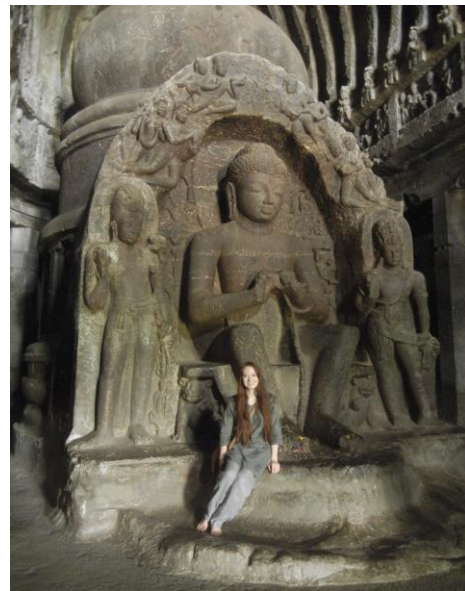
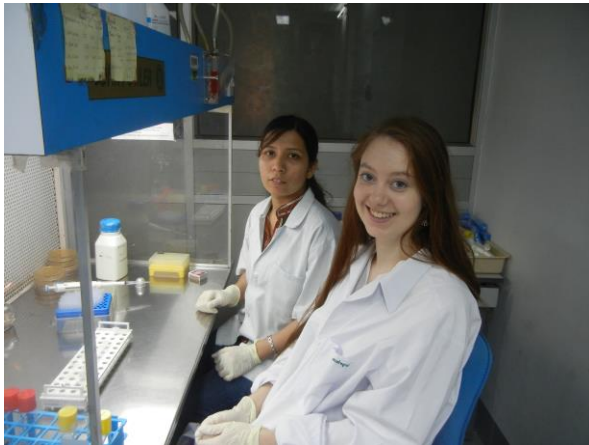


SCIENCE AND CULTURE IN MAHARASHTRA: MY SUMMER AT MAHYCO



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2013 Borlaug-Ruan International Internship
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Introduction

"Hello, my name is Rivkah Gardner-Frolick, I'm from Des Moines, Iowa and this summer I will be going to the Maharashtra Hybrid Seed Company in Jalna, India." I can still remember the excitement I felt as I said this during orientation for the 2013 Borlaug-Ruan Interns. It had been a long journey, but I was so close to arriving at my destination. My freshman year of high school I read an article in the Des Moines Register about the World Food Prize, the Global Youth Institute and the Borlaug-Ruan Internship and I knew I wanted to be involved. It combined the two things I love, science and global awareness. Unfortunately, my school already had their spots in the Global Youth Institute filled for that year, but they told me I could attend the new Iowa Youth Institute in 2012. As I began research for my paper on no-till farming in India, I grew more and more anxious for the Institute to roll around. I found listening to other students talk about their research papers and the variety of topics they had chosen the most interesting part of the Institute and I was excited to discuss my research paper and the meaning of my findings at the Global Youth Institute. Participating in the Global Youth Institute only confirmed my desire to apply for the internship and to my delight, I was selected. Only once I arrived in India did I realize, this internship was simply the start of my adventure, not the end.

Acknowledgements

First of all I would like to thank Norman Borlaug, who was a great pioneer of food security and inspired so many people to work towards the goal of global food security. He had a great love of youth and educational programs and without him I would not have had the opportunity to research viruses in India.

I would also like to thank Ambassador Quinn for his work with the World Food Prize. It continues to grow and prosper under his leadership. A big thank you goes to Lisa Fleming for her tireless dedication to all of the interns. She made our internships run as smoothly as possible and was always available for us, even when she herself was traveling. It is because of her that this program is so successful. Thank you also to Catherine Swoboda and Keegan Kautzsky who continually work to educate young people across the country about food security.

My third round of thanks goes out to all of the people at Mahyco that made my internship possible. Thank you to Dr. Radha for welcoming me into her lab and always striking up a conversation about India or our research. Thank you to Dr. Suresh for answering any questions I had with great patience and making sure my experience at the lab was as fulfilling as possible. Thank you to Kalyani Sarwadnya for taking time out of her schedule to be my mentor, Thank you to Nivrutti Sonone, Surrendra Reddy, Ramesh Karnawar and Babasaheb Berdapatil for taking me out to field and farm visits and explaining everything so thoroughly.

Thank you to my amazing host families, Dr. Zehr and Ben, as well as Dr. Prashar, Ms. Prashar, Akanksha and Saumya. They took me in during my internship taught me so much about Indian culture.

Thank you to Scott Schoneberg, for being a wonderful teacher, mentor and CAS coordinator at Central Academy. I cannot express the extent of my gratitude for the time he has put in to help me be successful in school, with our charitable fundraiser the Sounds of Sympathy Concert, the Central Academy Hunger banquet, and of course the Global Youth Institute. Thank you to Sara Karbeling for being an amazing teacher and was always available for conversation. Thanks to her I am now a better physicist and more knowledgeable about classic movies.

Finally, my family deserves the biggest thank you of all. Without their love and support I would never have been able to achieve so much. I know that if I ever need anything I can ask them. It is from my family that I got my love of science, art and travel and my life would not be the same without these influences. I would like to especially thank my parents. They always knew how to encourage me and have given me all of the support I could ask for, as well as independence to make my own decisions.

