TERMINAL REPORT

Training Workshop on Diagnostics of Plant Viruses

(Project No. AGF/CRO/11/007/REG)

IPB, University of the Philippines Los Baños | 17-28 August 2015

EXECUTIVE SUMMARY

This **"Training Workshop on the Diagnostic of Plant Viruses"** was implemented by the Institute of Plant Breeding – Crop Science Cluster, College of Agriculture, University of the Philippines – Los Baños through the ASEAN Plant Health Cooperation Network (APHCN) of ASEANET project on **"Taxonomic capacity building to support market access for agricultural trade in the ASEAN region"**. The said project is funded by the Japan – ASEAN Integration Fund (JAIF) that will be implemented for two years covering several activities related to training and attachment programs.

This two-week training workshop (August 17-28, 2015) was participated by 19 plant pathologists from Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Thailand and Vietnam. Majority of them are connected to the Plant Quarantine Centre and Plant Protection Division under the Department of Agriculture and one from Bogor Agricultural University.

The ultimate goal of the training workshop is to develop capacity building among plant virologists across the ASEAN region in addressing virus diseases existing in each country and diseases that may pose potential threats (emerging or invasive) in the exchange of crops or planting materials. The training workshop caters the need to equip our plant virologists who are working in universities, research institutions and plant quarantine and plant protection offices with basic knowledge on disease identification, detection and characterization using available tools (symptomatology, transmission, serology and molecular assay).

The training course utilized the combination of interactive 14 lectures and 14 laboratory practical, 1 demo activity and 2-day field visit to Southern and Central Luzon areas. Five sessions namely; Introduction, Plant viruses of agricultural crops, Transmission of common plant viruses, Detection of plant viruses using serological and molecular assays and Strategies of protecting crops from viruses were designed to cover the basic plant virology course.

Pre- and post-evaluations were also administered to assess the individual and over-all performances of participants as well as to determine the efficiency of the training or organizing team.

Session I. Opening Program and Introduction

The 19 participants from 9 different countries in Southeast Asian Region were welcomed by IPB headed by Prof. Teresita H. Borromeo, OIC-IPB-CSC. This was followed by a short message from Dr. Lum Keng Yeang, Chairperson of APHCN-ASEANET who introduced the organization's mandate and project in which conducting training workshop on capacity building is one of the activities. Then, Ms. Lolita M. Dolores gave the introduction and overview of the training course including the objectives, course outline and methodology. Consequently, Dr. Marita S. Pinili introduced each participant, resource persons and the training team.

Pre-evaluation test was given to the participants to gauge their level of knowledge on basic plant virology as well as to determine their expectations from the training workshop. Also, during this session, two lectures were given. Dr. Keiko T. Natsuaki, Professor from Tokyo University of Agriculture (Tokyo NODAI) introduced the world of plant viruses through historical facts, discovery and researches. Her

second lecture which basically tackled the classification of plant viruses gave insights on the general morphology, hosts, DNA/RNA viruses and characteristics of major genera of plant viruses. After the lectures, each representative from participating countries presented their country reports which introduced their organization, nature of work and status of plant viruses present in their country.

A welcome dinner was held at Kamayan, Bay, Laguna that showcased the typical Filipino cuisine and the traditional 'harana' or serenade to entertain the diverse culture of the participants.

Session II. Plant viruses of agricultural crops

During this session, three lectures were given; lectures 3 & 4 (Plant viruses infecting vegetable crops in the Philippines and Symptomatology, sampling and handling of plant samples for virus detection). Both lectures were presented by Ms. LM Dolores. She emphasized the significance of common and major plant viruses infecting vegetables including cucurbits and solanaceous crops in the Philippines. Ms. LM Dolores also mentioned the common symptoms induced by plant viruses, proper way of sampling, handling and storage of fragile plant samples. The 5th lecture which was presented by Dr. KT Natsuaki discussed rice viruses in Asia and Africa. Dr. KT Natsuaki emphasized the losses on rice in Africa and Asia due to *Rice tungro bacilliform virus* (RTBV) or *Rice tungro spherical virus* (RTSV) and *Rice yellow mottle virus* (RYMV), respectively. Virus vectors and mode of transmission were also mentioned in the lecture.

In the afternoon session, actual preparation of buffers and other materials for serological and molecular assays were performed in the laboratory. Participants were grouped into four, consisting of 4 to 5 members each. Each group was able to prepare stock solutions and extraction buffers for their next activities.

Session III. Transmission of common plant viruses

Another 3 lectures were given during the morning session. These include the General concept in transmission of plant viruses (Lecture 6) by Dr. KT Natuaki, Transmission of cucurbits and other vegetable viruses via insect-vectors and mechanical inoculation (Lecture 7) by Ms. LM Dolores and a supplementary lecture on transmission of plant viruses via plant-parasitic nematodes which was discussed by Dr. MS Pinili. Participants were able to gain knowledge on mechanical mode of transmitting plant viruses as well as the use of different vectors such as insects and nematodes.

In the afternoon session, participants were tasked to perform mechanical inoculation of *Tobacco mosaic virus* (TMV) and *Zucchini yellow mosaic virus* (ZYMV) on hosts and indicator plants. Extraction of infected plants as inoculum source was prepared prior to mechanical inoculation. Participants were also able to demonstrate non-persistent mode of virus, *Papaya ringspot virus - P* (PRSV-P) transmission using *Aphis gossypii* on papaya (*Carica papaya*), cucumber (*Cucumis sativus*), squash (*Cucurbita maxima*), *Chenopodium amaranticolor* and *C. quinoa*. At the same time, persistent mode of transmission of Begomovirus, *Tomato yellow leaf curl virus* (TYLCV) using whitefly, *Bemisia tabaci* on 6 different host plants was conducted in the screenhouse. *Banana bunchy top virus* (BBTV) inoculation was also performed using aphids, *Pentalonia nigronervosa*. Each participant was allowed to do aphid

starvation and virus acquisition then followed by inoculation to healthy tissue-cultured banana cv. 'Lakatan'. All inoculated plants were kept under screenhouse condition for symptom development.

Results were confirmed after 1 week of incubation. Mechanical inoculation of TMV on *Nicotiana glutinosa* showed local lesions whereas early wilting was observed from PRSV-inoculated papaya.

