

Screening for Viral Disease in the OSU Tomato Breeding Program

by
Alexandria Kershner

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

Presented May 30, 2018
Commencement June 2018

AN ABSTRACT OF THE THESIS OF
Alexandria Kershner for the degree of Honors Baccalaureate of Science in Biology presented on
May 30, 2018. Title: Screening for Viral Disease in the OSU Tomato Breeding Program.

Abstract approved: _____

Jim Myers

Disease control is a crucial aspect of plant breeding for researchers as well as commercial producers. After samples from OSU's tomato breeding program tested positive for Columnnea latent viroid in a screen done by a private company, secondary testing was done by the USDA laboratory in Corvallis which suggested that the samples were infected with tobamovirus instead. This project was set up to screen symptomatic plants for both Columnnea latent viroid as well as tobamovirus using reverse transcription polymerase chain reaction (RT-PCR). Out of the 42 samples tested, one sample from the P321-16-1B-1 line tested positive for tobamovirus, and one sample from the S261 line tested positive for Columnnea latent viroid. However, sequencing of the S261 positive indicated that the primer used in that test had amplified tomato DNA and was therefore a false positive.

Key Words: Tomatoes, Virus, Viroid, RT-PCR

Corresponding e-mail address: kershnea@oregonstate.edu

©Copyright by Alexandria Kershner
May 30, 2018
All Rights Reserved

Screening for Viral Disease in the OSU Tomato Breeding Program

by
Alexandria Kershner

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

Presented May 30, 2018
Commencement June 2018

Honors Baccalaureate of Science in Biology project of Alexandria Kershner presented on May 30, 2018.

APPROVED:

Jim Myers, Mentor, representing Department of Horticulture

Karen Keller, Committee Member, representing USDA ARS HCRL Plant Virology Lab

Michael Burgett, Committee Member, representing Department of Horticulture

Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

Alexandria Kershner, Author

Screening for Viral Disease in the OSU Tomato Breeding Program

Introduction

Statement of the problem

In August 2015 some samples from the OSU tomato breeding program tested positive for Columnnea Latent Viroid after being screened for use in a greenhouse by a private company evaluating these materials for commercial production. They were originally tested using reverse transcription polymerase chain reaction (RT-PCR) by Agdia as well as the USDA Vegetable Laboratory in Charleston, South Carolina. However, neither group sequenced their positive results and the USDA laboratory in Corvallis was unable to replicate their results. The Corvallis lab tests suggested the presence of a tobamovirus rather than a pospiviroid. This project was established to screen the OSU tomato breeding material for both pospiviroid and tobamovirus presence.

Literature review

Viroids are tiny pathogens made of single stranded, circular RNA. They do not code for any proteins and must infect plant cells to reproduce. Similarly to many viruses they are highly contagious and can be transferred by a variety of ways such as grafting, use of contaminated tools, seed transmission, and insect transmission (Singh 2014). They were discovered to be different from conventional viruses in 1967 when research on potato spindle tuber disease indicated that it was caused by free, rather than coated, RNA (Diener and Raymer 1967). Despite consisting of unprotected RNA, viroids are able to survive in dried leaves or seeds for years (Singh 1977, Singh et al. 1991).

Viroids of the family *Pospiviroidae* replicate in the nucleus of a cell, while viroids in the *Avsunviroidae* family replicate in the chloroplasts (Flores et al. 2005). Columnnea latent viroid is a Pospiviroid that originated in ornamental plants, but is often asymptomatic (Singh and Teixeira 2006). However, it can also infect tomatoes and cause symptoms of stunted growth, chlorosis of leaves, bronzing of leaves, or necrosis of tissues. There have not been any recent cases in the United States, however it was reported in the United Kingdom in 2007 (Nixon et al 2010).

Since viroids do not produce proteins, enzyme-linked immunosorbent assay (ELISA), which is commonly used as a diagnostic tool for many types of plant diseases, including viruses, cannot be used for detection (Hammond and Owens 2006). Instead,

Return-Polyacrylamide Gel Electrophoresis (R-PAGE) or RT-PCR are typically used for viroid detection (Singh 2014).