Background

Maharashtra Hybrid Seed Company

I spent my internship at Maharashtra Hybrid Seed Company (Mahyco) in Jalna, India. It is situated on a compound about ten minutes outside the town of Jalna, in the state of Maharashtra. The compound includes lab facilities, management offices, houses for employees, a canteen and a gym. The grounds of the compound are well maintained and many plants were in bloom while I was there. During my time at Mahyco I had the opportunity to learn about the mission of the company and its relationship with the community.

Mahyco was founded in 1964 by Dr. Badrinarayan Barwale, the 1998 World Food Prize Laureate. He started off as a farmer in the Maharashtra region of India, where he owned land. With this land he took a risk and planted hybrid seeds. He experimented with different varieties and had success with hybrid okra seed supplied by the Indian Agricultural Research Institute. He was chosen to receive seeds from the Rockefeller Foundation in partnership with the Indian Council of Agricultural Research. After this, Mahyco was born. (Legacy of Mahyco)

The early vision of newly independent India was to become agriculturally self-sufficient. Mahyco was created to provide agricultural support to farmers through enabling them to gain access to better seeds that were needed to realize their dream of self-sufficiency. Since then, Mahyco's emphasis on research and development distinguishes it as a biotechnology company. It operates with "state-of-the-art laboratories for seed health, molecular biology, cytogenetics, pathology, entomology, molecular virology and plant transformation" Their commitment to obtaining a quality final product that will aid farmers is encapsulated in their motto "enhance cultivation with the art of science." (Our Commitment)

In addition to the production of seeds, Mahyco also has a philanthropic emphasis. The Barwale Foundation was created to serve the needs of the population in and around Jalna. It sponsors Ganapati Netralaya eye hospital, Golden Jubilee School, and Barwale College. Each of these serves a vital need in the community. Netralaya eye hospital attracts thousands of patients from all over the state of Maharashtra, as well as other areas of India. They come to Netralaya because of the affordable price of care, with the same standard of quality found at expensive hospitals. For those patients who can't pay for operations, the fees are waived. The hospital also makes its own eyeglasses, which allows them to be accessible to all. The two schools sponsored by the Barwale Foundation focus on providing excellent education to members of the future generation in Jalna, especially in areas of science and biotechnology. Mahyco realizes that its contribution to bettering India does not end with the production of seeds and always strives to better the community.

Plant-Virus Interactions Laboratory

Upon arriving to Jalna, I found out the Plant-Virus Interactions Lab in the Research and Development division of Maharashtra Hybrid Seed Company Limited had agreed to take me under their wing. The goal of the lab is to study viruses and how they interact with plants, since this is an important first step in producing seeds that can resist virus infections. I had little prior experience with viruses and I enjoyed learning more about them from my wonderful co-workers.

The lab is headed by Dr. Radha Andanalakshmi. She earned her BS in Genetics and Masters in Plant Breeding from the University of Agricultural Sciences in Bangalore, India. She then went on to receive her Ph.D in Plant Molecular Biology from the University of South Carolina, mainly working on HC-Pro interacting proteins. Her post doctoral work at Yale centered around R-gene mediated resistance and plant host factors involved in virus resistance pathways, as well as suppressors of gene silencing. She has been the Principle Investigator in the Plant-Virus Interactions lab since 2006 and the Group Leader since 2009.

The Plant-Virus Interactions lab is managed by Dr. Suresh Kunkalikal. He received his Masters in Plant Pathology from the University of Agricultural Sciences in Karnataka, India. His thesis consisted of work on powdery mildew of Greengram caused by *Erysiphe polygoni*. Afterwards he graduated with a Ph.D in Plant Pathology from the same University with thesis work on epidemiology and management of *Papaya ring spot virus*.

Both Dr. Andanalakshmi and Dr. Kunkalikal took large amounts of time out of their schedules to introduce me to my project, as well as answer any questions I had along the way. They planned out my internship incredibly well and selected an appropriate project for me, which fit both my skill level and the time constraints of my internship.

What is Okra?

Okra (*Hibiscus esculentus*) is cultivated to produce seedpods, which can be harvested when immature and eaten as a nutritious vegetable. Though this is the common reason for growing okra, it is certainly not the only use for the okra plant. In addition to the seedpods, other parts of the okra plant can be useful (*Abelmoschus Esculentus*). Like the seedpods, the leaves can also be consumed as a vegetable. The seeds themselves can be used in dishes as a pulse or they can be roasted and ground into a coffee substitute. The stems provide a fiber that is used to make a variety of products.

Okra's origins are unknown but many believe that it is originally from a region that includes modern day Ethiopia (Okra, or "Gumbo"). It then spread out to other parts of Africa and the Mediterranean, but the routes it took are not clear. Later on, French colonists brought it to the New World. The introduction of okra in India likely came from conquering Christians since it is

not found wild and there is no name for okra in any of the old Indian languages. Okra is now widely seen in cuisines throughout the world.

Okra has an unfair reputation in the United States. It's usually cooked in a way that renders it slimy and unappealing. Often it is considered best when deep-fried, but this has more to do with America's obsession with fried foods rather than the qualities of okra. In the US it is most commonly used in gumbo, a Creole dish, or other soups that use okra as a thickener. The real tragedy is that this little vegetable can be so much more. In India, okra is usually eaten cooked with a mixture of spices and sautéed, shallow fried, or added to soups. Many dishes cook okra without added water and include raw mango powder, or some other acidic spice, which cuts down on the mucilage it produces. The tasty meal that results is only one of the reasons why okra is such a popular vegetable in India. One cup of raw okra has 2.0g protein, 3.2g fiber, and is a good source of essential vitamins and minerals such as Vitamin A, Vitamin C, Vitamin K, Thiamin, Vitamin B6, Folate, Magnesium, and Manganese (Okra, Raw).