On the other hand, the persistent mode of transmission on Begomovirus and BBTV did not show early symptoms as expected due to their long incubation period. However, aphids inoculated on banana have multiplied and produced nymphs.

Field visit

Field trips were conducted basically to identify symptoms of possible virus-infected crops, collect samples for virus detection and identification and gain supplementary knowledge on different farm practices under organic and conventional farming systems. Field visit was conducted in Silang, Cavite where established cut flower farm planted to Gerbera and Chrysanthemum was visited. This was followed by field observation in one of the oldest organic farms in the Philippines, Gourmet Farms, where participants were able to interview farm staff on how to manage an organic farm planted to lettuce and variety of herbs and how to utilize their products or harvests for local and foreign markets.

The second field tour was held in Muñoz, Nueva Ecija particularly in Ramon Magsaysay Centre for Agricultural Resources and Environmental Studies (RM-CARES) in Central Luzon State University (CLSU) and in the Philippine Centre for Postharvest Development and Mechanization (PhilMech). RM-CARES introduced how to establish organic farm from the conventional farming system. Participants showed enthusiasts and curiosity on the challenges faced by organic farmers from the tedious transition period, accreditation and certification of organically-grown crops and its relationship in managing pests and diseases. Participants were able to collect diseased samples from organically-grown plots for symptom identification and virus detection. On the other hand, the field visit in PhilMech showed recent technologies and discoveries in addressing postharvest problems on diseases and processing. The use of biological control agents (BCA) developed by Dr. Dionisio G. Alvindia, Supervising Scientist Research Specialist is one of the breakthroughs of their organization.

Session IV. Detection of plant viruses using serological and molecular assay

Basic concept on detecting RNA and DNA viruses using serological and molecular techniques were discussed in this session. Dr. Sri Hendrastuti Hidayat, Professor of Bogor Agricultural University explained the antibody-antigen interaction and various serological and molecular methods available for virus detection and identification. These lectures were followed by specific example *i.e.* case study of BBTV from the Philippine abaca and banana and the status and phylogenetic analyses of BBTV in Bali, Indonesia.

Under this session, 10 practical/lab activities dealing with virus nucleic acid extraction and detection using ELISA and PCR assay and gel electrophoretic analyses were conducted. A novel finding was generated from these activities, *i.e.*

the detection of *Cucumber mosaic virus* (CMV) from kangkong (*Ipomoea aquatica*), which is the first report in the Philippines.

In addition, simple demonstration on impregnating plant virus nucleic acid on FTA plant card was performed.

Session V. Strategies of protecting crops from viruses

Disease management strategies such as cultural methods, attenuated virus, virus – free planting materials and deployment of resistant varieties were discussed on this session. Specific example on the significance of plant quarantine on potato viruses in Syria was emphasized to avoid the geographic distribution of infected planting materials.

Technical and organizational evaluations

The overall evaluation showed that majority of the participants achieved their expectations from the training workshop. This outstanding rating was reflected from the very satisfactory results of their post-evaluation test. Their level of judgement in assessing possible virus-infected crops using the symptomatology was increased from fair to good rating scale. Thirty percent (30%) got excellent level of confidence in performing the nucleic acid extraction from plant samples and 32% indicated good level of confidence.

The training team including the resource persons and logistics also received high percentage of overall rating (4 – Good to 5- Excellent) from the participants.

TERMINAL REPORT

I. Basic Information

A. Project Title: Training Workshop on Diagnostics of Plant Viruses

Project Coordination:

Dr. Lum Keng Yeang – Chairperson, APHCN – ASEANET
Dr. Soetikno S. Sastroutomo – Secretary, APHCN – ASEANET
Dr. Marita S. Pinili – Regional Training Coordinator/Collaborator (IPB-UPLB)

Training Coordinators:

Ms. Lolita M. Dolores – Local Training Coordinator – Technical (IPB – UPLB)
Ms. Virma Rea G. Lee – Local Training Coordinator – Administrative (IPB – UPLB)
Ms. Maricel C. Gonzales – Local Training Secretary (IPB – UPLB)
Mr. Raol P. Pamiloza – Technical Training Team Member (IPB – UPLB)
Mr. Yron M. Retuta – Technical Training Team Member (IPB – UPLB)

B. Proponent and Address

Institute of Plant Breeding – Crop Science Cluster (IPB – CSC)
College of Agriculture (CA)
University of the Philippines Los Baños (UPLB)
College, Laguna 4031
Philippines

C. Implementing Agencies

Lead Agencies:

ASEAN Plant Health Cooperation Network of ASEANET (APHCN – ASEANET)
Building A-19 MARDI Complex, Serdang, Selangor 43400 Malaysia

Institute of Plant Breeding – Crop Science Cluster (IPB – CSC)
College of Agriculture (CA)
University of the Philippines Los Baños (UPLB)
College, Laguna 4031

Funding Agency:

Japan – ASEAN Integration Fund (JAIF)

D. **Project Duration:** Two (2) weeks

a. Date Project Started: 17 August 2015
b. Expected Date of Completion: 28 August 2015

E. **Period Covered by this Report:** 17 – 28 August 2015

II. Technical Description

A. Background

Diseases due to major groups of pathogen such as fungi, bacteria, viruses and nematodes continuously hamper crops yield and directly influence farmers/producers income in tropical and sub-tropical agriculture. To directly address the devastating effects of plant diseases, a correct and reliable diagnosis is the ultimate pre-requisite. Diagnosis is the forefront of an efficient implementation of an effective disease management system or tactics. Aside from this, early diagnosis prevents possible entry and establishment of potential 'invasive' or 'emerging' pathogens/diseases in one country. As we move towards the ASEAN integration, participating countries are expecting influx of plants/plant materials as we engage in a free-trade system. Therefore, knowledge, skills and know-how in diagnosis will play a vital role in addressing the impact of this ASEAN integration on the exchange of goods (plants in particular).

Diseases can be due to fungal attacks, bacterial invasion, parasitism of nematodes and or virus infection, or worst the combination and complex association of these pathogens. Among these, diseases due to plant viruses are difficult to control or manage due to the pathogen's ability to infect the plant systemically, rapid and wide dissemination via insect and nematode vectors, mechanical means and infected planting materials, and the ability of the virus genome to mutation or recombination, thus leading to complexity of detection.

