERIALS

RNA/DNA Extraction

CTAB Buffer

2%CTAB

100mM Tris-HCl, pH 8.0

20mM EDTA

1.4M NaCl

1.0% NA Sulfite

2.0% PVP-40

Chloroform IAA (24:1)

4M LiCl

75% Ethanol

PCR MIX

Nuclease Free Water (NFW)

Buffer 5X

$MgCl_2$ 25mM

dNTPs 10mM

Primers 10 µM

Go Taq Flexi Polymerase 5U/µl

METHODOLOGY

CTAB RNA Extraction Method

Adapted from Lodhi et. Al., 1994 PMBR 12: 6-13. Taken from Segundo Fuentes

1. In a 500 gauge polythene bag or similar, grind leaf tissue, add 10X (V/W) of CTAB buffer and grind again. Pour into microfuge tubes.
2. Microfuge 5 minutes room temperature, remove 700µl liquid to fresh microfuge tube.
3. Add equal volume of Chloroform IAA (24:1), finger vortex to mix thoroughly then microfuge 5 mins. Carefully remove aqueous layer to fresh microfuge tube, do not transfer any interphase.

4. Repeat Step 3.
5. Add an equal volume of 4M LiCl. Inverst tube to mix and leave overnight in fridge.
6. Microfuge 20 minutes to collect RNA.
7. Carefully remove liquid leaving pellet. Add 200µl of 75% ethanol, microfuge 10 minutes at 14,000rpm.
8. Remove ethanol, leaving pellet. Air dry pellet 30 minutes.
9. Resuspend pellet in NFW

Reverse Transcription

Prepared by Segundo Fuentes

1. Denaturation

Annealing Mix	1X
Sample (RNA Extraction)	5µl
NFW	4µl
Random Primer (250ng/ul)	1µ
Total	10µl

Denaturation Conditions	
65º C	10 min
10º C	Forever

2. RT Mix

RT Mix	1X
NFW	7
RT Buffer 5X	5
DTT 0.1M	1
dNTPs 10 mM	1
RNAse out Inhib. 40U/µl	0.5
M-MLV 200U/µl	0.5
Total	15µl

3. RT (cDNA)

RT Mix	15µl
Annealing Mix	10µl
Total	25µl

RT Condition	
40º C	60 min
95º C	5 min
10º C	Forever

RT PCR – Potyvirus (SPFMV, SPVC, SPVG, SPV2)

Mix	1X
NFW	5.5
Buffer 5X	5
$MgCl_2$ 25mM	3
dNTPs 10mM	2
Primer SPG-F 10µM	2.5
Primer SPC-F 10µM	0.4
Primer SPF-F 10µM	2
Primer SP2-F 10µM	0.2
Primer SPFCG2-R 10µM	2
Go Taq Flexi Pol 5U/µl	0.4
Total	23µl

PCR Mix	23
cDNA	2
Total	25µl

Conditions		
94º C	2 min	
94º C	30 sec	30 Cycles
60º C	30 sec	
72º C	80 sec	
72º C	10 min	
10º C	Forever	

RT PCR – SPMMV-SPCSV-Carla

Mix	1X
NFW	8.5
Buffer 5X	5
$MgCl_2$ 25mM	4
dNTPs 10mM	2
Primer SPMMV-Fn5 10µM	0.5
Primer SPMMV-Rn5 10µM	0.5
Primer SPCSV-HSP70h F1 10µM	0.5
Primer SPCSV-HSP70h R2 10µM	0.5
Primer Calra-RdRpF 10µM	0.5
Primer Carla-RdRpR 10µM	0.5
Go Taq Flexi Pol 5U/µl	0.5
Total	23µl

PCR Mix	23
cDNA	2
Total	25µl

Conditions		
94ºC	2min	
94ºC	30sec	7 Cycles
(60-n)ºC	30sec	
72ºC	90sec	
94ºC	30sec	
55ºC	30sec	35 Cycles
72ºC	90sec	
72ºC	10min	
10ºC	Forever	

RT-PCR Carlaviruses (SPCFV, SPC6V)