Why is Okra Important to Indian Farmers?

The high demand for okra from the Indian people necessitates similarly high levels of okra production. Smallholder farmers grow okra either for consumption by their family or for selling at market. In both of these cases the farmer depends on his okra crop to feed his family. The fact that okra accounts for 60% of India's export of fresh vegetables shows the importance of it as a cash crop. Since okra production is significant to both India and Indian farmers, the viruses that infect okra need to be carefully researched and controlled to improve production.

Viruses in Okra

The two viruses known to infect okra used in this study, *Okra yellow vein mosaic virus* (OYVMV) and *Tobacco streak virus* (TSV), are of enormous economic importance because of the devastating effects they have on an infected okra crop. OYVMV causes both lower yield and poor fruit quality (Shetty, et. al). Infected okra plants will produce seedpods that are deformed, discolored and smaller than healthy seedpods (Balamurugan). These okra pods are not marketable and return no profit for the farmer. One of the worst aspects of the virus is the easy transmission from plant to plant. It infects plants through the whitefly vector *Bemisia tabaci*, which are common in the summer months (Balamurugan). They are almost impossible to see and the large numbers of them can easily infect whole fields. The other virus, TSV, causes chlorosis and necrosis in the plant, which damage its ability to produce fruit. Farmers affected by these viruses receive only a few, poor quality okra pods from their fields, causing them a great loss of both food and profit.

Background on Study

This project is a study on the interactions between viruses when their infections are synchronous or sequential and the effects this has on the viruses' multiplication and spread in the host. The three viruses selected for study were *Okra yellow vein mosaic virus* (OYVMV), *Tobacco streak virus* (TSV), and *Groundnut bud necrosis virus* (GBNV). Okra yellow vein mosaic disease is widespread in Indian okra fields, while TSV only recently began to infect okra. GBNV has also started to be seen in okra samples taken from fields, but only in mixed infections with OYVMV and TSV. This raises the concern that infections of OYVMV and TSV make okra plants more susceptible to infection by GBNV. The aim of the study is to explore the effect infections of OYVMV and TSV have on infection by GBNV. There are three major possible outcomes for a mixed infection of these viruses. One, that one virus will turn out to be the dominant virus and there will be suppression of the other virus. Two, both viruses will grow at a slower rate. Finally, the third possibility is a synergistic effect will take place. Using what has been found in previous studies on mixed infections of these viruses in different plants, the hypothesis is that infection by OYVMV and/or TSV will make okra more susceptible to infection by GBNV.

With the goal of studying the interactions between viruses, the following steps will be performed. Okra seedlings will be infected with OYVMV, TSV, and GBNV individually, in pairs and all three at once. Once symptoms begin to appear an enzyme-linked immunosorbent assay (ELISA) test will be performed to serodiagnose the presence of virus. The next step is DNA or RNA extraction, then PCR and RT-PCR to amplify the gene of interest, in this case the coat protein gene, from DNA and RNA respectively. The PCR/RT-PCR products will be run on an agarose gel and the expected size band corresponding to the gene of interest will be cut out of the gel. It will be eluted, ligated and transformed into *E.coli*. Plates containing antibiotic will be streaked with the bacteria that contain the vector/plasmid with the gene of interest of the virus, as well as an antibiotic resistance gene. White (positive) colonies will be inoculated into LB medium and from that the plasmids will be isolated. They will then be digested to release the gene of interest from the plasmid and run on a gel. The positive plasmids will then be purified and the gene of interest sequenced. From the sequence the virus can be identified using the BLAST tool from the National Center for Biotechnology Information.

Procedure

Plants Selected

Susceptible okra plants were grown from seed in individual containers in the greenhouse. Susceptible cowpea plants were grown in the same conditions to be used as controls because of their high level of expression of virus symptoms.

Inoculation

Sl no.	Virus Inoculated	Sequence of Inoculation	No. of Plants	1st Date of Inoculation	2nd Date of Inoculation
1	OYVMV		5	6/25/13	NA
2	TSV		5	6/24/13	NA
3	GBNV		5	6/24/13	NA
4	OYVMV+TSV	OYVMV -> TSV	5	6/25/13	6/26/13
5	OYVMV+GBNV	OYVMV -> GBNV	5	6/25/13	6/26/13
6	GBNV+TSV	GBNV -> TSV	5	6/24/13	NA
7	OYVMV+GBNV+TSV		5	6/26/13	NA

Chart A. Set A inoculated plants.

Sl no.	Virus Inoculated	Sequence of Inoculation	No. of Plants	1st Date of Inoculation	2nd Date of Inoculation
4	OYVMV+TSV	OYVMV -> TSV	5	6/27/13	6/29/13
5	OYVMV+GBNV	OYVMV -> GBNV	5	6/27/13	6/29/13
7	OYVMV+GBNV+TSV		5	6/29/13	NA

Chart B. Set B inoculated plants.

The plants were inoculated with the appropriate viruses. There were two sets of plants. Set A had much younger plants for experiments 4, 5 and 7 while experiments 1, 2, 3 and 6 were older. Set B consisted of older plants used for experiments 1, 2, 3, and 6. Chart A shows the viruses that were inoculated in Set A and Chart B shows the plants in Set B.

