Toward the Effective Integrated Pest Management of Plant Disease Caused by Viruses in Developing Countries: Detection and Diagnosis, Capacity Building and Training, and Formulation of IPM Packages

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Summary:

Plant virus diseases transmitted by insect vectors and through seed or germplasm are one of the major contraints to vegetable production in countries of the IPM CRSP. Approaches to management of viral diseasesdesigned from information gained are intended to be applicable throughout the IPM CRSP and incorporated into IPM packages. Within the IPM-CRSP, the program collaborates mainly with the Diagnostics Global Themes and with all Regional Centers. Host country collaborators with scientific expertise in virology have been identified in most countries in all six regions. Efforts in the first year were focused on establishing contacts to enable initiation of work under the objectives. Host countries reporting viruses identified in this IPVDN report include Uzbekistan, Indonesia, Cambodia, India, Bangladesh, Dominican Republic, Guatemala, Honduras, and Mali, representing five of the six regions. Some of the identifications were performed in the host countries by the use of commercial test kits or sample submission for commercial testing, and some by collection of samples by US scientists for testing in their labs by PCR from membranes. The main crops tested were tomato, pepper, potato, cucurbits, bean, okra, and sweetpotato. Viruses from were identified belonging to nine different genera, with whitefly-transmitted begomoviruses and aphid-transmitted potyviruses being the most prevalent. A psyllid-transmitted bacterium, Liberibacter, has been identified in Central America as causing virus-like symptoms in potato and tomato. These findings will be used to prioritize future research on ecology and epidemiological research to study the ecology of virus-vector-host interactions in selected vegetable cropping systems and to recommend management packages. The aim of the second objective is to increase in-country capacity for virus diagnosis and conducting research on management approaches through training. A week-long workshop attended by scientists from the three Asian regions covered principles of virology, diagnosis, epidemiology, and field observation of tomato production practices. Samples collected were used for demonstration of ELISA, TBIA, and PCR diagnostic methods. Appraisals of virus ecology and temporal and spatial dynamics of aphid and whitefly vector species and populations are being conducted in some locations in order to design IPM approaches. Data are presented on efficacy of host-free periods to reduce incidence of whitefly transmitted begomoviruses in parts of the Dominican Republic. Programs are being conducted to produce clean potato and sweetpotato planting material, to reduce seed transmission of virus in okra and yardlong bean, to reduce virus incidence and impact by early roguing of symptomatic tomato, and to select for resistance in pepper, tomato, and other crops.

Important plant virus diseases and their vectors

Southeast Asia: Indonesia

Detection and diagnosis of viruses in vegetable crops in Indonesia

Detection and diagnosis of viruses in Indonesia was initially implemented by collecting field samples from West Java - mainly three vegetable crops, i.e. tomatoes, chilli pepper, and yard long bean. Serological techniques were routinely conducted at Bogor Agricultural University using ELISA as the initial detection method. Further diagnosis using PCR and nucleic acid sequence analysis of the PCR product was undertaken in some cases. Diagnosis of tomato and chilli pepper was usually conducted using 3 antisera, i.e. tobacco mosaic tobamovirus (TMV), cucumber mosaic cucumovirus (CMV), and general potyvirus, and one additional antiserum for chilli pepper, i.e. chilli veinal mottle potyvirus (ChiVMV). Detection of geminivirus of the Begomovirus genus was conducted by PCR methods as a routine activity since geminivirus infection has

become epidemic in tomato and chilli pepper in Indonesia for the last few years. In collaboration with the activities of Southeast Asia Regional Project, tomato and chilli pepper samples were collected from fields at Cipanas, Bogor, West Java in 2010. No positives of TMV, CMV, and ChiVMV were detected, but geminivirus was positively detected in 50% of the chilli pepper samples. This result was not surprising since previous surveys, conducted on a yearly basis since 2006, have shown that geminivirus is the predominant virus in tomato and chilli pepper. Survey of viral diseases on chilli pepper was part of the research activities under ACIAR project since 2006 whereas the survey on tomatoes was part of research activities funded by ABSP 2– USAID. Tests have not yet been done to identify the specific geminivirus(es).

Determining a causal agent of the yellow mosaic disease of yard long bean

Specific detection and diagnosis study was conducted to identify a virus associated with the yellow mosaic disease in yard long bean. This research was partially funded by Bogar Agricultural University through an International linkage Research Activity (PUI) to Tri Asmira Damayanti. The serological and transmission tests were conducted in the Laboratory of Plant Virology, Bogor Agricultural University (BAU), and the molecular detection, cloning, and sequencing were done at Washington State University (WSU). Based on serological tests, transmission tests and RT-PCR detection, yellow mosaic disease in Bubulak, Bogor (West Java) was associated with mixed infections of Bean common mosaic potyvirus (BCMV) and CMV. Further field surveys were conducted to collect samples from several districts in West Java, including Jatisari Karawang, Subang (Ciasem, Ciasem Tengah, Pegaden), Indramayu (Jatibarang, Bongas, Cadangpinggan) and Cirebon (Kaliwulu, Dawuan, Babakan, Saraban). Samples were then subjected to serological tests against antiserum (obtained from DSMZ (German Collection of Microorganisms and Cell Cultures), of *Bean common mosaic poytvirus* strain Peanut stripe – BCMV-PSt, *Bean common mosaic necrotic poytvirus* – BCMNV, *Bean yellow mosaic virus* – BYMV, *Bean leaf roll luteovirus* - BLRV, *Bean golden mosaic begomovirus* – BGMV, *Cowpea aphid-borne mosaic potyvirus* – CaBMV, *Cowpea severe mosaic comovirus* – CPSMV, *Cucumber mosaic virus* – CMV and genus specific *Potyvirus*. Samples reacted positively only against antisera to the potyvirus genus and to CMV, revealing both single and double infections. RT-PCR results showed that samples from Karawang (Jatisari), Subang (Ciasem), and Cirebon (Dawuan) were successfully amplified by a universal primer for Potyvirus. Samples from Subang (Ciasem Tengah, Pegaden), Indramayu (Cadang pulang) only showed weak DNA band with the same primer. Samples from Subang (Pegaden), Cirebon (Dawuan) and Indramyu (Cadang Pulang) reacted differently because they were successfully amplified using primer for CMV. Thus it appears that at least two viruses, a potyvirus and CMV, are associated with yard long bean showing yellow mosaic symptoms. Further tests are in progress.