Unlike viroids, tobamoviruses are classical single-stranded RNA viruses with a rod-shaped protein coat. There are many kinds of tobamovirus and they are often divided into subgroups based on whether they infect solanaceous species, cucurbits and legumes, or crucifers (Lartely et al. 1996). Symptoms vary based on strain but can include necrosis, leaf distortion, and mosaic patterning.

Tobacco mosaic virus is the most well known of the tobamoviruses as it was the first virus ever discovered, and it has long played an important role as a test subject in the field of molecular biology (Okada 1999). It infects solanaceous plants, as well as other plant families, and can cause losses of up to 20% of yield in tomatoes (Scholthof 2005). Similar to the viroids, it is highly contagious and is quickly spread by the use of contaminated equipment. It is also transmissible through seed as well as by aphids or bees (Tošić et al. 1980, Lojek & Orlob 1972, Okada et al. 2000).

Tomato mosaic virus is another tobamovirus that is very closely related to tobacco mosaic virus, to the point that they are often impossible to distinguish between based on symptoms alone (Brunt 1986). This is partially due to the fact that tomato mosaic virus and tobacco mosaic virus were originally thought to be the same virus, so older descriptions of tobacco mosaic virus in tomatoes may actually have been cases of tomato mosaic virus.

In recent years a new tobamovirus, tomato mottle mosaic virus, that is very closely related to tomato mosaic virus has also been reported. It was first reported in Mexico in 2013 and some evidence suggests that tomatoes that have Tm-2² resistance against tomato mosaic virus may also be resistant to tomato mottle mosaic virus (Bajet et al. 2016). A 2014 report that Florida tomato samples from 2010 and 2012 were identified to be infected with tomato mottle mosaic virus indicates the presence of this virus in the United States (Webster et al. 2014). Due to its very close relationship to tomato mosaic virus, it is possible that it has been underreported in the past and it may actually be fairly widespread.

Objectives of research

This study aimed to determine the cause of viral symptoms in OSU tomato breeding material. The hypothesis was that columnnea latent viroid was not present, but that the tomatoes might have been infected with a tobamovirus instead. To test this hypothesis, samples were taken from plants in the field that appeared to be suffering from viral symptoms.

Materials and Methods

Field

As this project was concerned with determining the cause of what appeared to a viral disease samples were taken from plants that showed potentially viral symptoms such as stunting, curling, and leaf discoloration (Figure 1). Leaf samples were taken in both 2016 and 2017 from tomatoes grown at the OSU research farm, the Lewis-Brown organic farm, and from tomatoes grown in the OSU greenhouses (Table 1). Samples were bagged and labelled at the sampling location then quickly transported back to the lab and stored in a refrigerator if not immediately used for testing. A portion of each sample was tested while fresh and the rest of the sample was preserved using freeze drying and stored in a freezer until needed.



Figure 1: Tomato plants collected from the OSU research farm in 2016 showing viral symptoms such as curling, discoloration, and necrosis.

Table 1. Accession, pedigree, date of sampling, number of samples taken, and location of sampling for each of the samples tested				
Accession	Pedigree (if experimental line)	Date Sampled	# of Samples	Location (O = OSU Research Farm, LB = Lewis Brown Farm, F+R = field and row at Veg Farm, G = Greenhouse)
S261	P19-2/aw	4-29-2016	2	G
P321-16-1 B-1	('Indigo Rose' x 'Ananas Noire')-16-1	4-29-2016	1	G

Table 1. continued				
Accession	Pedigree (if experimental line)	Date Sampled	# of Samples	Location (O = OSU Research Farm, LB = Lewis Brown Farm, F+R = field and row at the Research Farm, G = Greenhouse)
P324-4-1	[(P185 x 'OR Star') x P185]-1	4-29-2016	1	G
P321-7-1-1	('Indigo Rose' x 'Ananas Noire')-7-1	6-20-2016	1	G
P321-6-2-1	('Indigo Rose' x 'Ananas Noire')-6-1	6-20-2016	1	G
P324-4-1	[(P18 5x 'OR Star') x P185]-1	6-20-2016	1	G
Saucy	'Santiam' x 'Roma'	6-20-2016	1	G (seed produced in the field at the Research Farm in 2015)
P325-1-1	('OR Spring' x 'Indigo Rose')-1	6-20-2016	1	G
S261	P19-2/aw	6-20-2016	1	G
P325-2-2-1	('OR Spring' x 'Indigo Rose')-1	7-13-2016	1	O F7 R5
P322-1-1-1	('Black Cherry' x 'Indigo Rose')-1	7-13-2016	1	O F7 R6
LA 3004	<i>hp-1</i> in 'Rutgers' background	7-13-2016	1	O F7 R4
Siletz	'Oregon Spring' x 'Pikred'	7-13-2016	1	O Perennial Field
F2	?	7-13-2016	3	O F7 R1
Iron Lady	N/A*	7-13-2016	1	LB (TOMI 2515)