Simple to advance approaches in diagnosis of plant viruses have been developed for several years and have been used worldwide. From the simple serological assay that tests the sensitivity of antigen-antibody to molecular detection using virus specific primers in Polymerase Chain Reaction (PCR) assay, the nature and identity of this nucleic acid encapsulated in coat protein has been fully characterized.

B. Course Description

This "Training Workshop on the Diagnostics of Plant Viruses" is coordinated by the Institute of Plant Breeding – Crop Science Cluster, College of Agriculture, and University of the Philippines Los Baños through the ASEAN Plant Health Cooperation Network (APHCN) of ASEANET project on **"Taxonomic capacity building to support market access for agricultural trade in the ASEAN region"**. The said project is funded by the Japan – ASEAN Integration Fund (JAIF) that will be implemented for two years covering several activities related to training and attachment programs.

This training course aims to provide basic and practical understanding of the concept of plant viruses, diagnosis of diseased crops infected with economically important genera of plant viruses, and existing technology, practices and strategies in relation to management of virus diseases. The topics to cover include the following: knowledge on the basic classification, morphology of major genera of plant

viruses, virus transmission, diagnosis based on symptoms, detection using serological (Enzyme-linked immunosorbent assay, ELISA) and molecular (Polymerase Chain Reaction, PCR) methods, importance of plant viruses on major agricultural crops in the tropics and sub-tropics, and the available management options in avoiding or suppressing disease development. The knowledge mentioned above will help participants in establishing standard protocols in sampling, handling, processing plants suspected to viruses and identifying major genera of viruses, and be able them to design appropriate management strategy.

C. Objectives

General Objectives

Lecture: At the end of the training, it envisioned that the participants will acquire knowledge on the global importance of plant viruses under tropical and sub-tropical agriculture; and how to mitigate/manage diseases caused by plant viruses; and some plant quarantine issues pertaining to protect from potential threat of planting materials harbouring such viruses.

Laboratory: At the end of the training, the participants will acquire diagnostic skills in recognizing symptoms induced by plant viruses; learn the techniques on detection and identification of plant viruses using serological and molecular assays; and learn how plant viruses transmit from the source to the target host(s).

Specific Objectives

Lecture:

1. To acquire knowledge on the taxonomy and classification of plant viruses; DNA/RNA, morphology and characteristics of major genera.
2. To become aware on the importance of plant viruses in tropical and sub-tropical crops.
3. To gain knowledge on how viruses are transmitted
4. To learn the symptoms of virus-infected crops and the procedures of proper sampling and handling of specimen.
5. To acquire knowledge on simple and recent advances in detecting plant viruses.
6. To gain knowledge on the phylogenetic analysis of *Banana bunchy top virus.*
7. To learn how to protect crops from viruses through cultural control, resistant varieties, use of virus-free planting materials, attenuated virus and genetically modified (GM) crops.
8. To acquire knowledge on the status of rice viruses in Asia and Africa and potato viruses in Syria with emphasis on significance of plant quarantine.

Laboratory:

1. To learn the typical symptoms expressed by different genera of plant viruses.

2. To learn the basic techniques in sample collection, proper handling and transporting of virus-infected plants.
3. To learn how to prepare buffer and other solutions used for serological and molecular assays.
4. To detect plant viruses from leaf samples using Enzyme-linked immunosorbent assay (ELISA) and Polymerase Chain Reaction (PCR) assay.
5. To demonstrate how plant viruses can be transmitted into host plants using insect vector(s), and mechanical inoculation.

D. Training Course Outline

SESSION 1. Opening Program and Introduction

- Opening/Welcome Program
- Introduction and Overview of the Training Course
- Introduction of Participants, Resource Persons and Training Team
- Lecture 1. Virus world: The history of virus discovery and research
- Lecture 2. Basic classification of plant viruses: : Morphology, hosts, DNA/RNA viruses and characteristics of major genera

SESSION 2. Plant viruses of agricultural crops

- Lecture 3. Plant viruses infecting vegetable crops in the Philippines
- Lecture 4. Symptomatology, sampling and handling of plant samples for virus detection
- Lecture 5. Rice viruses in Asia and Africa
- Practical 1. Preparation of buffer and other materials for serological assay

SESSION 3. Transmission of common plant viruses

- Lecture 6. General concept in transmission of plant viruses
- Lecture 7. Transmission of cucurbits and other vegetable viruses via insect-vectors and mechanical inoculation (Pre-lab lecture)
- Lecture 8. Plant-parasitic nematodes as vectors of plant viruses: NEPO and TOBRA groups
- Practical 2. Transmission of RNA viruses through mechanical inoculation and aphids, *Aphis gossypii*
- Practical 3. Transmission of DNA virus (Begomovirus) Part I. Whitefly, *Bemisia tabaci*
- Practical 4. Transmission of DNA virus (*Banana bunchy top virus*) Part II. Aphids, *Pentalonia nigronervosa*
- Viewing of Results: Mechanical inoculation and insect-vector transmission

SESSION 4. Detection of plant viruses using serological and molecular assays

- Lecture 9. Detection of RNA and DNA viruses using serological assay
- Lecture 10. Detection of RNA and DNA viruses using molecular assay

- Lecture 11. Status, detection and phylogenetic analysis of *Banana bunchy top virus*
- Practical 5. Assessment of collected samples
- Practical 6. Extraction of RNA viruses from cucurbits and other crops
- Practical 7. Detection of RNA viruses using Enzyme-linked immunosorbent assay (ELISA)
- Practical 8. Extraction of DNA virus (*Banana bunchy top virus*) from banana
- Practical 9. Detection of DNA virus (*Banana bunchy top virus*) using ELISA
- Practical 10. Detection of RNA viruses using RT-PCR assay
- Practical 11. Gel electrophoresis and analysis
- Practical 12. Extraction of DNA viruses (BBTV, Begomovirus)
- Practical 13. Detection of DNA viruses using PCR assay
- Practical 14. Gel electrophoresis and analysis
- Demo 1. Trapping of plant virus nucleic acid using FTA plant card