Southeast Asia: Cambodia

Mr. Chou Cheythyrith from the National IPM Program of Cambodia and Ms. Hong Chanvibol, Plant Protection Sanitary and Phytosanitary, attended the Virus Workshop in Coimbatore, India and provided information on crops and viruses in a presentation to the group. Images were shown on tomato and pepper showing severe symptoms and identified simply as Yellow leaf curl virus and Mosaic virus in tomato and pepper. Leaves of Common bean and Yardlong bean had leaves with mosaic and were much smaller in size. Naidu has identified the virus on ribbed gourd and cucumber to be *Luffa yellow mosaic begomovirus* (LoYMV). There seemed to be little known as to exact virus identities,

although it was recognized that whitefly and aphids were main vectors.

South Asia and Southeast Asia:

Detection of viruses in several countries from plant tissue imprinted on FTA cards and nitrocellulose membranes (Rayapati, WSU-IAREC)

Plant samples suspected for virus infections, based on visual symptoms, were collected from tomato, chilli peppers, okra, bitter gourd, bottle gourd, cucumber, pumpkin, ridge gourd, sweet potato, okra and tomato in farmers' fields in India, Bangladesh, Nepal, Cambodia, Indonesia and Thailand, and Honduras. Various types of tissue (leaf, stem, petiole and fruit) were directly pressed gently on FTA® cards or nitrocellulose membrane (NCM) in the field, which were allowed to air dry prior to shipment to the Rayapati lab (WSU) for further processing and testing for different viruses. In addition, samples from non-vegetable crops, such as amaranthus, jasmine, papaya, passiflora growing in the vicinity of vegetable fields in the above countries and sweet potato from Honduras, were also imprinted on FTA® cards and sent for virus testing.

A simplified 3-step nucleic acid extraction protocol, initially developed for the detection of grapevine viruses, was used to elute nucleic acids captured on the FTA® cards and NCMs. Four to five discs (~2 mm in diameter) were punched from sap-stained spots on the FTA® cards or NCMs using the Harris Micro Punch (Sigma-Aldrich, St. Louis, MO). These discs were transferred into an eppendorf tube and nucleic acids bound to the discs were eluted using an appropriate buffer. Nucleic acids in the eluent were subjected to polymerase chain reaction (PCR) or reverse transcription (RT)-PCR using species-specific or broad spectrum primers for the detection of viruses infecting vegetable and non-vegetable crops. Degenerate primers specific to a portion of the cylindrical inclusion body of potyviruses, for the common region of geminiviruses or the replicase gene of tospoviruses, or primers specific to the coat protein of Cucumber mosaic cucumovirus (CMV) were used for PCR/RT-PCR detection of viruses in each sample. DNA fragments amplified by RT-PCR or PCR were subsequently cloned and sequenced. The derived nucleotide sequences were compared with corresponding sequences in GenBank. An analysis of the sequence data indicated the presence of potyviruses, geminiviruses and CMV listed in Table 1. None of these samples tested positive for tospoviruses. Further research is being conducted to gain additional molecular data for accurate identification of these viruses. This information will be used subsequently to develop diagnostic assays for virus identification at the field level in varietal evaluations and IPM trials in host countries.

Table 1. Viruses detected in samples from South and Southeast Asia.

Crop	Country	High similarity with:
Bitter gourd	India	*Squash leaf curl Philippines begomovirus* isolate P133; *Tomato leaf curl New Delhi begomovirus* (ToLCNDV)
Bottle gourd	India	ToLCNDV-[Pakistan:Solanum] isolate Rahim Yar Khan 2 clone PT6
Tomato	India	ToLCNDV isolate ToLCNDV-CTM
Amaranthus	India	*Basella rugose mosaic virus isolate BR*
Passiflora	India	*Chili veinal mottle begomovirus* (ChiVMV)
Tomato	Bangladesh	*Tomato leaf curl Patna begomovirus*
Tomato	Bangladesh	*Tomato leaf curl Bangladesh begomovirus*
Tomato	Bangladesh	ToLCNDV
Okra	Bangladesh	*Bhendi yellow vein mosaic begomovirus*
Papaya	Bangladesh	*Papaya ring spot potyvirus*
Tomato	Nepal	ChiVMV
Cucumber	Nepal	ChiVMV
Pumpkin	Nepal	*Zucchini yellow mosaic potyvirus*
Papaya	Nepal	*Papaya ring spot potyvirus*
Chilli pepper	Indonesia	*Cucumber mosaic cucumovirus*
Ridge gourd	Cambodia	*Luffa yellow mosaic begomovirus* (LoYMV)
Cucumber	Cambodia	LoYMV
Jasmine	Thailand	*Plum pox potyvirus isolate 48-922 from Canada*
Sweet potato	Honduras	*Sweet potato feathery mottle potyvirus*
Pepper	Honduras	*Pepper golden mosaic begomovirus*

South Asia: Bangladesh

Survey and diagnosis of virus diseases of vegetable crops using commercial ELISA kits

A survey was conducted on the status of virus diseases of vegetables in five districts (Narsingdi, Bogra, Chittagong, Gazipur and Jessore) of Bangladesh during summer (June-August) 2010. Only farmer's fields were included in the survey. Four to five plots (minimum 0.1 ha) were visited. Disease incidence and severity of virus disease were assessed visually, using a 1-4 rating (Table 2). Samples were collected in poly bags, kept cool over ice during transport, and then refrigerated until laboratory analysis by enzyme-linked immunosorbent assay (ELISA) was conducted, using commercial kits and protocols from ADGEN Phytodiagnostics (NEOGEN Europe Ltd. UK).

Among the cucurbitaceous vegetables, cucumber had the highest incidence of plants showing symptoms of virus disease and severity, followed by sponge gourd, ridge gourd and bottle gourd. Bitter gourd expressed no symptoms except in one area (Jessore). Teasle gourd and pointed gourd were apparently virus free. In other categories of vegetables, okra and yardlong bean had highest incidence of virus symptoms, followed by summer tomato. Country bean was apparently virus free, as no symptoms were observed. Disease incidence was fairly constant across different locations in crops except in okra. In most of the locations incidence of virus in okra was very high (more than 75%) but it was unusually low (around 10%) in Chittagong. In spite of higher incidence of virus disease, the in-field population of potential aphid or whitefly vectors was negligible at the time of observation, which was when the crop was mostly in mid to late fruiting stages and may be one of the reasons for low vector activity.