Table 1. continued				
Accession	Pedigree (if experimental line)	Date Sampled	# of Samples	Location (O = OSU Research Farm, LB = Lewis Brown Farm, F+R = field and row at the Research Farm, G = Greenhouse)
	404-1-WA x 421-1-OR	7-13-2016	1	LB (TOMI 2514)
LB 8-3-1-2-1	CULB PT A48-1x (S193-3 x L3683)	7-13-2016	1	LB (TOMI 16)
Saucy	'Santiam' x 'Roma'	7-13-2016	1	O F7 R8
P321-7-1-1	('Indigo Rose' x 'Ananas Noire')-7-1	7-18-2016	1	G
P321-6-2-1	('Indigo Rose' x 'Ananas Noire')-6-1	7-18-2016	1	G
P324-4-1	[(P185 x 'OR Star') x P185]-1	7-18-2016	1	G
Saucy	'Santiam' x 'Roma'	7-18-2016	1	G (seed produced in the field at the Research Farm in 2015)
P325-1-1	('OR Spring' x 'Indigo Rose')-1	7-18-2016	1	G
S261	P19-2/aw	7-18-2016	1	G
Mountain Rouge	N/A	7-26-2016		LB TOMI 3
Iron Lady	N/A	7-26-2016	1	LB TOMI 2515
	404-1-WA x 421-1-OR	7-13-2016	1	LB TOMI 2514
LB 8-3-1-2-1	CULB PT A48-1x (S193-3 x L3683)	7-13-2016	1	LB TOMI 16

Table 1. continued				
Accession	Pedigree (if experimental line)	Date Sampled	# of Samples	Location (O = OSU Research Farm, LB = Lewis Brown Farm, F+R = field and row at the Research Farm, G = Greenhouse)
Crimson Sprinter	N/A	7-13-2016	1	LB TOMI 4
Oroma	'Santiam' x 'Roma'	7-13-2016	1	LB TOMI 13
S195-1 (Legend)	'Centennial' x ('Oregon Spring' x 'Pikred')	7-13-2016	1	LB 2000
Siletz	'Oregon Spring' x 'Pikred'	8-8-2016	3	LB
Plum Regal	N/A	8-8-2016	4	LB
LB 80	?	8-8-2016	1	LB

*Pedigree not available.

Laboratory

All extractions were performed by hand following the protocol of the Bob Martin USDA ARS HCRL plant virology lab. Samples were ground into 1mL extraction buffer (see Appendix) either using mortar and pestle or with a plant tissue grinding machine. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was added at the grinding stage at a ratio of 10µL per 1mL of extraction buffer. In later extractions 20µL TCEP per 1mL of extraction buffer was used. Early extractions used sample sizes of about 0.02g of dried plant tissue, however in later extractions, sample size was reduced to a range of 0.006-.01g, in order to attempt to get cleaner final product. In extractions of fresh tissue, leaf area, not weight was used, with about 1.5 cm² of tissue tested.

The samples were then transferred to 1.5mL Eppendorf tubes and centrifuged at 13,500 rpm for 3 min. After centrifugation, 500µL of supernatant was added to a new 1.5mL tube containing 600µL potassium acetate (KoAc). The tubes were mixed by inverting and then centrifuged for 10 minutes, although in later extractions the centrifugation time was lengthened to up to 30 minutes in order to increase purity of end product. New tubes with 750µL 100% Isopropyl alcohol were prepared and 700µL of

supernatant was added after centrifugation finished. After this step samples were stored at -20°C for approximately 24 hours.