Okra yellow vein mosaic virus is transmitted through whitefly inoculation only. Note from Chart A that the plants were inoculated with OYVMV one day prior to their inoculation with a secondary infection. To allow the whiteflies to acquire the virus they were left to feed on plants infected with the OYVMV. After a feeding period of 24 hours the whiteflies had the ability to infect any plant they fed on. About twenty whiteflies were then distributed into each caged plant and were left to feed on the plants for 24 hours. They were then sprayed with insecticide to kill the whiteflies.

For mechanical inoculation with TSV and GBNV, leaf samples infected with a known TSV or GBNV isolate were used. About one gram of frozen tissue was ground in phosphate buffer to extract the sap, and with it, the virus. A fine layer of carbon powder was sprayed onto the leaves of the plants. A piece of cotton was dipped into the sap mixture and rubbed gently on the top of the leaves. The rubbing motion caused the carbon powder to create abrasions on the surface of the leaf, through which the virus could enter. The sap mixture was left on the plant for ten minutes, then washed with water. For inoculation with both TSV and GBNV the two sap mixtures were centrifuged to remove plant debris and the supernatant was mixed in equal amounts. The new sap mixture was applied to the plants in the same manner as detailed above for individual infections.

There was always a positive and negative control kept. In this case, the positive control was a cowpea plant inoculated with the virus sap and the negative control was a healthy plant. The positive controls were infected with GBNV, TSV, and GBNV+TSV from the same sap mixtures that were used to infect the okra plants.

Symptom Observation

Severity of symptoms of TSV and GBNV (Figure 1) were recorded four to six days post inoculation (dpi), while those of OYVMV were recorded twelve days post inoculation.

Symptoms:

OYVMV – vein clearing, mosaic, vein thickening.

TSV – yellow lesions, mosaic and necrotic spots on leaves.

GBNV – yellow lesions, necrotic spots on leaves.

Sample Collection

Samples were collected from symptomatic leaves. For RNA extraction about 100 mg of leaf tissue from each plant was collected into Eppendorf tubes in order to perform an ELISA test. The samples for RNA extraction were dipped into liquid nitrogen and kept at -80° C immediately after they were taken. The samples for ELISA testing were kept at 4° C.

ELISA Testing

An ELISA test was used to check the virus presence in the samples. In this test the virus is trapped in the well plates and antibodies that bind to the virus are attached tagged with alkaline phosphatase. An added substrate, PNPP, reacts with the alkaline phosphatase to create a yellow color in proportion to the amount of virus bound to the well.

A Direct Antigen Coating ELISA was used to determine the presence of GBNV. Protocol devised by Clark and Adams, 1977 was followed starting with 100 mg of sample. The concentration of each well was read at 405 nm with an ELISA reader.

A DAS ELISA was performed to determine the presence of TSV. Protocol devised by Clark and Adams, 1977 was followed starting with 100 mg of sample. The concentration of each well was taken with 405 nm light in an ELISA reader.

Cloning of TSV and GBNV CP Gene

RNA extraction of was performed using Sigma kit as per the directions in the manual. RNA samples were run on a 1.2% Formaldehyde gel. Six samples infected with TSV and six with GBNV, plus the positive controls, were chosen for RT-PCR.

Since TSV and GBNV are RNA viruses, Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was needed to make cDNA copies of the RNA, which can be used like DNA in the subsequent processes. PCR was carried out in a GeneAmp 9700 machine. The PCR products of TSV and GBNV were loaded onto a 1% agarose gel. The gel was run in 1X TAE buffer for 20 minutes at 120 volts. The band where the CP gene was expected appeared on the samples tested for TSV, but the expected gene for GBNV did not appear.

Since the gel electrophoresis process separates DNA fragments by their sizes it can be used to isolate one fragment of a known size or number of base pairs, which in this case is the CP gene of TSV and GBNV. To extract DNA from the gel it needs to be melted and filtered until only the DNA remains. To elute only the CP gene, the bands containing the genes for TSV and GBNV were cut away from each other and the rest of the gel. The elution was performed according to the Qiagen Gel Extraction Kit.

To see the concentration of the elute 2 μL of it was loaded onto a 1% agarose gel. It was determined that using 2.0 μL of elute would provide a sufficient amount of DNA to use for ligation.

A ligation was made to insert the gene of interest into the pTZ57R/T vector's DNA. This was done so that it can be transformed into bacteria and grown, in order to increase the amount of the gene of interest. The ligation was carried out according to the Thermo-scientific Kit. The ligation was prepared by adding 4.5 μL Sterile molecule water, 2.0 μL 5X buffer, 1.0 μL pTz57 R/T, 0.5 μL T4 DNA ligase, and 2.0 μL of elute to total a 10 μL reaction. The ligation was left to incubate at 4°C overnight.

The bacteria were grown on a selective media of ampicillin. The ligation inserted the gene of interest as well as an antibiotic resistance gene. This means that if the ligation were successful then only the bacteria containing the plasmid with the gene of interest would grow. Plates were prepared by melting 100 mL LB agar, cooling it to 60°C, then adding 100 μL ampicillin. The mixture was poured into four plates and left to solidify in the Laminar.

TOPO/DH5 α *E.coli* competent cells were transformed through the KCM method. A mixture of 10 μL ligation, 20 μL 5X KCM, and 70 μL competent cells was made. All of this was performed in a Laminar. The mixture was put on ice for 20 minutes, and subsequently kept at room temperature for 10 minutes. After this 1 mL LB media was added and it was incubated in a rotor box at 37°C for 1.5 hours. It was then centrifuged and most of the supernatant was pipetted off. The bacterial pellet was dispersed into the remaining liquid to provide a higher concentration of bacteria. This liquid was spread evenly on the plate. Then it was incubated at 37°C.