Table 2. Incidence and severity of virus diseases of some vegetables in several districts of Bangladesh during summer 2010

Crops	Locations of Surveyed Area									
	Narsingdi		Bogra		Chittagong		Gazipur		Jessore	
	Dis.Inc	Sev	Dis.Inc	Sev	Dis.Inc	Sev	Dis.Inc	Sev	Dis.Inc	Sev
Cucumber	75	3	80	3	70	2	*	*	30	2
Sponge gourd	50	3	*	*	30	2	30	2	*	*
Ridge gourd	30	2	*	*	20	2	*	*	15	2
Bottle gourd	*	*	30	2	20	2	15	2	*	*
Bitter gourd	0	0	*	*	0	0	*	*	50	3
Teasle gourd	0	0	0	0	0	0	*	*	*	*
Pointed gourd	*	*	0	0	*	*	*	*	*	*
Okra	80	3	80	4	10	2	*	*	*	*
Tomato	*	*	*	*	*	*	*	*	20	2
Country Bean	*	*	*	*	*	*	*	*	0	0
Yardlong Bean	*	*	80	3	25	2	*	*	*	*

Dis.Inc: Disease Incidence (%), Sev: Disease Severity (1= Only mild symptom observed on few leaves, 2 = More severe symptom than 1 but no apparent yield loss, 3 = Severe symptom with some degree of yield loss, 4 = Severe stunting and high yield loss). * No fields were observed.

Testing cucurbitaceous crops by ELISA revealed only one virus, Watermelon mosaic potyvirus (WMV), in only one of six sponge gourd and one of three ridge gourd samples. The remaining 23 samples tested negative to CMV and three potyviruses, papaya ringspot, zucchini yellow mosaic, and WMV. This was viewed as very unusual because symptoms in the field were visually very much like those recognized as viral diseases. Failure to identify one of the viruses tested as the cause of symptoms was attributed to the cause being a nutritional disorder or undetectable low titers of the virus in the portions tested because of age of the plants. Alternatively, other viruses might have been present that could not be tested for due to unavailability of appropriate kits. Virus-like symptoms were also observed on Okra and yardlong bean, but identities could not be assessed because of lack of test kits. Further investigations are planned to identify the viruses in these crops.

Latin America and the Caribbean: Dominican Republic

Incidence and distribution of Tomato spotted wilt virus in tomato

Last year, Tomato spotted wilt tospovirus (TSWV) had been detected in protected crops of tomato and pepper at two locations in the northern part of the country, Jarabacoa and Constanza. This year TSWV was documented in tomato growing in open fields in the southern area of Sabana. ELISA kits and immunostrips from Agdia were used for the virus diagnosis. A survey was performed to determine the distribution of TSWV in Nizao, Las Auyamas, Sabana Larga, Rancho arriba, las Caobas, Carretera Ocoa-Azua and Carretera Palenque San Cristobal of Ocoa Valley. Positive samples were found only in Sabana Larga with an incidence of 10%. This thrips-transmitted virus is more prevalent under protected rowing conditions than in open fields.

New virus-like symptoms found on tomato grown under protected conditions

A new symptom typified by apical chlorosis (Fig. 1) was found on tomato grown under protected conditions at San Josè de Ocoa in the localities of Sabana Larga, La cienega, El Pinal and Rancho Arriba. The pathogen has not been characterized, but it is causing around 90% incidence in those facilities. IDIAF ran ELISA tests for PVY, CMV, TEV, and TMV. The results were negative for those viruses. Samples were taken and sent to Dr. Margarita Palmieri at the Universidad del Valle in Guatemala, where she ran the PCR and obtained some bands that need to be sequenced to identify the virus.

Latin America and the Caribbean: Guatemala

Detection of begomoviruses and Candidatus liberobacter in tomatoes and peppers

To gain insight into the nature of the viruses and other pathogens causing disease symptoms in peppers and tomatoes in Guatemala, representative samples were collected from peppers and tomatoes showing symptoms of virus infection in March 2010. Sap was prepared from these samples and applied to AgDia absorption strips, and the strips were sent to UC Davis where DNA extractions and PCR analyses were performed. Vilmori, a French seed company, contributed funds to assist in processing of the samples from Guatemala.

Table 3. Results of virus and Candidatus liberobacter tests conducted on tomato samples collected in Guatemala in March 2010

Sample	Symptoms	General begomovirus	TYLCV	ToMHV	ToSLCV	Liberobacter 1	Liberobacter 2	Phytoplasma
T1	severe distortion, stunting (broccoli) yellowing/purpling	+	--	+	+	+	+	--
T2	Upcurled leaves, purple vein, distorted growth	+	--	+	+	--	--	--
T3	Upcurled leaves, purple vein, crumple, mild distorted growth	+	--	+	+	--	--	--
T4	Strong upcurling, purple vein, new growth stunted	+	--	+	+	--	--	--
T5	Strong upcurling, purple vein (curly top-like) similar to T3	+	--	+	--	--	--	--
T6	Strong interveinal Chlorosis of old lvs, Upcurling, purple vein (=184 sb6)	+	--	+	+	+	+	--
T7	Strong yellow mottle Crumple, light grn Yellow mottle (milder)	+	--	--	+	--	--	--
T8	Older leaves interveinal yellows and brittle/young leaves upcurled w/purple vein	+	--	+	+	--	--	--
T9	General yellowing on older grwth and brittle new growth with yellow And purple veins	+	--	+	+	+	+	--
T10	Strong upcurling, crumple, yellowing TYLCV-like	+	+	+	+	--	--	--
T11	Yellowing of older leaves and brittle stunted growth, purple	+	+	+	+	+	+	--
T12	Strong yellow mottle crumple on old leaves severe distortion/crumple on new growth	+	+	+	+	--	--	--

Results based on PCR analysis with general begomovirus primers, primers specific for TYLCV (2560c/1480c), ToMHV DNA-A (2200v/900c) and ToSLCV DNA-A (520v/1860c). Two primer pairs were used for Liberobacter: 1=OA2/OI2c and 2=CL514F/R and the PI/Tint primers were used to detect phytoplasmas.