SESSION 5. Strategies in protecting crops from viruses

- Lecture 12. Potato viruses in Syria with emphasis on the significance of plant quarantine
- Lecture 13. How to protect plants from viruses? Part I. Management strategies through cultural methods, attenuated virus and use of virus-free planting materials
- Lecture 14. How to protect plants from viruses? Part II. The use of resistant varieties in virus disease management

E. Training Content and Schedule

Week 1

Date/Venue/Time	Topic/Activity	Resource Person(s)/ Facilitator
PRE-TRAINING		
SUNDAY 16 August 2015		
	Arrival and billeting at BP-International Makiling (El Cielito Hotel)	Ms. Virma Rea G. Lee *Training Coordinator (Administrative)*
TRAINING PROPER		
MONDAY 17 August 2015		
SESSION 1: OPENING PROGRAM AND INTRODUCTION Venue: IPB Seminar Room		
08:00 – 08:15	Registration	Ms. Maricel C. Gonzales *Secretariat*
	Group Photo	
	Welcome Address	Prof. Teresita H. Borromeo *OIC, IPB-CSC*
	Short Message	Dr. Lum Keng Yeang *Chairperson, APHCN-ASEANET*
09:00 – 09:15	Training Introduction and Overview	Ms. Lolita M. Dolores *Training Coordinator (Technical)*
09:15 – 09:30	Introduction of Participants, Trainers and Training Team	Dr. Marita S. Pinili *Regional Training Coordinator*
09:30 – 10:00	Pre-evaluation test	
10:00 – 10:15	Tea/Coffee Break	
10:15 – 12:00	Lecture 1. Virus world: The history of virus discovery and research	Dr. Keiko T. Natsuaki *Tokyo University of Agriculture*
12:00 – 13:00	Lunch Break	
13:00 – 15:00	Lecture 2. Basic classification of plant viruses: Morphology, hosts, DNA/RNA viruses and characteristics of major genera	Dr. Keiko T. Natsuaki *Tokyo University of Agriculture*
15:00 – 15:15	Tea/Coffee Break	
15:15 – 17:00	In-country Report	All participants
18:00 – 20:30	Dinner Reception *Venue: Kamayan, Bay, Laguna*	Participants, Resource Persons, Training Team, Guests

Date/Venue/Time	Topic/Activity	Resource Person(s)/ Facilitator
TUESDAY 18 August 2015		
SESSION 2: PLANT VIRUSES OF AGRICULTURAL CROPS *Venue: IPB Seminar Room*		
08:00 – 09:30	Lecture 3. Plant viruses infecting vegetables in the Philippines	Ms. Lolita M. Dolores
09:30 – 09:45	Coffee/Tea Break	
09:45 – 10:45	Lecture 4. Symptomatology, sampling and handling of plants for virus detection	Ms. Lolita M. Dolores
10:45 – 12:00	Lecture 5. Rice viruses in Asia and Africa	Dr. Keiko T. Natsuaki
12:00 – 13:00	Lunch Break	
Venue: Plant Pathology Lab		
13:00 – 15:00	Practical 1. Preparation of buffer and other materials for serological assay	Ms. Maricel C. Gonzales Dr. Marita S. Pinili Mr. Yron M. Retuta Mr. Raol P. Pamiloza Ms. Araceli L. Alcachupas
15:00 – 15:15	Tea/Coffee Break	
15:15 – 17:00	Practical 1. Preparation of buffer and other materials for serological assay (ELISA)…*continuation*	Ms. Maricel C. Gonzales Dr. Marita S. Pinili Mr. Yron M. Retuta Mr. Raol P. Pamiloza Ms. Araceli L. Alcachupas
WEDNESDAY 19 August 2015		
SESSION 3: TRANSMISSION OF COMMON PLANT VIRUSES *Venue: IPB Seminar Room*		
08:00 – 09:30	Lecture 6. General concept in transmission of plant viruses	Dr. Keiko T. Natsuaki
09:30 – 09:45	Tea/Coffee Break	
9:45 – 11:00	Lecture 7. Transmission of cucurbits and other vegetable viruses via insect-vectors and mechanical inoculation	Ms. Lolita M. Dolores
11:00 – 12:00	Lecture 8. Plant-parasitic nematodes as vectors of plant viruses; NEPO and TOBRA groups	Dr. Marita S. Pinili
12:00 – 13:00	Lunch Break	
Venue: Plant Pathology Lab		
13:00 – 15:00	Practical 2: Transmission of RNA viruses through mechanical and aphid, *Aphis gossypii* inoculation	Ms. Maricel C. Gonzales Ms. Araceli L. Alcachupas Mr. Yron M. Retuta Ms. Diane A. Biglete
15:00 – 15:15	Tea/Coffee Break	

Date/Venue/Time	Topic/Activity	Resource Person(s)/ Facilitator
15:15 – 17:00	Practical 3: Transmission of DNA viruses (Begomovirus) Part I –Whitefly, *Bemisia tabaci*	Ms. Araceli L. Alcachupas Mr. Noel M. Lawas Mr. Raol P. Pamiloza
THURSDAY 20 August 2015		
SESSION 5: STRATEGIES IN PROTECTING PLANTS FROM VIRUSES *Venue: IPB Seminar Room*		
08:00 – 09:30	Lecture (12). Potato viruses in Syria with emphasis on the significance of plant quarantine	Dr. Keiko T. Natsuaki
09:30 – 09:45	Tea/Coffee Break	
09:45 – 10:45	Lecture (13). How to protect plants from viruses? Part I. Management strategies through cultural methods, attenuated virus and virus-free planting materials	Dr. Keiko T. Natsuaki
10:45 – 12:00	Lecture (14). How to protect plants from viruses? Part 2. The use of resistant varieties in virus disease management.	Ms. Lolita M. Dolores
12:00 – 13:00	Lunch Break	
Venue: Plant Pathology Lab		
13:00 – 15:00	Practical 4. Transmission of DNA virus (*Banana bunchy top virus*) Part II. Aphids, *Pentalonia nigronervosa*	Dr. Marita S. Pinili Ms. Maricel C. Gonzales Ms. Araceli L. Alcachupas Mr. Raol P. Pamiloza Ms. Amalia R. Ilagan
15:00 – 15:15	Tea/Coffee Break	
15:15 – 17:00	Practical 4. Transmission of DNA virus (*Banana bunchy top virus*) Part II. Aphids, *Pentalonia nigronervosa…continuation*	Dr. Marita S. Pinili Ms. Maricel C. Gonzales Ms. Araceli L. Alcachupas Mr. Raol P. Pamiloza Ms. Amalia R. Ilagan
FRIDAY 21 August 2015		
Field visit in Silang, Cavite		
07:00	Leave UPLB	
09:00 – 10:30	Field visit in Green Farm	Mr. Toto Faner *Farm Owner*
10:30 – 12:00	Field visit in Gourmet Farms	Gourmet Farms staff
12:00 – 13:30	Lunch (Gourmet Farms)	
13:30 -15:30	Visit to Tagaytay	
15:30 – 17:00	Sta. Rosa	
18:00	Arrive UPLB	