Five positive (white) colonies from the plate were taken and added to LB Media containing Ampicillin. This was left overnight to incubate at 37°C. The plasmid was isolated following instructions from the Sigma Kit. The elute obtained contained plasmid DNA. The DNA was then restriction digested to isolate the gene of interest for confirmation.

Two clones were selected for sequencing. Sequencing PCR was performed with the BigDye terminator cycle sequencing kit (Applied Biosystems) using an automated DNA sequencing system (3130 Genetic Analyzer – Applied Biosystems).

Cloning and Sequencing for OYVMV

DNA extraction of 100mg okra leaf samples was performed by the cetyl trimethyl ammonium bromide (CTAB) method. The pellet produced was then dissolved in 30 μ L distilled water.

PCR - A PCR mixture was made with 2 μ L sample DNA, 2 μ L 2mM dNTPs, 0.8 μ L 50 mM MgCl₂, 0.5 μ L (10 pMol) forward primer, 0.5 μ L (10 pMol) reverse primer, 0.1 μ L Taq. DNA Polymerase, and 12.1 μ L sterile molecule water for a total of 20 μ L reaction. The PCR products were loaded onto a 1% agarose gel. The gel containing the band was cut and then eluted using the same procedure as used for TSV. To test the concentration, 2 μ L of the elute were loaded onto a gel. It was determined that 2 μ L of the elute would be sufficient and the ligation was carried out in the same method as previously stated. The ligation was kept at 4°C overnight.

The rest of the process for sequencing the gene of interest in OYVMV was the same as previously performed for TSV.

Results

The ELISA results for GBNV and TSV are as follows.

Sl.No	Sample No (by PVIL)	Okra Samples inoculated with virus	GBNV	TSV
			DAC A450	DAS A450
1	A-2-1	Okra inoculated with TSV	-	0.773
2	A-2-2	Okra inoculated with TSV	-	0.431
3	A-2-3	Okra inoculated with TSV	-	0.459
4	A-2-4	Okra inoculated with TSV	-	0.810
5	A-2-5	Okra inoculated with TSV	-	0.961
6	A-3-1	Okra inoculated with GBNV	-0.010	-
7	A-3-2	Okra inoculated with GBNV	-0.049	-
8	A-3-3	Okra inoculated with GBNV	-0.036	-
9	A-3-4	Okra inoculated with GBNV	-0.046	-
10	A-3-5	Okra inoculated with GBNV	-0.046	-
11	A-4-1	Okra inoculated with OYVMV + TSV	-	0.262
12	A-4-2	Okra inoculated with OYVMV + TSV	-	0.053
13	A-4-3	Okra inoculated with OYVMV + TSV	-	0.176
14	A-4-4	Okra inoculated with OYVMV + TSV	-	0.066
15	A-4-5	Okra inoculated with OYVMV + TSV	-	0.026
16	A-5-1	Okra inoculated with OYVMV + GBNV	-0.009	-
17	A-5-2	Okra inoculated with OYVMV + GBNV	-0.007	-

18	A-5-3	Okra inoculated with OYVMV + GBNV	-0.042	-
19	A-5-4	Okra inoculated with OYVMV + GBNV	0.000	-
20	A-5-5	Okra inoculated with OYVMV + GBNV	-0.030	-
21	A-6-1	Okra inoculated with GBNV+ TSV	-0.032	0.674
22	A-6-2	Okra inoculated with GBNV+ TSV	-0.042	0.811
23	A-6-3	Okra inoculated with GBNV+ TSV	-0.037	1.069
24	A-6-4	Okra inoculated with GBNV+ TSV	-0.043	0.523
25	A-6-5	Okra inoculated with GBNV+ TSV	-0.039	0.369
26	A-7-1	Okra inoculated with OYVMV + GBNV + TSV	-0.048	0.151
27	A-7-2	Okra inoculated with OYVMV + GBNV + TSV	-0.014	0.328
28	A-7-3	Okra inoculated with OYVMV + GBNV + TSV	-0.050	0.175
29	A-7-4	Okra inoculated with OYVMV + GBNV + TSV	-0.033	0.144
30	A-7-5	Okra inoculated with OYVMV + GBNV + TSV	-0.050	0.103
31	B-4-1	Okra inoculated with OYVMV + TSV	-	0.725
32	B-4-2	Okra inoculated with OYVMV + TSV	-	0.531
33	B-4-3	Okra inoculated with OYVMV + TSV	-	0.388
34	B-4-4	Okra inoculated with OYVMV + TSV	-	0.531
35	B-4-5	Okra inoculated with OYVMV + TSV	-	0.473
36	B-5-1	Okra inoculated with OYVMV + GBNV	-0.029	-
37	B-5-2	Okra inoculated with OYVMV + GBNV	-0.050	-
38	B-5-3	Okra inoculated with OYVMV + GBNV	-0.036	-
39	B-5-4	Okra inoculated with OYVMV + GBNV	-0.046	-
40	B-5-5	Okra inoculated with OYVMV + GBNV	-0.017	-
41	B-7-1	Okra inoculated with OYVMV + GBNV + TSV	-0.047	0.344
42	B-7-2	Okra inoculated with OYVMV + GBNV + TSV	-0.044	0.406
43	B-7-3	Okra inoculated with OYVMV + GBNV + TSV	-0.045	0.509
44	B-7-4	Okra inoculated with OYVMV + GBNV + TSV	-0.037	0.526
45	B-7-5	Okra inoculated with OYVMV + GBNV + TSV	-0.046	0.603
		Healthy Okra	-0.003	-0.004
		Buffer Control	0.000	0.000
		Positive for GBNV	1.166	
		Positive for TSV		0.653

Chart C. ELISA results. The highlighted values represent positive results.