The peppers had typical symptoms of begomovirus infection. PCR analyses confirmed all four samples were infected with the begomovirus *Pepper golden mosaic virus* (PGMV). All twelve tomato samples were infected with one or more begomoviruses (Table 3). Four (T1, T6, T9 and T11) were also infected with *Liberobacter* (based on PCR with 2 primer pairs), whereas none were infected with phytoplasma (P1/Tint primers used). Tomatoes infected with *Liberobacter* showed general yellowing, including older leaves, leaf brittleness, stunted growth, and new growth was yellow with purple veins. Most of the tomato samples were infected with *Tomato mosaic Havana virus* (ToMHV) and *Tomato severe leaf curl virus* (ToSLCV), which are commonly found infecting tomatoes in Guatemala and cause severe leaf curl symptoms and purpling and stunting. *Tomato yellow leaf curl virus* (TYLCV) was detected in only three plants (T10, T11 and T12), all of which had a strong degree of yellowing, consistent with infection with this virus, which was introduced into Guatemala two or three years ago. Thus, these tomato samples had varying degrees of mixed infection with begomoviruses and *Liberobacter*. This explains

the variety of symptoms observed in the field and why begomovirus-resistant varieties may be showing variable responses as well. This complex of viruses and non-culturable bacteria pose a major threat to the capacity to produce peppers and tomatoes in the field in Guatemala and increase the urgency for the development of IPM packages for insect-transmitted viruses and virus-like agents.

Latin America and the Caribbean: Honduras

Initial testing for viruses in solanecous crops and weeds in potato fields

Ninety-six foliar samples from symptomatic potato plants were collected representing the three main potato cropping regions of the country and the two main cropping seasons. Additionally, 21 samples of tomatoes, 6 of peppers and 6 of weeds, were also collected for analysis to total 129 samples collected of solanaceous crops and associated weeds. In addition to funding from the IPVDN, the costs of these activities were met using leveraged financial support from the local offices of FAO and FINTRAC. The following results have been obtained.

Group A Samples. Between June 2009 and February 2010, a total 98 samples were collected for analyses, of which 89 were of potatoes and 4 and 5 samples, respectively, were of tomatoes and weeds growing in the neighborhood of potato fields. They were analyzed at Agdia Incorporated (Elkhart, IN, USA), subjecting them to a battery of 23 tests that included specific detection of 20 distinct species of viruses or viroids belonging to 13 genera included in at least 6 distinct families of viruses, and also for genus-specific detection of members of the Potyvirus, Begomovirus and Closterovirus genera. The analytical procedure used was ELISA, except for nucleic acid hybridization for viroids and PCR for Begomovirus and Closterovirus genera tests.

The selection of the tests to be performed on each sample (Table 4) was made following a thorough literature review and consultation, and was based at the end on the following selection criteria: a) reported occurrence of the virus in the region or in Honduras; and b) occurrence of the virus in the countries from which Honduras has historically imported potato seed.

Reactions positive to presence of at least one virus occurred in 36% of the samples; 3% were confirmed positives infection with more than one virus. The species detected represented four different genera of viruses as follows.

A. Potyvirus genus. 15% tested positive to this genus, and 13% were positive specifically to PVY; no positives were positive to the other potyviruses tested, namely, PVA, PVV and TEV. This suggests that other unidentified potyvirus species may occur at a lower prevalence.

B. Potexvirus genus. 10% tested positive to PVX, the only species of this genus tested for.

C. Begomovirus genus. 10% samples tested positive, of which 6 were potato and 4 tomato.

D. Closterovirus genus. 4% tested positive, all potato.

Of the insect-transmitted viruses detected, aphid-transmitted potyviruses, mainly PVY, were most prevalent followed by whitefly-transmitted Begomovirus. Neither group appears to be of much importance considering their frequency; nevertheless, they have the potential to cause extensive damage because of the characteristics of their vectors and the concurrent effect of climatic change on their range of activity. PVX, which has no insect vector, was detected at a frequency similar to Begomovirus. This virus can also become a problem as it is transmitted via tubers, which the local growers customarily keep as seed for next cropping season.

Table 4. Viruses selected for testing in solanaceous crops in Honduras

Name of species or virus genus tested for	Initials	Family	Genus
Alfalfa mosaic virus	AMV	Bromoviridae	Alfamovirus
Cucumber mosaic virus	CMV	Bromoviridae	Cucumovirus
Tomato spotted wilt virus	TSWV	Bunyaviridae	Tospovirus
Andean potato mottled virus	APMoV	Comoviridae	Comovirus
Tomato black ring virus	TBRV	Comoviridae	Nepovirus
Potato aucuba mosaic virus	PAMV	Alphaflexiviridae	Potexvirus
Potato latent virus	PotLV	Betaflexiviridae	Carlavirus
Potato virus X	PVX	Alphaflexiviridae	Potexvirus
Potato virus M	PVM	Alphaflexiviridae	Carlavirus
Potato virus S	PVS	Alphaflexiviridae	Carlavirus
Potato leaf roll virus	PLRV	Luteoviridae	Polerovirus
Potato spindle tuber viroid	PSTVd	Pospiviridae	Pospiviroid
Potato virus A	PVA	Potyviridae	Potyvirus
Potato virus V	PVV	Potyviridae	Potyvirus
Potato virus Y	PVY	Potyviridae	Potyvirus
Tobacco etch virus	TEV	Potyviridae	Potyvirus
Andean potato latent virus	APLV	Tymoviridae	Tymovirus
Potato mop top virus	PMTV	Virgaviridae	Pomovirus
Tobacco mosaic virus	TMV	Virgaviridae	Tobamovirus
Tobacco rattle virus	TRV	Virgaviridae	Tobravirus
Potyvirus group	-	Potyviridae	-
Begomovirus group	-	Geminiviridae	Begomovirus
Closterovirus group	-	Closteroviridae	-

Group B Samples. A group of 31 samples (7 potato, 17 tomato, 6 pepper and 1 weed) was collected in July and August 2010, and divided in two sets for analysis. A first set of 4 tomato samples was sent to AGDIA for ELISA testing for TMV and group PCR analysis for begomovirus, closterovirus and potyvirus. These samples (TOM-F-01, TOM-F-02, TOM-F-03, and TOM-F-04) tested negative to TMV, closterovirus and potyvirus These tomato samples were found to be positive in the commercial testing lab (AGDIA) using a set of PCR primers that amplify a non-coding with coding region fragment of begomoviruses. Sequences from this region matched most closely to *Tomato severe leaf curl virus* (~93%) and *Tomato mosaic Havana virus* (~91%). These two begomoviruses have been found to be prevalent in Guatemala as well.