Liquid samples were removed from the freezer and mixed by inversion then centrifuged 30 minutes at 13,500 rpm before pouring off the supernatant while keeping the RNA pellet in the tube. The samples were then mixed with 500µL of a 50/50 mix of ethanol and wash buffer and 10µL of silica glass milk (see buffers) and vortexed until the pellet was resuspended. They were pulsed twice in the centrifuge at 5,000 rpm then the supernatant was again poured off while keeping the pellet. This process was repeated twice with 500µL of a 50/50 mix of ethanol and wash buffer per sample in order to wash the RNA pellet. After washing, samples were put into a vacuum for 20 minutes to remove all traces of ethanol. Once ethanol was fully evaporated, 55µL of elution buffer was added to samples and vortexed until the pellet resuspended. The samples were then incubated at 70°C for 4 minutes. Samples were then spun down for 10 minutes and 40µL of supernatant was removed and stored at -80°C.

RNA was extracted from the tobamovirus positive control in the same manner as the plant tissue samples as described above. (The tobamovirus positive was obtained late in the testing process and was only used in the testing of the samples taken on 8-8-2016.)

After extraction, RNA quality was assessed using gel electrophoresis. Denaturing formaldehyde gels with 1% agarose were run at 60V for about 2 hours to determine if RNA was suitable quality for further testing. (These gels were run at the Myers' lab only; at the USDA lab quality was tested after reverse transcription or checked using a Nanodrop spectrophotometer.)

Reverse transcription was performed either using the Promega GoScript Reverse Transcription kit (Myers' lab), following the protocol or by hand (recipe in Appendix).

At the USDA lab, PCR was performed with housekeeping genes (NAD5, NAD2, ndhB, or 18s) to determine quality of DNA before proceeding. After determining that the DNA was of acceptable quality, PCR was performed with two pospiviroid primers and three tobamovirus primers (see Appendix Table S1). Agarose gels with ethidium bromide were run to visualize results. Band stab PCR procedure was used to isolate and amplify positive results which were then sent to MacroGen, a commercial lab, for sequencing.

Results

S261, P321-16-1B-1, P324-4-1, S276, P279-05-1-1, Siletz, Plum Regal, and LB80 were evaluated for the presence of pospiviroids and tobamoviruses. The other samples were subjected to multiple extractions but we were unable to obtain clean and usable RNA. Samples appeared to have degraded in storage. From the samples evaluated, two positive results were observed. One was a positive for pospiviroids in

sample #1 of S261 (Figure 2) and the other was a positive result for tobamovirus in the sample from line P321-16-1B-1 (Figure 3).

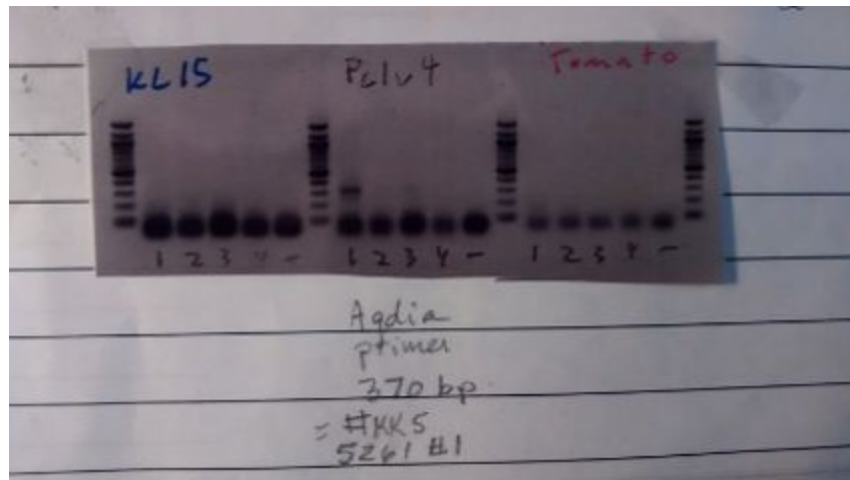


Figure 2: Results of PCR test with pospiviroid primers. 1: S261 #1 2: S261 #2 3: P321-16-1B-1 4: P324-4-1

The positive result was sent in for sequencing but BLASTN analysis indicated that the sequence matched noncoding tomato chromosomal DNA rather than any viral sequences.