Mix	1X
NFW	10.9
Buffer 5X	5
$MgCl_2$ 25mM	2.5
dNTPs 10mM	0.5
Primer Calra-RdRpF 10µM	0.5
Primer Carla-RdRpR 10µM	0.5
Go Taq Flexi Pol 5U/µl	0.1
Total	20µl

PCR Mix	20
cDNA	5
Total	25µl

Conditions		
94º C	2 min	
94º C	30 sec	35 Cycles
50º C	30 sec	
72º C	1 min	
72º C	10 min	
10º C	Forever	

RT-PCR Begomovirus, SPCV, SPVCV

Mix	1X
NFW	7.3
Buffer 5X	5
$MgCl_2$ 25mM	7
dNTPs 10mM	1.5
Primer SPG1 10µM	0.5
Primer SPG2 10µM	0.5
Primer SPVCV F 10µM	0.4
Primer SPVCV R 10µM	0.4
Primer SPCV F 10µM	0.4
Primer SPCV R 10µM	0.4
Go Taq Flexi Pol 5U/µl	0.6
Total	24µl

PCR Mix	24
cDNA	1
Total	25µl

Conditions		
94ºC	40sec	11 Cycles (n=-1.7ºC per cycle)
(72-n)ºC	40sec	
72ºC	70sec	
94ºC	40sec	24 Cycles
53ºC	40sec	
72ºC	70sec	
72ºC	10min	
10ºC	Forever	

RESULTS

SAMPLES

July 14th 2015

CFFP First Collection

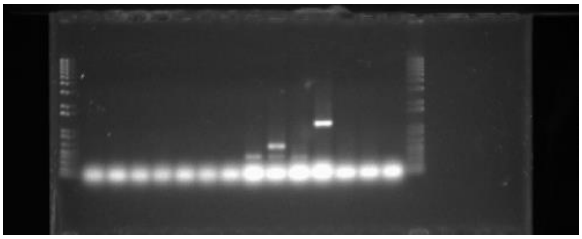
Field: Cañete San Luis

Date Planted: 12 March 2014

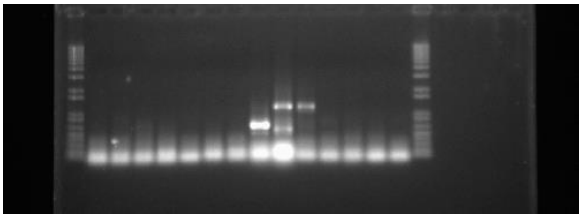
Date Collected: 1 August

Sample	
1. 20mg	Extraction Sample # 1
2. 20mg	
3. 20mg	
4. 20mg	
5. 20mg	
6. 20mg	Extraction Sample # 2
7. 20mg	
8. 20mg	
9. 20mg	
10. 20mg	
11. 20mg	Example Sample # 3
12. 20mg	
13. 20mg	
14. 20mg	
15. 20mg	
16. 20mg	Extraction Sample # 4
17. 20mg	
18. 20mg	
19. 20mg	
20. 20mg	
21. 20mg	Extraction Sample # 5
22. 20mg	
23. 20mg	
24. 20mg	
25. 20mg	
26. 20mg	Extraction Sample # 6
27. 20mg	
28. 20mg	
29. 20mg	
30. 20mg	
31. 20mg	Extraction Sample # 7
32. 20mg	
33. 20mg	