Results of PCR

TSV

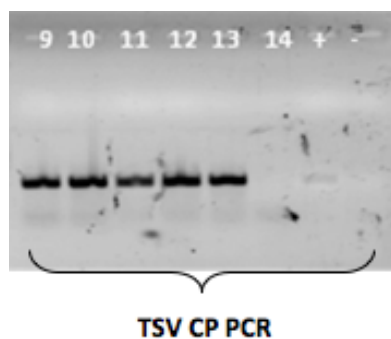


Figure 2. Gel results of TSV CP PCR.

Well No.	Plant No.
9	A-2-1
10	B-4-1
11	A-6-3
12	B-7-3
13	B (GBNV+TSV control)
14	B (GBNV control)

Chart D. Key to TSV gel results.

The coat protein gene of TSV matched those of known sequences with 91% accuracy.

Images of the plants infected with TSV that were used in the above gel electrophoresis.



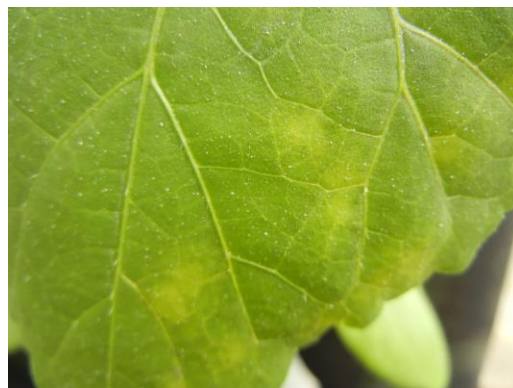
A-2-1



B-4-1



A-6-3



B-7-3

GBNV

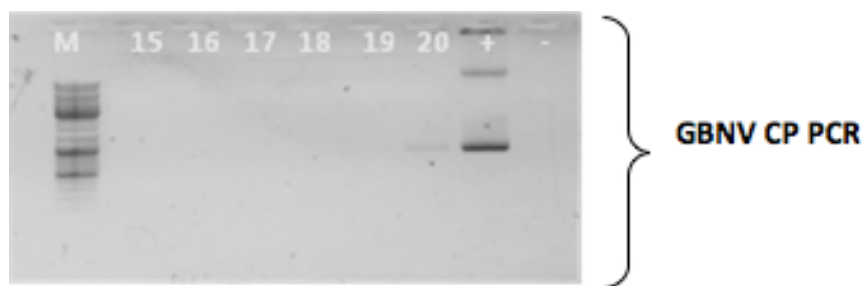


Figure 3. Gel results of GBNV CP PCR.

Well No.	Plant No.
15	A-3-1
16	B-5-1
17	A-6-3
18	B-7-3
19	B (GBNV+TSV control)
20	B (GBNV control)
M	Gene Rular DNA Ladder

Chart E. Key to GBNV gel results.

No GBNV infections were found in any of the plants tested.

OYVMV

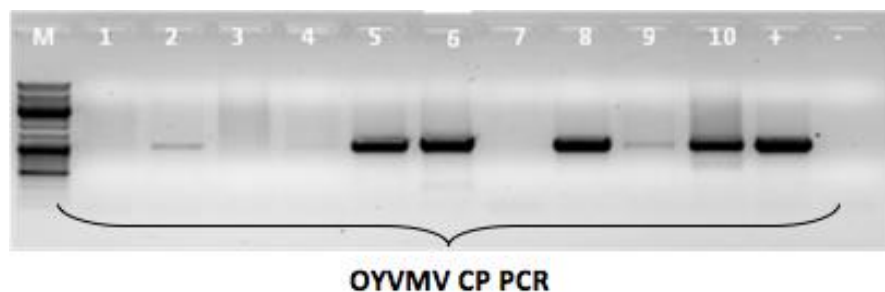


Figure 4. Gel results for OYVMV CP PCR.

Well No.	Plant No.
1	A-1-1
2	A-1-2
3	B-4-1
4	B-4-2
5	B-5-1
6	B-5-2
7	B-7-1
8	B-7-3
9	B-7-2
10	B-1-1
11	positive control
12	negative control
M	Gene Ruler DNA Ladder

Chart F. Key to OYVMV gel results.

Discussion and Conclusion

It is common for different viruses to concurrently infect a host. When more than one virus infects a plant they can interact with each other and either one becomes dominant, they both suffer, or they can both thrive. In samples taken from the field there have been cases where GBNV can infect and thrive in okra already infected with either OYVMV or TSV or infected with OYVMV and TSV. This experiment seemed to indicate a synergistic action of the okra viruses. The results of this experiment can help support this hypothesis. However, since the experiment was performed in lab conditions we cannot definitively say field conditions would induce the same response. It was decided that the experiment would be repeated with fresher GBNV inoculum to see if different results are found.