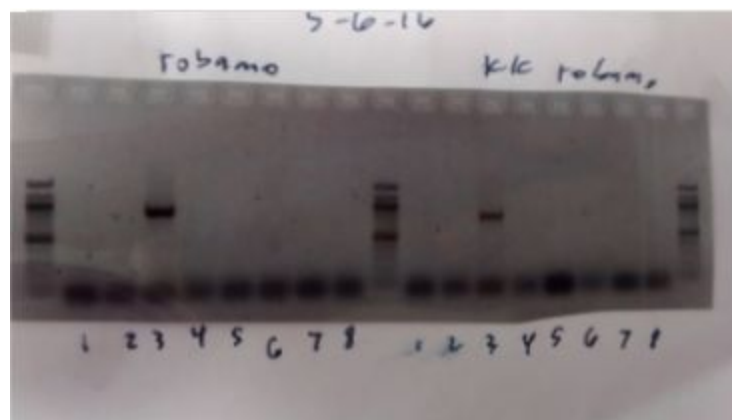


Figure 3: Results of PCR test with tobamo primers
1: S261 #1 2: S261 #2 3: P321-16-1B-1 4: P324-4-1
5-8: Quality controls for RT and PCR

The other positive result was found in the sample from line P321-16-1B-1. It tested positive with both sets of tobamovirus primers.

Discussion and Conclusions:

Although one sample (S261) tested positive with the Pclv4 primer for pospiviroid, it seems likely that it was a false positive. The fact that only one of the three pospiviroid primers had a positive result suggests that the Pclv4 primer (the same one originally used by Agdia) may be prone to false positives. Sequencing analysis of the positive result indicated that the primer had amplified tomato DNA rather than a virus. While insertion of viral genetic material into the host genome is not common outside of retroviruses, there have been rare cases of gene fragments of exclusively RNA non-retroviruses being added to host genomes so it may be theoretically possible for a viroid to be inserted (Fort et al. 2011). However, in this case it seems more likely that the primer amplified a section of tomato DNA with coincidental similarity. BLAST comparison of the entire columnnea latent viroid sequence to the entire tomato genome indicated that there were several short sequences of moderate similarity. Comparison of the primers to the sequenced positive showed a high level of stringency, indicating that the design of the primer itself is problematic and is likely to lead to false positives.

The other positive result (tobamovirus in the P321-16-1B-1 sample) is more clear cut, as both primers indicated the same result. However, as discussed in the literature review section, there are multiple types of tobamovirus and future research may want to determine exactly which type caused this infection. As the positive was not sequenced, it is currently unknown which tobamovirus caused the infection. Knowing which tobamoviruses tomato lines are currently vulnerable to could be very important in breeding for resistance in the future, so it's possible that this could be a valuable area to consider for future research projects. However, even without knowing the exact type of tobamovirus, general best practices, such as sterilizing equipment between uses should be followed. There are also several methods of seed treatment that are effective against tobamoviruses and viruses in general, such as using Virkon, bleach, or nonfat dry milk as disinfectant agents (Li et al. 2015)

As there were several plants that were symptomatic but did not test positive for either pospiviroid or tobamovirus, future screening for other potential causes of disease may be warranted. One potential disease causing agent to look into is *Candidatus Liberibacter solanacearum*, which is a bacteria that is transmitted by the potato psyllid (*Bactericera cockerelli*). It causes zebra chip disease in potatoes, which led to huge crop losses in Oregon in 2011 (Horton et al. 2015). It is also capable of infecting tomatoes, where it causes psyllid yellows. Typical symptoms of psyllid yellows include stunting, curling, mottling, chlorosis, and purpling of leaflets (as seen in Figure 4) (Dufault 2014).



Figure 4: Comparison of an OSU tomato plant (left) to a tomato plant infected with *Ca. Liberibacter solanacearum* (right photo by Dr. Lia Lieferting)

These symptoms are consistent with the symptoms that were seen in many sickly plants in the field. (The purpling of leaflets is more common in the high anthocyanin lines, however they are still able to be compared to other plants of the same line.) An insect vectored disease could also explain the somewhat scattered pattern of diseased plants that was seen in the field.