Date/Venue/Time	Topic/Activity	Resource Person(s)/ Facilitator
SATURDAY 22 August 2015		
Field visit and sample collection in Nueva Ecija, Central Luzon		
05:00	Leave UPLB	
10:00	Muñoz, Nueva Ecija	
10:00 – 10:30	Briefing at Ramon Magsaysay Center for Agricultural Resources and Environmental Studies	Dr. Jonathan L. Galindez *Deputy Director, RM-CARES*
10:30 – 12:00	Field visit and sampling, tour to experimental plots, biofertilizer, composting facilities	
12:00 – 13:00	Briefing and presentation of research accomplishments at Philippine Center for Postharvest Development and Mechanization, PhilMech	Dr. Dionisio G. Alvindia *Supervising Science Research Specialist, PhilMech*
13:00 – 14:00	Lunch	
14:00 – 15:00	Tour in PhilMech	
15:00	Leave Nueva Ecija	
21:00	Arrive UPLB	
SUNDAY 23 August 2015 *REST DAY*		

Week 2

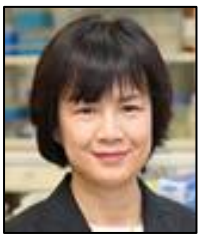
Date/Venue/Time	Topic/Activity	Resource Person(s)/ Facilitator
MONDAY 24 August 2015		
SESSION 4. *DETECTION OF PLANT VIRUSES USING SEROLOGICAL AND MOLECULAR TECHNIQUES* *Venue: IPB Seminar Room*		
08:00 – 09:00	Lecture 9. Detection of RNA and DNA viruses using serological assay	Dr. Sri Hendrastuti Hidayat *Bogor Agricultural University*
09:00 – 09:15	Tea/Coffee Break	
09:15 – 10:15	Lecture 10. Detection of RNA and DNA viruses using molecular technique	Dr. Sri Hendrastuti Hidayat
10:15 – 11:00	Lecture 11. Status, detection and phylogenetic analysis of *Banana bunchy top virus*	Dr. Marita S. Pinili
11:00 – 12:00	Practical 5. Assessment of collected samples	Participants
12:00 – 13:00	Lunch Break	
Venue: Plant Pathology Lab		
13:00 – 15:00	Practical 6. Extraction of RNA viruses from cucurbits and other crops	Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Ms. Araceli L. Alcachupas Mr. Yron M. Retuta Mr. Raol P. Pamiloza Ms. Diane A. Biglete
15:00 - 15:15	Tea/Coffee Break	
15:15 – 17:00	Practical 7. Detection of RNA viruses using Enzyme-linked immunoassay (ELISA)	Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Ms. Araceli L. Alcachupas Mr. Yron M. Retuta Mr. Raol P. Pamiloza Ms. Diane A. Biglete
TUESDAY 25 August 2015		
SESSION 4. *DETECTION OF PLANT VIRUSES USING SEROLOGICAL AND MOLECULAR TECHNIQUES…continuation* *Venue: Plant Pathology Lab*		
08:00 – 09:30	Practical 7. Detection of RNA viruses using Enzyme-linked immunoassay (ELISA)…*continuation*	Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Ms. Araceli L. Alcachupas Mr. Yron M. Retuta Mr. Raol P. Pamiloza Ms. Diane A. Biglete
09:30 – 09:45	Tea/Coffee Break	

Date/Venue/Time	Topic/Activity	Resource Person(s)/ Facilitator
09:45 – 12:00	Practical 6. Detection of RNA viruses using Enzyme-linked immunoassay (ELISA)…*continuation*	Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Ms. Araceli L. Alcachupas Mr. Yron M. Retuta Mr. Raol P. Pamiloza Ms. Diane A. Biglete
12:00 – 13:00	Lunch Break	
13:00 – 15:00	Practical 8. Extraction of DNA virus (BBTV) from banana	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
15:00 – 15:15	Tea/Coffee Break	
15:15 – 17:00	Practical 9. Detection of DNA virus (BBTV) from banana using Enzyme-linked immunoassay (ELISA)	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
WEDNESDAY 26 August 2015		
SESSION 4. *DETECTION OF PLANT VIRUSES USING SEROLOGICAL AND MOLECULAR TECHNIQUES…continuation* *Venue: Plant Pathology Lab*		
08:00 – 09:30	Practical 9. Detection of DNA virus (BBTV) from banana using Enzyme-linked immunoassay (ELISA)…*continuation*	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
09:30 – 09:45	Tea/Coffee Break	
09:45 – 12:00	Practical 10. Detection of RNA viruses using RT-PCR	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
12:00 – 13:00	Lunch Break	
13:00 – 15:00	Practical 10. Detection of RNA viruses using RT-PCR…*continuation*	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
15:00 – 15:15	Tea/Coffee Break	
15:15 – 17:00	Practical 11. Gel electrophoresis	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza

Date/Venue/Time	Topic/Activity	Resource Person(s)/ Facilitator
THURSDAY 27 August 2015		
SESSION 4. *DETECTION OF PLANT VIRUSES USING SEROLOGICAL AND MOLECULAR TECHNIQUES…continuation* *Venue: Plant Pathology Lab*		
08:00 – 09:30	Practical 12. Extraction of DNA viruses (BBTV, Begomovirus)	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Lolita M. Dolores Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
09:30 – 09:45	Tea/Coffee Break	
09:45 – 12:00	Practical 13. Detection of DNA viruses using PCR assay	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Lolita M. Dolores Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
12:00 – 13:00	Lunch Break	
13:00 – 15:00	Practical 13. Detection of DNA viruses using PCR assay…*continuation*	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Lolita M. Dolores Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
15:00 – 15:15	Tea/Coffee Break	
15:15 – 16:15	Practical 14. Gel electrophoresis and analysis	Dr. Marita S. Pinili Dr. Sri Hendrastuti Hidayat Ms. Lolita M. Dolores Ms. Maricel C. Gonzales Mr. Yron M. Retuta Mr. Raol P. Pamiloza
16:15 – 18:00	DEMO 1. Trapping of plant virus nucleic acid using FTA plant card	Dr. Marita S. Pinili
18:00 – 21:00	Dinner courtesy of Bureau of Plant Industry (BPI) – Plant Quarantine Center *Venue: Isdaan, Bay, Laguna*	
Date/Venue/Time	**Topic/Activity**	**Resource Person(s)/ Facilitator**
FRIDAY 28 August 2015		
Venue: IPB Seminar Room		
08:00 – 9:00	Viewing of Results: Virus	Dr. Sri Hendrastuti Hidayat

	transmission through insect-vector and mechanical inoculation experiments	Dr. Marita S. Pinili Ms. Araceli L. Alcachupas
09:00 – 10:30	Post-test evaluation	Dr. Marita S. Pinili *Regional Training Coordinator*
10:30 – 10:45	Coffee/Tea break	
CLOSING PROGRAM		
	Remarks	Dr. Soetikno S. Sastroutomo Dr. Sri Hendrastuti Hidayat Ms. Lolita M. Dolores
	Presentation of Certificates	Dr. Soetikno S. Sastroutomo Dr. Marita S. Pinili Ms. Lolita M. Dolores Dr. Sri Hendrastuti Hidayat
	Responses from 2 participants	Ms. Preyapan Pongsapich (Thailand) Mr. Tran Van Chien (Vietnam)
	Closing message	Dr. Marita S. Pinili
12:00 - 13:00	Lunch	
SATURDAY 29 August 2015 DEPARTURE		

F. Resource Persons



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J. List of Participants

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K. Participant Groupings

Group 1

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Ms. Sari Nurulita (Indonesia)
Ms. Normawati binti Lanisa (Malaysia)
Mr. Tran Van Chien (Vietnam)
Ms. Myint Myint Khaing (Myanmar)

Group 2

Mr. Kang Sareth (Cambodia)
Ms. Khonesavanh Chittarath (Lao PDR)
Ms. Geronima P. Eusebio (Philippines)
Ms. Dinh Thi Anh Tuyet (Vietnam)
Ms. Layla Syaznie binti Abdullah Lim (Brunei Darussalam)

Group 3

Ms. Sri Setiyawati (Indonesia)
Ms. Sengsathith Phalakhone (Lao PDR)
Mr. Darwin M. Landicho (Philippines)
Ms. Yaowapa Tantiwanich (Thailand)
Ms. Su Myat Thwe (Myanmar)

Group 4

Ms. Nur Fitriawati MSi (Indonesia)
Ms. Norhayati binti Madiha (Malaysia)
Ms. Preyapan Pongsapich (Thailand)
Ms. Adi Lisea binti Mohd Addly (Brunei Darussalam)

L. General Information

Accommodation

Participants were accommodated at BP-International Makiling (El Cielito Hotel).

Meals

Breakfast – complement of the hotel
Snacks and lunch –served at the training venue
Dinner – at your pleasure and choice, except during Dinner Reception on 17 August 2015

Local Transportation

The Training Coordinator – Administrative Group and Logistics arranged the transportation from Ninoy Aquino International Airport (NAIA) to UPLB as well as back to the NAIA.

UPLB Location/Address

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II. Methodology

The 2-week training workshop utilized the interactive lectures, laboratory activities, short demonstration and field tour. The entire training course was divided into 5 major sessions such as Session 1. Opening Program and Introduction, Session 2. Plant viruses of agricultural crops, Session 3. Transmission of common plant viruses, Session 4. Detection of plant viruses using serological and molecular assays and Session 5. Strategies of protecting crops from viruses. Each session consisted of 1 to 2-hour lectures done prior to laboratory activities. Lectures were presented by Dr. Keiko T. Natsuaki from Tokyo University of Agriculture, Tokyo, Japan, Dr. Sri Hendrastuti Hidayat from Bogor Agricultural University, Bogor, Indonesia, Ms. Lolita M. Dolores from Institute of Plant Breeding, University of the Philippines – Los Baños, Laguna, Philippines (IPB-UPLB) and Dr. Marita S. Pinili from IPB-UPLB.

Prior to the training proper, each participant was provided with a Training Workshop Manual (*See attached Manual*) which includes the training schedules and laboratory protocols and training paraphernalia like kit and lab gown. A pre-evaluation test was also administered to determine the participants' expectations and level of familiarity on basic plant virology. A country report was also initiated to assess the participants' professional background, nature of work and status of plant viruses in their country. A 2-minute oral presentation was done and presented by a representative from each participating country (*See attached country report*).

Lecture hand-outs (*See attached papers presented during the training workshop*) were also provided after the workshop prior to the post – evaluation test. A 2-way lecture discussion was imposed during each session to be able to disseminate and exchange variety of information among countries. A total of 14 lectures starting from the virus history to management strategies were presented by the resource persons. Each lecturer was given 1 to 2 hours per topic and an open discussion at the end of each lecture.

Pre-laboratory lectures were also given as further instructions and reminders before performing the actual activity. In performing laboratory activities except the BBTV transmission, participants were grouped into 4 consisting of 4 to 5 members. A total of 14 laboratory sessions were conducted inside the laboratory and screenhouse. Post-discussion in the form of group reports was done to present the output of their experiments and to further discuss the principles, results and to give recommendations.