The remaining samples were sent to Dr. J. Brown (U Arizona) for specific PCR analysis of

begomovirus and the fastidious bacteria *Candidatus Liberibacter solanacearum*. Laboratory tests were conducted at the Brown lab for begomovirus detection, using a different set of primers that amplify the coat protein (core Cp PCR, updated 2007). Symptomatic tomato and pepper plants mostly positive for whitefly-transmitted geminiviruses. Samples of four tomato and two bell pepper were prepared and from DNA sequencing determined to be *Tomato severe leaf curl virus* (TSLCV). Potato samples gave PCR products, but sequences obtained were not of begomoviruses indicating false positives.

Detection of Sweet Potato Viruses

Between July and August 2010, a group of 20 samples was collected and divided in two sets for analysis. The samples represented different fields and also plants held by Zamorano in greenhouse and tissue culture laboratory as part of an effort to produce virus-free propagative material. One set of 14 samples was sent to AGDIA where they were analyzed for TMV using ELISA and for Begomovirus, Closterovirus and Potyvirus using group PCR analysis.

Tests to be performed were selected using criteria as used for potato. PCR analyses at AGDIA showed that leaf sample CAM-F-01 tested weakly positive for Potyviridae and also for Closteroviridae. Sequences from this sample showed 73% similarity with the Closterviridae species *Sweet potato chlorotic stunt virus* (SCSV), a crinivirus. The samples CAM-F-02 and CAM-Z-4 also tested weakly positive for the presence of Closteroviridae.

Results of commercial lab PCR-testing of some of the sweet potato samples showing virus-like symptoms indicated the presence of a closterovirus giving a weak positive using the 'group test'. One of those two plants (1) also was weakly positive for potyvirus using the 'potyvirus group test'. No significant shared identity was found for the PCR product amplified from sample 4 suggesting that the amplicon was possibly not viral in origin. Sample 1 shared 73% nt identity with *Sweet potato chlorotic stunt virus*, while sample 2 shared 92% identity with *Sweet potato chlorotic stunt virus*. 'Beauregard' (orange) contained both viruses and 'Bushbock' (red) was infected with SCStV only (2 samples each, were tested). The phytosanitary certificate that accompany sweet potato germplasm purchased as seed potatoes for planting in Honduras did not indicate which if any plant viruses were tested for. Ten of the 14 samples sent to AGDIA, including the Closteroviridae-positive CAM-Z-4, came from the Zamorano greenhouse mother plants and tissue culture plantlets. Based on these results, Zamorano proceeded to eliminate the positive mother plant and the plantlets derived from it by tissue culture.

The other 6 samples were sent to Dr. J. Brown of the U. of Arizona for specific PCR analysis of begomovirus. Design of PCR primers for detection of well-studied plant viruses that infect sweet potato, as well as for close relatives of the potyvirus and closterovirus detected in two varieties. Positive controls may be difficult to obtain, but requests are out to several laboratories to obtain known virus cultures or mini-circles (oligos) or cloned amplicons, to use as positive controls to optimize RT-PCR and PCR assays that will be established at the UA-Brown lab and then transferred to Zamorano for local use. In work described above by Rayapati (WSU), *Sweetpotato feathery mottle virus* was detected in a sample from Honduras by PCR from FTA-card eluted RNA.

West Africa: Mali

Detection and identification of viruses infecting pepper, okra and weeds in Mali

During a trip to Mali by Gilbertson (UC-Davis) in May 2010, collected samples of peppers from a variety trial conducted in Baguineda, as well as samples of okra, *Sida* spp. *Physalis* sp. and

cucurbits. Sap, was prepared from representative leaves and applied to AgDia absorption strips. These were returned to UC Davis where they were tested for infection by begomoviruses (using DNA extracts from one strip) or RNA viruses (using RNA extracts from another strip). General primer pairs for whitefly-transmitted begomoviruses or for betasatellites were used for DNA viruses. For RNA viruses, general primer pairs for the potyvirus genus and for Cucumber mosaic virus (CMV) were used. Amplified DNA was sequenced to determine and confirm virus identity.

Peppers in the variety trial in Baguineda were collected to represent symptoms ranging from a light green-yellow mosaic/mottle to leaf distortion and up-curling (Table 5). Plants showing mottle/mosaic symptoms were PCR positive only for CMV and generic potyvirus, the sequence of which was 89% similar to Pepper veinal mottle virus (PVMV). Those showing leaf curling, distortion and stunting were infected with a begomovirus that was subsequently determined by PCR-specific primers to be Tomato yellow leaf crumple begomovirus (ToLCrV). Peppers with severe stunting and distortion were found to be infected by all three viruses. These results indicate that a complex of insect-transmitted viruses can infect peppers in Mali, that management strategies will have to take into account both aphid- and whitefly-transmitted viruses, and that viruses cannot be diagnosed by visual symptoms.

Okra plants with severe or mild leaf curl and crumple symptoms were found to be infected with begomoviruses and the associated betasatellite. By sequence analysis, the begomoviruses detected were either Cotton leaf curl Gezira virus (CLCuGV) or Okra yellow crinkle virus (OYCrV), and the associated betasatellite was Cotton leaf curl Gezira betasatellite. Four Okra plant samples from Bamako were all PCR-positive, with CLCuGV-okra and OYCrV co-infecting 3 severe and 1 mild symptom samples. One okra plant with mild symptoms was infected by OYCrV alone. The detection of this complex of begomoviruses and betasatellite is consistent with previous results, showing these agents are responsible for okra leaf curl disease in Mali and other countries in West Africa.

Interestingly, the *Sida* spp. with upcurling and yellowing was found to be infected with a begomoviruses and a betasatellite. The *Sida* spp. from Sotuba was infected with a *Sida* strain of CLCuGV and a betasatellite, whereas the *Sida* from Baguineda was infected with OYCrV and the okra strain of CLCuGV and a betasatellite. A collection of *Physalis* was weakly positive for begomovirus and beta, but a mixed weed sample was negative. These results suggest that the *Sida* spp., a very common perennial weed, is a reservoir for begomoviruses with the potential to infect okra. However, more research needs to be conducted to prove that the viruses in *Sida* can infect okra. Finally, the virus infecting cucurbits was determined to be Zucchini yellow mosaic potyvirus (ZYMV), a virus that we have previously detected infecting cucurbits in Mali.