Citations:

Bajet, N. B., Kurowski, C., Himmel, P. (2016). GUIDELINES FOR IDENTIFICATION OF TOMATO MOSAIC VIRUS STRAINS USING DIFFERENTIAL HOSTS. <http://cppsi.ucanr.edu/files/225901.pdf> .

Batuman, O., & Gilbertson, R. L. (2013). First Report of *Columnea latent viroid* (CLVd) in Tomato in Mali. *Plant Disease*, 97(5), pp. 692.3.

Brunt, A. A. (1986). Tomato mosaic virus. In *The plant viruses* (pp. 181-204). Springer, Boston, MA.

Diener, T. O., & Raymer, W. B. (1967). Potato spindle tuber virus: a plant virus with properties of a free nucleic acid. *Science*, 158(3799), 378-381.

Dufault, N. (2014). Monitoring for a Potential Pathogen in Florida Potato and Tomato. <http://nwdistrict.ifas.ufl.edu/phag/2014/10/31/monitoring-for-a-potential-pathogen-in-florida-potato-and-tomato/>

Flores, R., Hernández, C., Alba, A. E. M. D., Daròs, J. A., & Serio, F. D. (2005). Viroids and viroid-host interactions. *Annu. Rev. Phytopathol.*, 43, 117-139.

Fort, P., Albertini, A., Van-Hua, A., Berthomieu, A., Roche, S., Delsuc, F., Pasteur, N., Capy, P., Gaudin, Y. & Weill, M. (2011). Fossil rhabdoviral sequences integrated into arthropod genomes: ontogeny, evolution, and potential functionality. *Molecular biology and evolution*, 29(1), 381-390.

Hammond, R. W., & Owens, R. A. (2006). Viroids: New and continuing risks for horticultural and agricultural crops. *APSnet Features*.
https://www.researchgate.net/publication/274162502_Viroids_New_and_Continuing_Risks_for_Horticultural_and_Agricultural_Crops

Horton, D. R., Cooper, W. R., Munyaneza, J. E., Swisher, K. D., Echegaray, E. R., Murphy, A. F., Rondon, S. I., Wohleb, C. H., Waters, T. D. & Jensen, A. S. (2015). A new problem and old questions: potato psyllid in the Pacific Northwest. *American Entomologist*, 61(4), 234-244.

Lartey, R. T., Voss, T. C., & Melcher, U. (1996). Tobamovirus evolution: gene overlaps, recombination, and taxonomic implications. *Molecular biology and evolution*, 13(10), 1327-1338.

Li, R., Baysal-Gurel, F., Abdo, Z., Miller, S. A., & Ling, K. S. (2015). Evaluation of disinfectants to prevent mechanical transmission of viruses and a viroid in greenhouse tomato production. *Virology journal*, 12(1), 5.

Li, Y. Y., Wang, C. L., Xiang, D., Li, R. H., Liu, Y., & Li, F. (2014). First report of tomato mottle mosaic virus infection of pepper in China. *Plant Disease*, 98(10), 1447-1447.

Lojek, J. S., & Orlob, G. B. (1972). Transmission of tobacco mosaic virus by *Myzus persicae*. *Journal of General Virology*, 17(1), 125-127.

Nixon, T., R. Glover, S. Mathews-Berry, M. Daly, E. Hobden, C. Lambourne, V. Harju, & A. Skelton. (2010). Columnea latent viroid (CLVd) in tomato: the first report in the United Kingdom. *Plant Pathology*, 59(2), 392-392.

Okada, Y. (1999). Historical overview of research on the tobacco mosaic virus genome: genome organization, infectivity and gene manipulation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 354(1383), 569-582.

Okada, K., Kusakari, S. I., Kawaratani, M., Negoro, J. I., Satoshi, T. O., & Osaki, T. (2000). Tobacco mosaic virus is transmissible from tomato to tomato by pollinating bumblebees. *Journal of General Plant Pathology*, 66(1), 71-74.