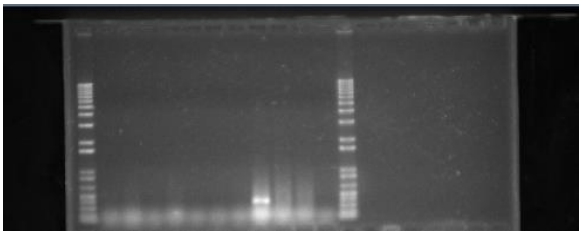
Potyvirus PCR Samples 1-7



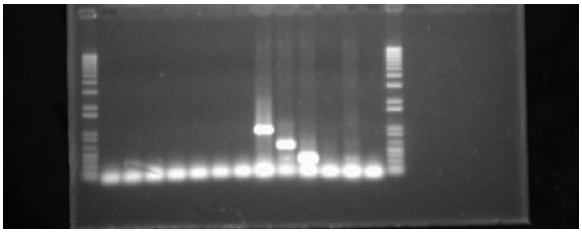
SPMMV-SPCSV-Carla PCR Samples 1-7



Carlavirus PCR Samples 1-7



Begomovirus-SPCV-SPVCV PCR Samples 1-7



July 15th 2015

CFFP Second Collection

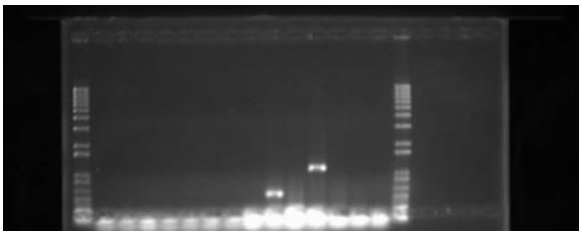
Field: Cañete San Luis

Date Planted: 12 March 2014

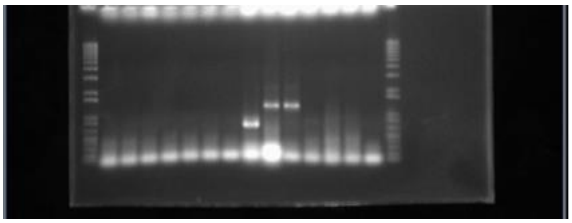
Date Collected: Unknown

Sample	
1. 20mg	Extraction Sample # 8
2. 20mg	
3. 20mg	
4. 20mg	
5. 20mg	
6. 20mg	Extraction Sample # 9
7. 20mg	
8. 20mg	
9. 20mg	
10. 20mg	
11. 20mg	Extraction Sample # 10
12. 20mg	
13. 20mg	
14. 20mg	
15. 20mg	
16. 20mg	Extraction Sample # 11
17. 20mg	
18. 20mg	
19. 20mg	
20. 20mg	
21. 20mg	Extraction Sample # 12
22. 20mg	
23. 20mg	
24. 20mg	
25. 20mg	
26. 20mg	Extraction Sample # 13
27. 20mg	
28. 20mg	
29. 20mg	
30. 20mg	
31. 20mg	Extraction Sample # 14
32. 20mg	
33. 20mg	