Experiment 2

Results

Sl. no.	Plant no	Virus	GBNV	TSV
			DAC A450	DAS A450
1	1	TSV	NA	+ ve
2	2	“	“	“
3	3	“	“	“
4	4	“	“	“
5	5	“	“	“
6	1	GBNV	+ ve	NA
7	2	“	“	“
8	3	“	“	“
9	4	“	“	“
10	5	“	“	“
16	1	OYVMV + GBNV	+ ve	NA
17	2	“	“	“
18	3	“	“	“
19	4	“	“	“
20	5	“	“	“
21	1	GBNV+ TSV	+ ve	+ ve
22	2	“	“	“
23	3	“	“	“
24	4	“	“	“
25	5	“	“	“
26	1	OYVMV + GBNV + TSV	+ ve	+ ve
27	2	“	“	“
28	3	“	“	“
29	4	“	“	“
30	5	“	“	“
31	1	OYVMV + TSV	NA	+ ve
32	2	“	“	“
33	3	“	“	“
34	4	“	“	“
35	5	“	“	“
41	1	OYVMV + GBNV + TSV	+ ve	+ ve
42	2	“	“	“
43	3	“	“	“
44	4	“	“	“
45	5	“	“	“
		Healthy Okra	-0.003	-0.004

	Buffer Control	0.000	0.000
	Positive for GBNV	1.166	
	Positive for TSV		0.653

Chart G. ELISA results for experiment 2.

TSV – An amplicon of expected size of 0.7 kb was observed in all the samples (Fig. 5).

GBNV – An amplicon of expected size of 0.85 kb was observed in all the samples (Fig. 6).

OYVMV – An amplicon of expected size of 1.0 kb was observed in all the samples (Fig. 7).

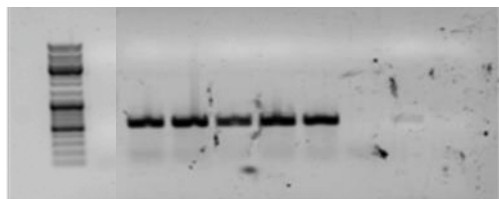


Figure 5. Gel results for TSV CP PCR.

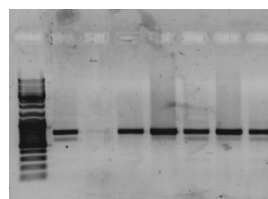


Figure 6. Gel results for GBNV CP PCR.

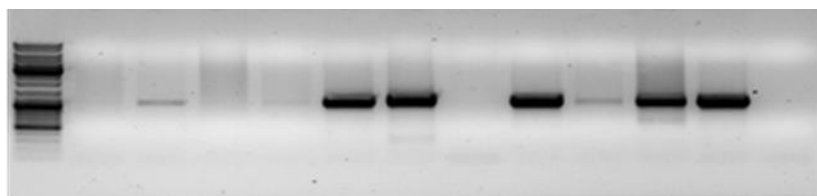


Figure 7. Gel results for OYVMV CP PCR.

Discussion and Conclusion

In the second experiment a fresher virus inoculum was used than in the first experiment. In Experiment 2 it was found that infections by other viruses do make the plant more susceptible to infection by GBNV, as GBNV was found in mixed infections with these viruses, but not by itself. The results lend support to our hypothesis that GBNV can infect okra when it is already infected with other viruses. This could lead to worsening of the initial infection, which would make a virus infection almost inevitably severe. The results of the experiment raise alarm with the possibility of a new virus expanding its host range to include okra. This would be detrimental to farmers as it adds another virus that has the potential to cause serious damage to their crop. The new ability of GBNV to infect okra needs to be carefully monitored.

Implications for Food Security

In India, okra is a commonly grown crop used for consumption as well as for selling on the market. It also makes up a significant portion of India's fresh vegetable exports, over sixty percent. Okra is vital, not only to the individual Indian farmer, but to the country. This study shows that *Ground nut bud necrosis virus* (GBNV) has recently widened its host range to include okra. Already, any infection by the two viruses previously known to infect okra, *Tobacco streak*

virus (TSV) and *Okra yellow vein mosaic virus* (OYVMV), can be devastating to a crop. With the addition of a third virus, this likelihood increases. While GBNV is currently only found in infections with TSV and OYVMV, it could evolve to be pathogenic without the aid of the other two viruses. This would present yet another challenge to smallholder farmers. Without a healthy okra harvest many farmers could see a significant drop in income as okra infected with these viruses produces small, malformed and discolored fruits, which has reduced marketability. Any loss of income would ultimately contribute to their family's food insecurity.

The results of this study can be used for developing our knowledge about viruses and their interactions in okra. The goal is to create molecular and breeding strategies to develop quality seeds that will either be able to continue to produce good quality okra pods even after infection by these viruses or be resistant. This would allow farmers to have a good okra crop, which would in turn lead to an increase in their income. Farmers and their families will be more food secure when they are able to earn the expected income from their fields.

Reflections

My Borlaug-Ruan Internship experience was amazing. I had the opportunity to travel to India, work to ensure food security, meet many incredible people and experience a unique culture that welcomed me with open arms. I still cannot believe my good fortune in being selected to be one of the twenty-two interns whose lives changed forever. From my research to my cultural excursions, I will never forget the time I spent in India.

I had the opportunity to explore many of the attractions around Jalna. I visited Bibi Ka Maqbara, commonly known as the “Mini Taj,” the Ellora Caves, and the Ajanta Caves. My favorite was the Ajanta Caves. Calling them magnificent would be an understatement. They consist of a string of twenty-nine caves carved by hand into the rock face. The caves are a collection of Buddhist monasteries and temples that are thought to have been built around 200 BC. I was stunned by the richly painted walls and ceilings of some of the caves, as well as the hundreds of Buddhas in all shapes and sizes. I was fascinated by the vibrant culture of India wherever I went.