Scholthof, K. B. G. (2005). *Tobacco mosaic virus: The Plant Health Instructor*. DOI:10.1094/PHI-I-2000-1010-01

Singh, R. P. (2014). The discovery and eradication of potato spindle tuber viroid in Canada. *Virusdisease*, 25(4), 415–424. <http://doi.org/10.1007/s13337-014-0225-9>

Singh, R. P., Boucher, A., & Wang, R. G. (1991). Detection, distribution and long-term persistence of potato spindle tuber viroid in true potato seed from Heilongjiang, China. *American Potato Journal*, 68(1), 65.

Singh, R. P., & Finnie, R. E. (1977). Stability of potato spindle tuber viroid in freeze-dried leaf powder. *Phytopathology*.

Singh, R. P. & Teixeira da Silva, J. A., (2006). *Floriculture, ornamental and plant biotechnology*. Global Science Books. 3:531-539

Tošić, M., Šutić, D., & Pešić, Z. (1980). Transmission of tobacco mosaic virus through pepper (*Capsicum annuum* L.) seed. *Journal of Phytopathology*, 97(1), 10-13.

Webster, C. G., Roskopf, E. N., Lucas, L., Mellinger, H. C., & Adkins, S. (2014). First report of tomato mottle mosaic virus infecting tomato in the United States. *Plant Health Prog*, 15(2).

Appendix:

Buffers

Extraction Buffer (final 1000mL)

24.2g Tris Base

12.66g Lithium Chloride

15g Lithium Dodecylsulphate

2.92g EDTA

10g Sodium Deoxycholate
14.3mL NP-40
14.33g TCEP-HCl (added separately)

Wash Buffer (500mL)

10mL 1M Tris-HCl pH 7.5
1mL .5M EDTA
10mL 5M NaCl
479mL sterile H₂O

Silica glass milk (100mL)

60 gm silica particles
500 mL sterile distilled H₂O (DDI) or depC water

Elution Buffer/.01M Tris pH 8.5(50 mL)

.5mL 1M Tris-HCl pH 8.5
49.5mL sterile water

MOPS Buffer 10X (1000mL)

41.9g MOPS
8.2g Sodium Acetate
3.72g EDTA

Reverse Transcription recipe (25uL):

18.5uL water
2.5uL 10X buffer w/ Mg⁺
.5uL dntp mix (10mM each)
.5uL F primer (50uM)
.5uL R primer (50uM)
.15uL Taq polymerase

Primers

Table S1: Name, specificity, size of expected band, sequence, and source for each primer used				
Primer Name	Specificity	Size	Primer Sequence	Source of Primer
KL15-282: R106-86	Columnnea Latent Viroid	368	5'-CCGGGGGC TCCTGAGAC CGCTC-3'	Adgia
KL15-283: F107-117	Columnnea Latent Viroid	368	5'-GGCAACTC AGACCGAGC GGGG-3'	Agdia
pCLV4 F	Columnnea Latent Viroid	374	5'-GGGGCTC CTGAGACCG CTCTTG-3'	Batuman, O., & Gilbertson, R. L (2013)
pCLV4 R	Columnnea Latent Viroid	374	5'-GGGGCAA CTCAGACCG AGC-3'	Batuman, O., & Gilbertson, R. L (2013)
KK Pospiviroid F	Universal Pospiviroid	193-225	5'-TCAGGGAT CCCCGGGGA A-3'	USDA ARS HCRL Plant Virology Lab
KK Pospiviroid R	Universal Pospiviroid	193-225	5'-CAGTTGTW TCCACCGGG TAG -3'	USDA ARS HCRL Plant Virology Lab
KK_tobamo F	Universal Tobamovirus	800	5'-GAAGMAG TTGTMGAYG AGTTCAT -3'	USDA ARS HCRL Plant Virology Lab
KK_tobamo R	Universal Tobamovirus	800	5'-CTTCGATT TAAGTGGAG GGAA-3'	USDA ARS HCRL Plant Virology Lab

Table S1. continued				
Primer Name	Specificity	Size	Primer Sequence	Source of Primer
Tobamo F	Universal Tobamovirus	880	5'-GCWAAGG TKGTWYTBG TRGAYGG -3'	Li et al. (2014)
Tobamo R	Universal Tobamovirus	880	5'-GTAATTGC TATTGDGTW CCWGC-3'	Li et al. (2014)