Field tour was also integrated in the training workshop with the main purpose of disease assessment, sampling, proper storage of diseased specimen and gain supplementary knowledge on field practices in relation to pest and disease management. The 2-day field tour was done in Southern and Central Luzon, where organic and conventional farming systems are being practiced. Field observations were conducted in a cut flower farm and organic farm in Silang, Cavite. Sampling was conducted in Ramon Magsaysay Centre for Agricultural Resources and Environmental Studies (RM-CARES) in Central Luzon State University (CLSU), Muñoz, Nueva Ecija. Tour was also done in the Philippine Centre for Postharvest Development and Mechanization (PhilMech) where machineries used in postharvest processing and researches on postharvest diseases like the biological control agents (BCA) were showcased.

Technical and organizational evaluations were also given in a form of sample questions provided by the resource persons and organizing team. Pre-evaluation test consisting of participants' expectations, basic questions in plant virology and overall assessment on their skills on symptomatology to nucleic acid extraction were given. The post-evaluation test was administered during the last day of the training workshop. Questions were designed to determine the level of confidence in performing the activities conducted during the training workshop, objectives met, future plans and recommendations. Each resource persons and overall logistics were also rated based on the set criteria.

III. Accomplishments and Major Findings

Session I. Opening Program and Introduction

The 19 participants representing Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Thailand and Vietnam were welcomed at the Institute of Plant Breeding (IPB) by Prof. Teresita H. Borromeo, Officer – in – Charge of IPB – CSC (Figs. 1 & 2). This was followed by a short message from Dr. Lum Keng Yeang, Chairperson of APHCN – ASEANET. Dr. Lum introduced APHCN – ASEANET as an organization and its role in the capacity building for agricultural trade by strengthening the collaboration among plant virologists in ASEAN region through organizing training workshops.

After the messages, Ms. Lolita M. Dolores, Training Technical Coordinator discussed the training overview, objectives, course outline and schedules and methodology (Fig. 3). This was followed by introduction of participants, training team including Dr.Soetikno S. Sastroutomo, Secretary of APHCN – ASEANET and invited resource persons such as Dr. Keiko T. Natsuaki, Tokyo University of Agriculture (TUA) and Dr. Sri Hendrastuti Hidayat, Professor in Bogor Agricultural University.

Majority of the participants were plant virologists who are working under the Plant Quarantine Centre and Plant Protection Centre under the Department of Agriculture.

Pre-evaluation test was given to the participants to gauge their level of knowledge on basic plant virology as well as their expectations from the training workshop (Fig. 4).

Also, during this session, 2 lectures were given. Dr. KT Natsuaki, introduced the world of plant viruses through historical facts, discovery and researches. Her second lecture which basically tackled the classification of plant viruses gave insights on the general morphology, hosts, DNA/RNA viruses and characteristics of major genera of plant viruses (Fig. 5). Dr. KT Natsuaki also asked each participant about crops and viruses of interest. Some of the preferred viruses included Tospovirus, Potyvirus, Allexivirus and Nanovirus.

Country Report

After the lectures, each representative from participating countries presented their country reports which introduced their organization, nature of work and status of plant viruses present in their country (*See attached Country Reports*). Ms. Adi Lisea binti Mohd Addly reported the main responsibilities of their Crop Protection Unit and Biodiversity Division under the Department of Agriculture and Agrifood, status of plant viruses in Brunei Darussalam and their field of interest (Fig. 6). Ms. Sor Sareka represented the General Directorate of Agriculture (GDA) which is under the Ministry of Agriculture, Forestry and Fisheries (MAFF) in Cambodia. She mentioned the plant diagnostics responsibilities, current status of project activities and challenges and issues related to pest and disease diagnosis including lack of equipment, facilities and staff. Ms. Sri Setiyawati of the Indonesian Agricultural Quarantine presented the works and the yield losses of major crops due virus diseases in Indonesia. She also mentioned the virus detection assays they used to prevent the introduction of plant quarantine virus diseases. Ms. Setiyawati also emphasized their action plan in eradicating Papaya ringspot disease which is the newly-introduced quarantine virus

disease of papaya in Indonesia. In Lao PDR, Ms. Sengsathith Phalakhone mentioned the lack of facilities, equipment and trained staff when it comes to virus disease diagnosis. Thus, plant samples suspected with virus infection were usually sent to Thailand and Australia for analysis. Ms. Norhayati binti Madiha reported the mission and services offered by Plant Biosecurity Division and the current status of plant viruses in Malaysia. She also presented the virus detection tools available in their department. In Myanmar as presented by Ms. Myint Myint Khaing, the lack of infrastructure, equipment for and knowledge on disease diagnosis makes their Plant Protection Centre incapable of effective disease detection and identification. On the other hand, the Philippine participant, Mr. Darwin Landicho presented the organizational structure of Post Entry Quarantine Station, National Plant Quarantine Services Division under the Bureau of Plant Industry and the nature of work such as laboratory services on pests, diseases, GMO testing and Pest Risk Analysis and others. In Thailand, the availability of serological assay kits such as NCM – ELISA, variants of Dot Blot and Gold labelling IgG Flow (GLIF) and molecular tools showed their capability in dealing with various plant viruses. As presented by Ms. Preyapan Pongsapich of the Plant Protection Research and Development Office (PPRDO), frequent survey of various crops in the field, assessment of virus diseases, monitoring and field inspection are the routine activities of their department. Mr. Tran Van Chien of Vietnam presented the list and status of virus diseases of major crops in their country including the devastating *Southern rice black-streaked dwarf virus* (SRBSDV). He also mentioned various methods of virus detection as well as their common problems in virus disease diagnosis such as lack of well-experienced diagnostic officers, facilities and costs of detection.

Reception Dinner

A welcome dinner was held at Kamayan, Bay, Laguna and showcased the typical Filipino cuisine and the traditional ‘harana’ or serenade to entertain the diverse culture of the participants. The said reception dinner was not only attended by the participants but the rest of the training team, resource speakers and visitor from former student of Dr. KT Natsuaki (Fig. 7).