Table 5. Association of viruses with symptoms in a pepper variety trial in Beguineda, Mali

Symptoms	Begomo	Beta	Poty	CMV	Sequence Similarity
Mottle/mosaic	-	-	+	+	
Mottle/mosaic	-	-	+	+	PVMV-89%; CMV- 97%
Mottle/mosaic	-	-	+	+	PVMV-89%; CMV- 97%
Mottle/mosaic	-	-	+	+	
Severe distortion/stunt 'broccoli;	+	-	+	+	PVMV-89%; CMV- 97%
Severe distortion/stunt	+	-	+	+	PVMV-89%; CMV- 97%
Severe distortion/stunt	+	-	-	-	
Severe distortion/stunt	+	-	-	-	
Upcurling, crumple	+	-	+	-	
Upcurling, crumple	+	-	+	+	
Upcurling, crumple	+	+/-	+	+	PVMV-89%; CMV- 97%
Upcurling, crumple	+	-	-	+	
Mosaic/mottle; Crumple, necrosis	+/-	+/-	+	+	
Mosaic/mottle; Crumple, necrosis	-	-	+	+	PVMV-89%; CMV- 97%
Mosaic/mottle; Crumple, necrosis	+	-	+	+	
Mosaic/mottle	+	-	+	+	PVMV-89%; CMV- 89%

Begomovirus: +=detected with a general primer pair for whitefly-transmitted begomoviruses and subsequently determined to be *Tomato yellow leaf crumple virus* by PCR with specific primers.

Beta: +=detected by PCR with a general primer pair for betasatellites

Poty: +=detected by PCR with a general primer pair for potyviruses

CMV: +=detected by PCR with a primer pair designed for CMV

Central Asia: Uzbekistan

Zarifa Kadirova from the Institute of Genetics and Experimental Biology of Plants of the Academy of Sciences of Uzbekistan, Tashkent, attended the Virus Workshop in Coimbatore, India and provided information on the status of viruses in potato and wheat, major crops in Uzbek. Potato viruses X, Z, M, S, and A as well as Potato leafroll virus have been detected in Tashkent and Samarqand growing regions. Identifications appeared to have been done by biological assays to indicator plants. She had received some training on virus diagnosis in the Rayapati lab (WSU) and reported the discovery of Tomato spotted wilt tospovirus

(TSWV) in market surveys of tomato fruits by the use of Immunostrips (Agdia).

Long-term institutional capacity building

A major, week-long workshop on virus diseases was held in Coimbatore, India at Tamil Nadu Agricultural University, July 12-17, 2010. Lectures were given on the nature of viruses, diagnostics, and management. Host country participants made presentations on their activities and capabilities in virology at their institutions. A field trip was taken to observe tomato infected with peanut bud necrosis, as well as cucurbit and legume crops with virus-like symptoms. Stops included plant nurseries, both contained and open, where tomato seedlings were grown to supply farmers. At vegetable markets, virus symptoms were observed on tomato fruits and okra. Samples were taken at various locations and used for demonstrations of ELISA by Rayapati and of tissue blot immunoassay (TBIA) by Tolin.

Tolin and Gilbertson met with the International Plant Diagnostics Network Global Theme at their planning meeting in Antigua, Guatemala and discussed the interaction between the two projects. Included in these discussions were the activities on increasing virus diagnostic capabilities in host countries. At this time, few locations can reliably conduct PCR and RT-PCR, but are able to conduct immunoassays. Restrictions in obtaining test materials are a major constraint, as well as the cost. Membrane-based methods to collect and store sap from plant samples for transport to a central lab, including those of the US collaborators, has enabled an increase in our knowledge of viruses present, but is not a sustainable model. The description below of the workshop in Indonesia is a good example of the activities and the interest of the countries.

Workshop on Plant Disease and Insect Pathogen Diagnostics was conducted in Bogor, Indonesia in July 22 – 23, 2010 as part of collaborative activities between IPM CRSP Southeast Asia Regional Project and IPDN Global Theme. This workshop was attended by 20 participants (11 males and 9 females) from Indonesia, Phillippines, and Cambodia. The participants have diverse knowledge in regard to plant disease diagnostic due to their different background and professions, i.e. scientists, extension agents, and graduate students from IPB. General plant disease diagnostics was discussed involving serological and polymerase chain reaction techniques for viruses, morphological based identification for fungi, biochemical and physiological based techniques for bacteria, and some basic techniques for insect pathogen diagnostics. Field visit to horticultural growing area in Ciloto was conducted a day prior to workshop to introduce disease type symptoms and also to collect samples for laboratory works during the workshop. Workshop materials (program, power point presentation, and pictures taken during the activities) was documented and saved in two CDs, which were distributed to each of the participants at the end of the workshop.

The diagnostic laboratory at Zamorano in Honduras has made important steps towards validating and adopting appropriate technologies for virus testing. These include ELISA with commercial kits, immunostrips for specific viruses, and PCR to detect DNA viruses such as begomoviruses and phyotoplasmas. In 2009, through funding from the Common Fund for Commodities that supports a research project on the Coconut Lethal Yellowing Diseases, a Real Time PCR thermocycler was acquired and the technology validated and adopted for CLY at the Zamorano lab. The use of FTA cards to by-pass laborious DNA extraction was also validated for CLY. It is expected that the use of RT-PCR and FTA cards will greatly support the diagnostic capabilities of the Zamorano lab for virus testing. The FHIA laboratory is also

increasing its capacity to perform testing on site.

The laboratory at University del Valle de Guatemala probably has the most advanced capabilities for virus diagnosis and has reliably conducted PCR and ELISA for several years. The IDIAF lab in Dominican Republic was just constructed in the last phase of the project, but is beginning to conduct tests. Tolin and Deom provided earlier training to this lab, and Martinez received further training in the Tolin lab at Virginia Tech.