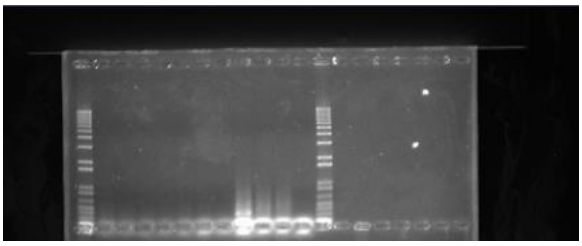
Potyvirus PCR Samples 8-14



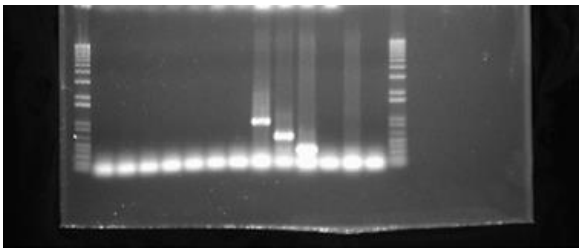
SPMMV-SPCSV-Carla PCR Samples 8-14



Carlavirus PCR Samples 8-14



Begomovirus-SPCV-SPVCV PCR Samples 8-14



July 16th 2015

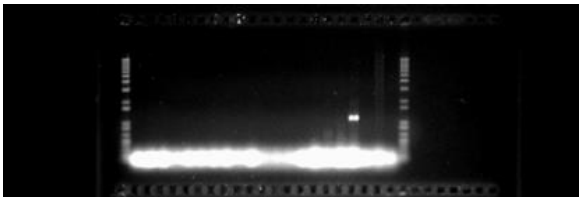
C11 Second Collection

Field: Exp. 2014 Cañete San Luis

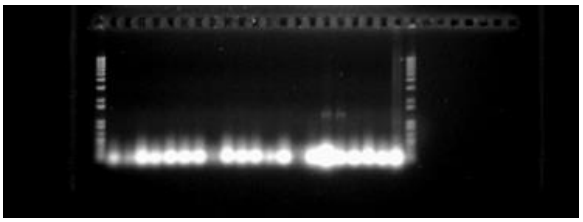
Date Planted: 12 March 2014

Date Collected: Unknown

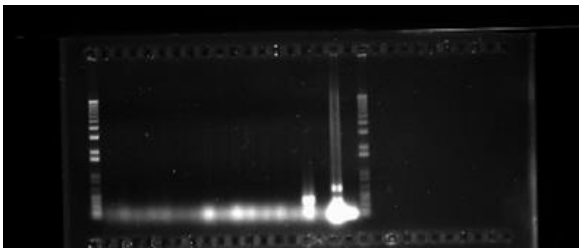
Potyvirus Samples 15-28



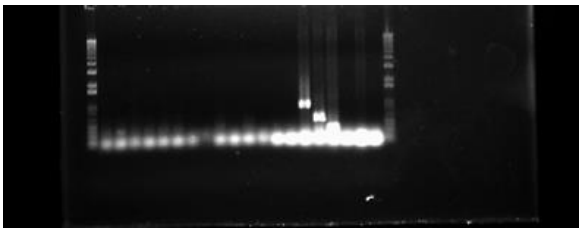
SPMMV-SPCSV-Carla Samples 15-28



Carlavirus Samples 15-28



Begomovirus-SPCV-SPVCV Samples 15-28



Sample	
1. 20mg	Extraction Sample # 15
2. 20mg	
3. 20mg	
4. 20mg	
5. 20mg	
6. 20mg	Extraction Sample # 16
7. 20mg	
8. 20mg	
9. 20mg	
10. 20mg	
11. 20mg	Extraction Sample # 17
12. 20mg	
13. 20mg	
14. 20mg	
15. 20mg	
16. 20mg	Extraction Sample # 18
17. 20mg	
18. 20mg	
19. 20mg	
20. 20mg	
21. 20mg	Extraction Sample # 19
22. 20mg	
23. 20mg	
24. 20mg	
25. 20mg	
26. 20mg	Extraction Sample # 20
27. 20mg	
28. 20mg	
29. 20mg	
30. 20mg	
31. 20mg	Extraction Sample # 21
32. 20mg	
33. 20mg	

July 17th 2015

C11 First Collection

Field: Exp. 2014 Cañete San Luis

Date Planted: 12 March 2014

Date Collected: 1 August 2014

Sample	
1. 20mg	Extraction Sample # 22
2. 20mg	
3. 20mg	
4. 20mg	
5. 20mg	
6. 20mg	Extraction Sample #23
7. 20mg	
8. 20mg	
9. 20mg	
10. 20mg	
11. 20mg	Extraction Sample # 24
12. 20mg	
13. 20mg	
14. 20mg	
15. 20mg	
16. 20mg	Extraction Sample # 25
17. 20mg	
18. 20mg	
19. 20mg	
20. 20mg	
21. 20mg	Extraction Sample # 26
22. 20mg	
23. 20mg	
24. 20mg	
25. 20mg	
26. 20mg	Extraction Sample # 27
27. 20mg	
28. 20mg	
29. 20mg	
30. 20mg	
31. 20mg	Extraction Sample # 28
32. 20mg	
33. 20mg	

July 23th 2015

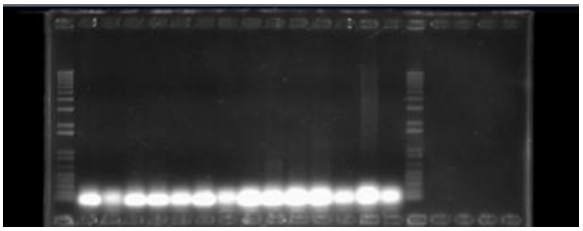
H-FFP First Collection "Cantinflas"