Near the end of my time in India, I had the opportunity to be able to travel to Mumbai with my host sister. I was thrown from the peaceful life in the countryside around Jalna into the hustle and bustle of a huge city. In Mumbai I got to go to the beach, a temple, a museum, a mall and a street market. Through this I got a taste of both sides of India.

Throughout my stay I discovered the importance of food to Indian culture and the everyday lives of Indians. Even though India is at the forefront of the modern world with all of its technological developments, Indians still take pride in “real food.” Overly processed food does not form an integral part of their diets. My host mom explained to me that it is considered better to make your own food, from the buying of the vegetables, to creating spice mixes to flavor dishes. I could see this readily in my meals with my host family, my host mom always made sure that her family had a freshly cooked meal made from scratch for lunch and dinner. I believe that many other countries that have forgotten this principle should learn from the Indian philosophy about food.

After two months of living and experiencing India, I reflected on how my internship had changed my views. Through my conversations with my fellow researchers, many of whom grew up on farms, and with farmers, I learned that food insecurity needs to be solved using science. Science is the key to our future success at sustainably feeding a growing population. Developing technology to aid us in our endeavors will not come quickly, but it will help the many food insecure people across the globe. My Borlaug-Ruan Internship reaffirmed my faith in science being used to change the world for good.

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Appendix

Figure 1

Symptom observation on Okra infected with OYVMV, TSV and GBNV				
Sl. No	Sample No (by PVIL)	TSV Symptoms 4 Days post inoculation	GBNV Symptoms 4 Days post inoculation	OYVMV Symptoms 12 Days post inoculation
1	A-2-1 (TSV Inoculated)	Sev	NA	NA
2	A-2-2 (TSV Inoculated)	Sev	NA	NA
3	A-2-3 (TSV Inoculated)	Sev	NA	NA
4	A-2-4 (TSV Inoculated)	Sev	NA	NA
5	A-2-5 (TSV Inoculated)	Sev	NA	NA
6	A-3-1 (GBNV Inoculated)	NA	VMLD	NA
7	A-3-2 (GBNV Inoculated)	NA	VMLD	NA
8	A-3-3 (GBNV Inoculated)	NA	VMLD	NA
9	A-3-4 (GBNV Inoculated)	NA	VMLD	NA
10	A-3-5 (GBNV Inoculated)	NA	VMLD	NA

11	A-4-1 (T+O Inoculated)	Sev	NA	MLD
12	A-4-2 (T+O Inoculated)	Sev	NA	MLD
13	A-4-3 (T+O Inoculated)	Sev	NA	MLD
14	A-4-4 (T+O Inoculated)	Sev	NA	MLD
15	A-4-5 (T+O Inoculated)	Sev	NA	MLD
16	A-5-1 (G+O Inoculated)	NA	VMLD	MLD
17	A-5-2 (G+O Inoculated)	NA	VMLD	MLD
18	A-5-3 (G+O Inoculated)	NA	VMLD	MLD
19	A-5-4 (G+O Inoculated)	NA	VMLD	MLD
20	A-5-5 (G+O Inoculated)	NA	VMLD	MLD
21	A-6-1 (G+T Inoculated)	Sev	No GBNV symptom	NA
22	A-6-2 (G+T Inoculated)	Sev	No GBNV symptom	NA
23	A-6-3 (G+T Inoculated)	Sev	No GBNV symptom	NA
24	A-6-4 (G+T Inoculated)	Sev	No GBNV symptom	NA
25	A-6-5 (G+T Inoculated)	Sev	No GBNV symptom	NA
26	A-7-1(G+T+O Inoculated)	Sev	No GBNV symptom	MLD
27	A-7-2 (G+T+O Inoculated)	Sev	No GBNV symptom	MLD
28	A-7-3 (G+T+O Inoculated)	Sev	No GBNV symptom	MLD
29	A-7-4 (G+T+O Inoculated)	Sev	No GBNV symptom	MLD
30	A-7-5 (G+T+O Inoculated)	Sev	No GBNV symptom	MLD
31	B-4-1 (T+O Inoculated)	Sev	NA	MLD
32	B-4-2 (T+O Inoculated)	Sev	NA	MLD
33	B-4-3 (T+O Inoculated)	Sev	NA	MLD
34	B-4-4 (T+O Inoculated)	Sev	NA	MLD
35	B-4-5 (T+O Inoculated)	Sev	NA	MLD
36	B-5-1 (G+O Inoculated)	NA	VMLD	SEV
37	B-5-2 (G+O Inoculated)	NA	VMLD	SEV
38	B-5-3 (G+O Inoculated)	NA	VMLD	MLD
39	B-5-4 (G+O Inoculated)	NA	VMLD	SEV
40	B-5-5 (G+O Inoculated)	NA	VMLD	MLD
41	B-7-1 (G+T+O Inoculated)	Sev	No GBNV symptom	MOD
42	B-7-2 (G+T+O Inoculated)	Sev	No GBNV symptom	MOD
43	B-7-3 (G+T+O Inoculated)	Sev	No GBNV symptom	SEV
44	B-7-4 (G+T+O Inoculated)	Sev	No GBNV symptom	MLD
45	B-7-5 (G+T+O Inoculated)	Sev	No GBNV symptom	MOD

Chart H. Symptoms observed in infected okra plants.