India: Ecological Research

Assessment of seed transmission of Tobacco streak virus (TSV) in okra

Based on initial field surveys of okra by Karthikeyan and Rayapati in farmers' fields in Tamil Nadu, dissemination of *Tobacco streak virus* (TSV) via hybrid seed obtained from commercial sources was suspected. Seedlings from virus-infected seed could serve as a source for secondary spread of the virus via pollen carried by thrips. To assess the potential of seed-transmission of TSV, we collected pods from TSV-infected, symptomatic okra plants in farmer's fields and commercial breeding plots in Dharmapuri and Krishnagiri areas of Salem and Coimbatore districts of Tamil Nadu. Seeds from dried pods were harvested from both local varieties as well as hybrids and sown in pots, and seedlings were maintained under greenhouse conditions. Plants were observed for symptoms on leaves and fruits at weekly intervals till harvest. The presence of TSV in symptomatic okra plants was ascertained by RT-PCR using primers specific to the coat protein. Cumulative results from three seasons are shown in Table 5. The data clearly shows high rates of seed transmission of TSV in commercial hybrids produced by self pollination than in local varieties, where seeds are produced by natural, open pollination. The mean rate of seed transmission of 11.4 per cent clearly highlights the risk of TSV spread via distribution of hybrid seed. TSV is known to be transmitted via seed in many crops, and outreach activities should be conducted to bring awareness to farmers and commercial seed companies on the risk of spreading the virus via distribution of commercial hybrid seed and to eradicate sources of infection by rouging symptomatic seedlings for minimizing secondary spread of the virus.

Table 6. Comparison of seed transmission of TSV in different okra seed sources

Sl. No.	Set of experiment	Seed from	Total no of seed tested	Number of infected plants	Per cent transmission
1	I	Local variety - 1	300	0	0
2	II	Local variety - 2	250	7	2.8
3	III	Commercial hybrid variety - 1	200	58	29.0
4	IV	Commercial hybrid variety - 2	100	19	19.0
5	V	Commercial hybrid variety - 3	300	47	15.7
Mean			**1150**	**131**	**11.4**

Implementation of applied research on specific virus diseases in selected crops

South Asia: India

Evaluation of 'roguing' as a tactic for management of Peanut bud necrosis virus in tomato

Results from previous seasons reported in the Tospovirus global theme have shown that roguing virus-infected tomato seedlings, during and/or soon after, transplanting could reduce the spread of *Peanut bud necrosis virus* (PBNV). To further validate this approach for incorporating into an IPM package for tomato, Karthikeyan and Rayapati conducted a field trial in a farmer's field near Thondamuthur of Coimbatore district using the tomato cultivar Vaishnavi. The field was divided into two equal halves at the time of transplanting, and one half was transplanted with seedlings that appeared healthy and the other half was transplanted with a mixture of seedlings with and without visual symptoms. All seedlings came from a single nursery and were the same age. After transplanting, any seedling that showed symptoms of PBNV was removed in the "with roguing" plot for up to 45 days post-transplanting. These plots were not treated with any pesticides for controlling thrips vectors. Weeds were removed at regular intervals. The number of tomato plants showing symptoms of PBNV was scored at biweekly intervals and are shown in Table 7.

The data indicated that incidence of PBNV was significantly higher in plots with no roguing when compared to plots with roguing in both locations (Table 7). In addition, cumulative yield of tomato harvested from the non-rogued plot was 11.1 t/ha versus 16.45 t/ha from the rogued plot, an increase of 48.2% . An analysis of benefit-cost ratio using tomato sale price at the time of final harvest (a low market price of Rs. 4/kg) concluded that farmers can gain an additional revenue of Rs. 21,400/= per hectare by adopting roguing of infected seedlings during transplanting and within the first 45 days of post-transplanting, without incurring additional costs for spraying pesticides to control thrips vectors.

Table 7. Effect of roguing on the incidence of PBNV in tomato
Location : Thondamuthur / Coimbatore **Tomato Cultivar : Vaishnavi**

Sl. No.	Days after planting	Per cent PBNV incidence*		Per cent PBNV disease increase over plot with roguing	CD (p=0.05)
		Plot with roguing	Plot with no roguing		
1	15	3.90 (11.93)	10.25 (18.65)	163	5.1
2	30	5.65 (13.12)	17.90 (20.04)	217	7.5
3	45	6.80 (15.20)	21.00 (28.10)	209	9.4
4	60	10.40 (18.88)	26.50 (32.22)	155	13.4
5	75	12.20 (20.34)	32.00 (33.24)	162	15.7
6	90	15.00 (22.90)	41.34 (39.80)	176	18.9

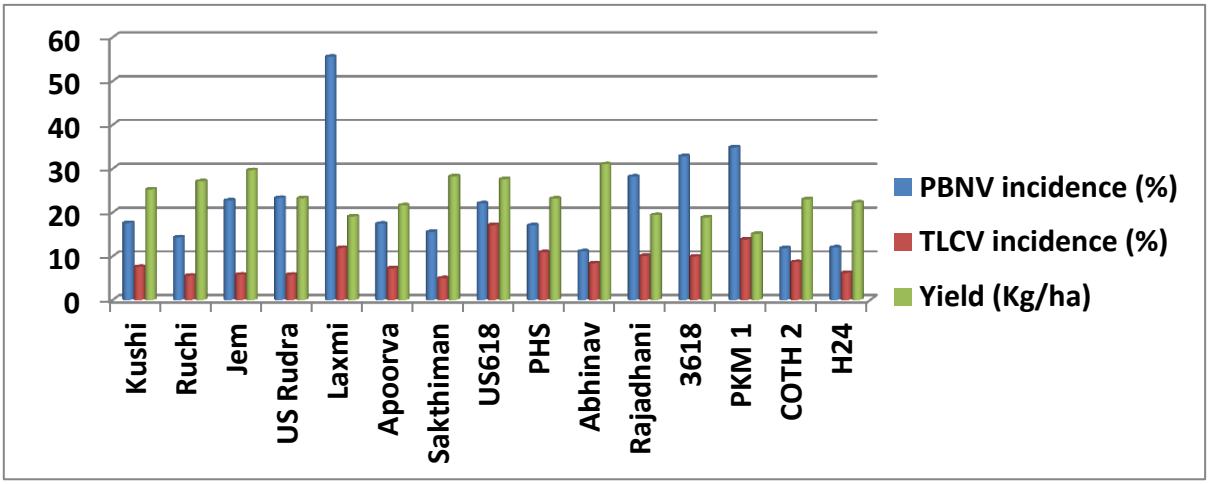
*Values in parentheses are arcsine transformed values

Evaluation of field performance of tomato cultivars and hybrids against Peanut bud necrosis virus and Tomato leaf curl virus

Field trials were conducted to evaluate performance of selected tomato cultivars and hybrids (developed by public institutions and commercial seed companies) commonly grown by farmers of Tamil Nadu. They are Ruchi, Kushi, JEM, US Rudra, Lakshmi, Apoorva, Sakthiman, US 618, PHS, Abinav, Rajadhani, 3618, PKM-1, COTH-2 and H24. The trial was established to evaluate field performance against *Peanut bud necrosis virus* (PBNV) at two different locations of Tamil Nadu endemic for the virus disease (Fig. 4). Healthy seedlings raised under controlled conditions were transplanted in a randomized block design with three replications. The plot size was $5m^2$ with 8 rows of planting. Plants were observed for symptoms caused by PBNV infection at biweekly intervals. In addition, these plots were observed for tomato leaf curl disease caused by *Tomato leaf curl virus* (TLCV) and spread by whiteflies, in order to obtain data on resistance/tolerance to two economically important viral diseases. The populations of thrips and whitefly were also recorded.