Field: Exp. 2013 Huaral Aucallama

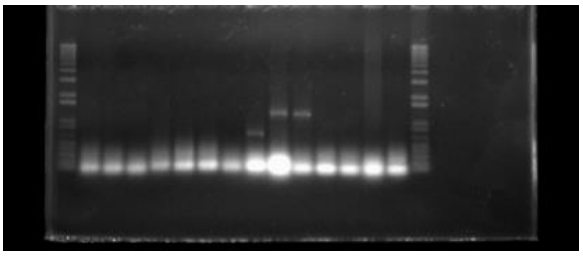
Date Planted: 21 January 2014

Date Collected: 21 April 2014

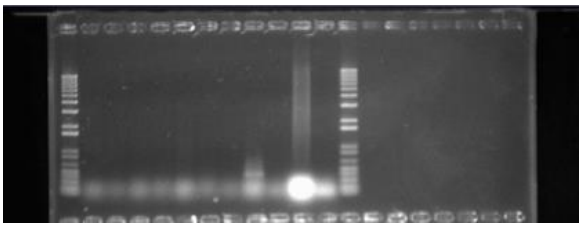
Potyvirus PCR Samples 29-35



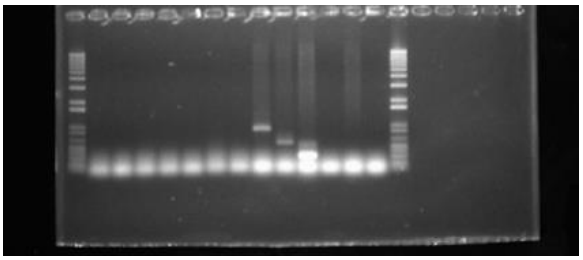
SPMMV-SPCSV-Carla PCR Samples 29-35



Carlavirus PCR Samples 29-35



Begomovirus-SPCV-SPVCV PCR Samples 29-35



Sample	
1. 20mg	Extraction Sample # 29
2. 20mg	
3. 20mg	
4. 20mg	
5. 20mg	
6. 20mg	Extraction Sample # 30
7. 20mg	
8. 20mg	
9. 20mg	
10. 20mg	
11. 20mg	Extraction Sample # 31
12. 20mg	
13. 20mg	
14. 20mg	
15. 20mg	
16. 20mg	Extraction Sample # 32
17. 20mg	
18. 20mg	
19. 20mg	
20. 20mg	
21. 20mg	Extraction Sample # 33
22. 20mg	
23. 20mg	
24. 20mg	
25. 20mg	
26. 20mg	
27. 20mg	Extraction Sample # 34
28. 20mg	
29. 20mg	
30. 20mg	
31. 20mg	Extraction Sample # 35
32. 20mg	
33. 20mg	
34. 20mg	
35. 20mg	
36. 20mg	

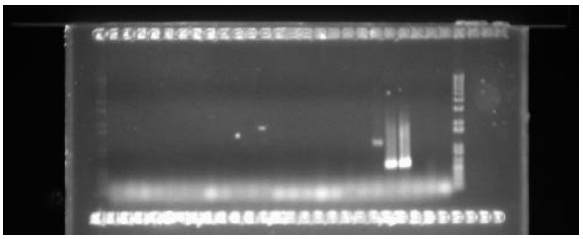
Aug. 3rd 2015

HFFP Second Collection

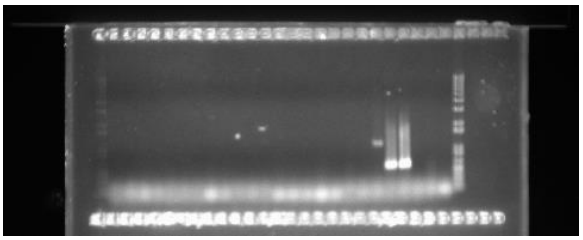
Field: Exp. 2013 Huaral Aucallama

Date Collected: July 3

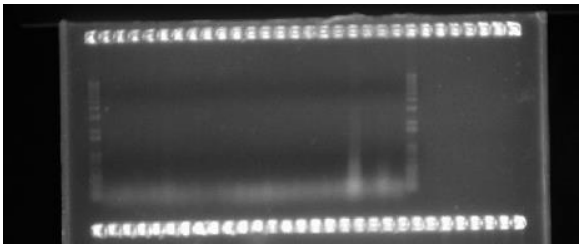
Potyvirus PCR Samples 36-52



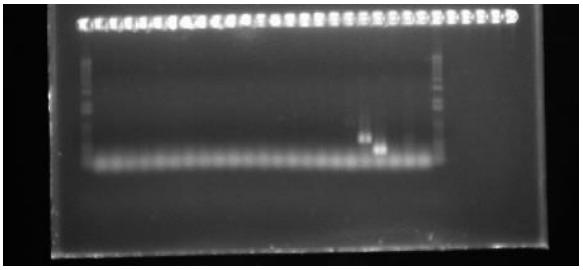
SPMMV-SPCSV-Carla PCR Samples 36-52



Carlavirus PCR Samples 36-52



Begomovirus-SPCV-SPVCV PCR Samples 36-52



Sample	
1. 20mg	Extraction Sample # 36
2. 20mg	
3. 20mg	
4. 20mg	
5. 20mg	
6. 20mg	Extraction Sample # 37
7. 20mg	
8. 20mg	
9. 20mg	
10. 20mg	
11. 20mg	Extraction Sample # 38
12. 20mg	
13. 20mg	
14. 20mg	
15. 20mg	
16. 20mg	Extraction Sample # 39
17. 20mg	
18. 20mg	
19. 20mg	
20. 20mg	
21. 20mg	Extraction Sample # 40
22. 20mg	
23. 20mg	
24. 20mg	
25. 20mg	
26. 20mg	Extraction Sample # 41
27. 20mg	
28. 20mg	
29. 20mg	
30. 20mg	
31. 20mg	Extraction Sample # 42
32. 20mg	
33. 20mg	
34. 20mg	
35. 20mg	
36. 20mg	Extraction Sample # 43
37. 20mg	
38. 20mg	
39. 20mg	
40. 20mg	

Aug. 6th 2015

H-FF1 First Collection

Field: Exp. 2013 Huaral Aucallama

Date Planted: 24 December 2013

Date Collected: 21 April

Sample	
1. 20mg	Extraction Sample # 44
2. 20mg	
3. 20mg	
4. 20mg	
5. 20mg	
6. 20mg	Extraction Sample # 45
7. 20mg	
8. 20mg	
9. 20mg	
10. 20mg	
11. 20mg	Extraction Sample # 46
12. 20mg	
13. 20mg	
14. 20mg	
15. 20mg	
16. 20mg	Extraction Sample # 47
17. 20mg	
18. 20mg	
19. 20mg	
20. 20mg	
21. 20mg	Extraction Sample # 48
22. 20mg	
23. 20mg	
24. 20mg	
25. 20mg	
26. 20mg	Extraction Sample # 49
27. 20mg	
28. 20mg	
29. 20mg	
30. 20mg	
31. 20mg	Extraction Sample # 50
32. 20mg	
33. 20mg	
34. 20mg	
35. 20mg	
36. 20mg	Extraction Sample # 51
37. 20mg	
38. 20mg	
39. 20mg	
40. 20mg	
41. 20mg	Extraction Sample # 52
42. 20mg	
43. 20mg	
44. 20mg	

Aug. 10th 2015

H-FF1 Second Collection

Field: Exp. 2013 Huaral Aucallama

Date Planted: 24 December 2013

Date Collected: 31 January 2014

Aug. 7th 2015 RNA Extractions for Controls

Sano	100mg
Sano I Set	100mg
SPVCV	84.5mg
SPCV	100mg
Begomo C54	102mg
SPCFV	100mg
SPVD	100mg
SPMMV	100mg
SPCSV	100mg
SPVG	100mg
SPV2	100mg
SPFMV RC	50mg
SPFMV C1 (N. Beut)	22mg
SPFMV C1 (I. Nil)	47mg