The results indicated that the overall incidence of PBNV was greater than of TLCV, and that entries varied in their response (Fig.1). There was no correlation between the number of vectors recorded in each cultivar/hybrid and disease incidence. PBNV incidence at 90 days post-transplanting was lowest in the cultivar Abhinav (11.1%) followed by the hybrid COTH-2 (11.82%) and H 24 (11.97%) . The disease incidence was moderate in hybrids Ruchi (14.31%), Sakthiman (15.54%) and PHS (17.04%), and higher in Jem (22.73%), UD Rudra (23.31), Laxmi (55.55%), PKM-1 (34.87%) and 3618 (32.83%). A low incidence of tomato leaf curl disease was observed in hybrids Sakthiman (4.95%), Ruchi (5.56%), US Rudra (5.78%), Jem (5.79%) and H24 (6.20%) at 90 days post-transplanting**.** The incidence of leaf curl was significantly higher in the hybrids US 618 (17.13%), PKM-1 (13.83%) and Laxmi (11.90%) during the same period (Fig. 1). The data obtained in this trial show that some cultivars and/or hybrids have field tolerance to diseases caused by PBNV and TLCV. Abhinav recorded significantly higher fruit yield of 31 t / ha followed by Jem with 29.65t/ha and Sakthiman with 28.25 t / ha.

Fig. 1. Field performance of different tomato cultivars / hybrids against diseases caused by PBNV and TYLCV; Values are mean of two trials, with incidence at 90 days post-transplant



Latin America and the Caribbean: Dominican Republic

Monitoring the base-line rate of infection of tomato with TYLCV in the Ocoa Valley

IDIAF scientists established an experimental plot designed to observe the behavior of the TYLV epidemic. A local variety, Floradade, was used because it is grown widely in the valley and is very susceptible to TYLCV. Five replicate plots were planted, each with 105 plants. The incidence of TYLV- infected plants was monitored weekly to observe the epidemiology of the disease in order to design an effective intervention. Plants were inspected for symptoms expression, beginning 15 days after transplanting. No application of insecticide against whitefly was done in order to expose the crop to high pressure of inoculum. Traps were placed to collect whiteflies, as reported in the LAC report.

Only 37 plants were infected 15 days after transplanting. After 4 weeks, the infected plants started to increase. By the end of growing season the incidence was 100%. The plants set fruits that were small and showing unripening symptoms, resulting in reduced yield. Of interest from the vector management standpoint, LAC data showed yellow traps were most efficient for trapping whiteflies. According to the numbers of individuals present, colonization of *Bemisia tabaci*, the vector of TYLCV, occurred after the second week. The population increased up to 20 times in the next 3 weeks. The combined results indicate that whitefly populations would need to be controlled by 2nd week to decrease the population explosion and concomitant transmission of TYLCV to all tomato plants.

Monitoring of Tomato yellow leaf curl virus (TYLCV) in whiteflies to assess the continued effectiveness of the 3 month host-free period

The implementation of a 3-month whitefly host-free period in the Dominican Republic (DR) continues to be a key component of a successful IPM program for the management of this damaging virus. As part of the Plant Viruses Global Theme activities, Gilbertson has collaborated with Transagricola to conduct continued monitoring whiteflies for TYLCV to assess the efficacy of the host-free period in the two major tomato-growing areas of the DR, the North (around Santiago) and the South (Azua Valley); as well as in Ocoa, an area where there is no host-free period.

As has been the case in previous years, little or no TYLCV was detected in whiteflies collected early in the tomato growing season (September and October 2009). The virus began to be detected in whiteflies collected in November. By December 2009, many samples from the North and the South were strongly positive for the virus and by January and February 2010, almost all of the whiteflies from both locations were strongly positive for TYLCV; this coincided with the development of TYLCV in tomatoes in the field in the DR. Many of the whiteflies collected in March, April and May 2010 also were positive for TYLCV; however, the viral titers began to decline as the harvest was completed and sanitation efforts were implemented. Following the implementation of the host-free period (June 2010), TYLCV was not detected in whiteflies from the North or the South that were collected in June 2010. TYLCV was not detected in most of the whiteflies collected in July 2010; however, two strong positives were obtained from two locations in the South (Tabara Abajo and Km 15), and these were collected from peppers, a host that should be included in the host-free period. This finding alerted Ministry of Agriculture personnel to visit this area and to make sure all growers were following the host-free period. In addition, it was determined that whitefly populations were relatively high on certain weeds, and sanitation efforts were implemented to decrease the populations of whiteflies. Whiteflies collected in August had very little TYLCV (only a couple samples had

very weak positives, and similar results were obtained for whiteflies collected in September. Overall, these results indicated that the host-free period was effective in reducing the amount of TYLCV in whiteflies and that the virus pressure should be low heading into the 2010 growing season. These results also demonstrate how the monitoring of the virus in whiteflies can alert government and industry personnel of possible violations of the host-free period (or other situations) that lead to the build-up of the virus, nd allow for implementation of practices to reduce or eliminate these outbreaks before the start of the growing season. Thus, the monitoring of TYLCV continues to be an important part of the effective IPM program for this virus in the Dominican Republic.

Latin America and the Caribbean: Honduras

Production of virus-free sweet potatoes propagative material through the use of tissue culture

Mother plants were established from material donated by a grower. Preliminary trials to culture sweet potatoes meristems in tissue culture were successful. In July 2010, samples from mother plants and lab microplants were sent to the U.S. for virus diagnostic. One mother plant resulted positive to Closterovirus. At this time, we have under culture 194 plants from the negative virus diagnostic plants. In six months or so, plants will be given to the growers after the acclimation process. The growers will use them to reproduce virus-free vegetative material.