Sample	
1. 20mg	Extraction Sample # 53
2. 20mg	
3. 20mg	
4. 20mg	
5. 20mg	
6. 20mg	Extraction Sample # 54
7. 20mg	
8. 20mg	
9. 20mg	
10. 20mg	
11. 20mg	Extraction Sample # 55
12. 20mg	
13. 20mg	
14. 20mg	
15. 20mg	
16. 20mg	Extraction Sample # 56
17. 20mg	
18. 20mg	
19. 20mg	
20. 20mg	
21. 20mg	Extraction Sample # 57
22. 20mg	
23. 20mg	
24. 20mg	
25. 20mg	
26. 20mg	Extraction Sample # 58
27. 20mg	
28. 20mg	
29. 20mg	
30. 20mg	
31. 20mg	Extraction Sample # 59
32. 20mg	
33. 20mg	
34. 20mg	
35. 20mg	
36. 20mg	Extraction Sample # 60
37. 20mg	
38. 20mg	
39. 20mg	
40. 20mg	

Discussion

In my experiments, there were a plethora of potential sources for error. I included many procedures to counteract potential mistakes or lab errors. The potential for contamination throughout the whole process, from extraction to electrophoresis, meant using the right protection, sanitary methods, and not mixing any materials. Loading a gel for electrophoresis posed a slight challenge because of the nature of the two liquids blending as soon as the tip of the micropipette was dipped into the buffer solution.

The majority of samples that were run through PCR amplification by my experiments were early generations of Sweet Potato plants. Because of this, not very many tested positive for any virus infections since not enough time was given for viruses to spread throughout fields of the sweet potato crops.

As CIP continues the workload, more samples will be found to be infected.

The experiments will reveal the nature of virus proliferation and hopefully provide insight for the rate at which what Peruvian virus spread. The results will also help to discover which viruses are present and transmitted or not transmitted.

If it is discovered that some viruses do not spread at significant rates, do not significantly impact yields, or are not significantly phenotypically expressed, then farmers will know which viruses to focus on. All of this information will aid in the protection of Sweet Potato crops in the Peruvian region.

Conclusion

Sweet potato viruses affect Peruvian crops and can lead to decreased yields. Among the more common sweet potato viruses are Sweet Potato Feather Mottle Virus (SPFMV) and other Potyviruses. SPFMV along with Sweet Potato Chlorotic Stunt Virus (SPCSV) can co-infect a crop to produce a disease called Sweet Potato Virus Disease (SPVD).

Data acquired by PCR assays will help to investigate the presence of viruses and will provide insight for the effects that viruses have on crops. The information will be used in accordance with current Sweet potato virus literature to explain synergistic virus interactions and their symptoms on sweet potato crops in Peru, and throughout Africa. Examining the spread of these viruses, along with less common strains, throughout Peru and determining what symptoms they cause are important for discovering resistances and helping farmers to protect their yields.

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Cultural Experiences

Living in Lima made for a very unique and interesting experience. I had no problem fitting in or even blending in with the local people and I believe that made the international experience that much better. Although I was a tourist in a sense, I was really a Peruvian for the eight weeks I spent there. The few people who asked where I was from were surprised to hear me say that I was an American. My Spanish was complimented by those who knew I was from the U.S. and people liked to hear I was of Latin American descent as well. Having things in common really created an atmosphere that was very friendly. I made some good friends, played soccer, and shopped at local shops as a Peruvian.

My colleagues in the lab accepted me as part of the family and were kind enough to treat me cultural insights and experiences out into the city. On all occasions I thankful for my understanding of my culture and language, and made a tremendous effort to tune into the Peruvian culture.

During my stay in Peru, I got to witness the country cheering on the national soccer team in the Copa America soccer tournament. I was able to watch the games with my host, Mr. Lindo, and learned a lot about the Peruvian analytics of the international game of soccer.

All of these experiences, plus many more, created a new perspective for me to see Latin America, and the world. I have come back from my internship as a Peruvian and I'm incredibly humbled by the beautiful nation and genuinely kind people.

Photos