Session II. Plant viruses of agricultural crops

Ms. LM Dolores delivered her lectures on “Plant viruses infecting vegetable crops in the Philippines” where she introduced the major and common plant viruses of cucurbits and solanaceous crops. This was followed by her topic on “Symptomatology, sampling and handling of plant samples for virus detection”. Here, she presented common symptoms induced by groups of viruses, proper way of collecting virus-suspected plants and handling and storage of fragile samples (Fig. 8). The next lecture which was presented by Dr. KT Natsuaki deals with rice viruses in Asia and Africa. In this lecture, Dr. KT Natsuaki showed the distribution of *Rice yellow mottle virus* in Africa, its manner of transmission (insect vectors and mechanical) and the control measures to reduce RYMV infection on major rice varieties. In the case of major rice viruses in Asia, Dr. KT Natsuaki mentioned the occurrence of SRBSDV which is very common in Southern China and North Vietnam. Other economically important rice viruses including the *Rice dwarf virus*

(RDV), *Rice stripe virus* (RSV), *Rice tungro spherical virus* (RTSV) and *Rice tungro bacilliform virus* (RTBV) and their mode of transmission were discussed.

In the afternoon session, actual preparation of buffers and other materials for serological and molecular assays were performed in the laboratory (Fig. 9). Participants were grouped into four, consisting of 4 to 5 members each. Each group was able to prepare extraction buffers for their next activities. Buffers such as PBS Buffer, TBS Buffer, stock solutions like 1M Tris-Base, 0.5 M EDTA and 5 M NaCl, Dellaporta Extraction Buffer and CTAB Extraction Buffer were prepared (Figs. 10 & 11).

Session III. Transmission of common plant viruses

The plant virus transmission lectures and laboratory activities were done during the first week of the training workshop. Three (3) lectures were given during the morning session. The general concept in transmission of plant viruses (Lecture 6) was given by Dr. KT Natsuaki. She mentioned the roles of various groups of insects, mite, nematodes and microorganisms like fungi as known vectors of plant viruses (Fig. 12). The mode of insect transmission *i.e.* persistent and non-persistent was compared. Transmission of cucurbits and other vegetable viruses via insect-vectors and mechanical inoculation (Lecture 7) was discussed by Ms. LM Dolores. Ms. LM Dolores gave specific examples of virus groups and their respective vectors and manner of transmission (mechanical or via insect vectors). This was followed by a supplementary lecture of Dr. MS Pinili on transmission of plant viruses via plant-parasitic nematodes. In this lecture, specific plant-parasitic nematodes and their associated plant viruses were discussed. Participants were able to gain knowledge on mechanical mode of transmitting plant viruses as well as the use of different vectors such as insects and nematodes.

In the afternoon session, participants were able to demonstrate non-persistent mode of virus, *Papaya ringspot virus - P* (PRSV-P) transmission using *Aphis gossypii* on papaya (*Carica papaya*), cucumber (*Cucumis sativus*), squash (*Cucurbita maxima*), *C. amaranticolor* and *C. quinoa* (Fig. 13).

Participants were also tasked to perform mechanical inoculation of *Tobacco mosaic virus* (TMV) and *Zucchini yellow mosaic virus* (ZYMV) on host plants, *Nicotiana tabacum* cv. 'Santi', *N. benthamiana* and *N. glutinosa* and indicator plants such as *Chenopodium amaranticolor, C. quinoa, Datura metel, Gomphrena globosa* and *Physalis floridana* (Fig. 14). Extraction of infected plants as inoculum source was prepared prior to mechanical inoculation.

Persistent mode of transmission of Begomovirus using whitefly, *Bemisia tabaci* on different host plants such as cotton (*Gossypium herbaceum*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), *N. glutinosa, C. amaranticolor* and *G. globosa* was conducted in the screenhouse. Inoculum source kept in Mylar cage with viruliferous whiteflies was transferred individually in screen cages to allow natural feeding of the vectors (Fig. 15).

Banana bunchy top virus (BBTV) inoculation was also performed using aphids, *Pentalonia nigronervosa*. Each participant was allowed to do aphid starvation and virus acquisition then followed by inoculation to healthy tissue-cultured banana cv. 'Lakatan' (Fig. 17). All inoculated plants were kept under screen house condition for symptom development.

Also at the end of this session, a certificate of appreciation was awarded to Dr. KT Natsuaki for being the resource person during the first week of the training workshop (Fig. 18). The certificate was given by Dr. Soetikono S. Sastroutomo.

Field visit

A 2-day field tour was conducted basically to; (1) identify symptoms of possible virus-infected crops, (2) collect samples for virus detection and identification and (3) gain supplementary knowledge on different farm practices under organic and conventional farming systems. The first field visit was conducted in Silang, Cavite where established cut flower farm planted to Gerbera and Chrysanthemum was visited. Mr. Toto Faner, owner of the Flower Farm explained the basic cut flower production practices and management of pests and diseases (Fig. 19). Participants were also able to observe and collect samples showing typical virus symptoms (Fig. 20). This was followed by field observation in one of the oldest organic farms in the Philippines, Gourmet Farms, where participants were able to interview farm staff on how to manage an organic farm planted to lettuce and variety of herbs and how to utilize their products or harvests for local and foreign markets (Figs. 21 & 22).

The second day of field tour was held in the Science City Muñoz, Nueva Ecija particularly in Ramon Magsaysay Centre for Agricultural Resources and Environmental Studies (RM-CARES) in Central Luzon State University and in the Philippine Centre for Postharvest Development and Mechanization (PhilMech). RM-CARES introduced how to establish organic farm from the conventional farming system. Dr. Jonathan L. Galindez, Deputy Director of RM-CARES presented their organization's mandate, activities and products through a 7-minute video presentation (Fig. 23). Participants showed enthusiasts and curiosity by asking questions on the challenges faced by organic farmers from the tedious transition period, accreditation and certification of organically-grown crops and its relationship in managing pests and diseases. Also, participants were able to collect diseased samples from organically-grown plots for symptom identification and virus detection (Fig. 24).

On the other hand, the field visit in PhilMech showed recent technologies and discoveries in addressing postharvest problems related to diseases and processing. PhilMech welcomed the participants and the rest of the training team by a short program and giving flyers and published handbook. The use of biological control agents (BCA) developed by Dr. Dionisio G. Alvindia, Supervising Scientist Research Specialist is one of the breakthroughs of organization (Fig. 25). After the lunch break, PhilMech staff introduced their technology like machineries for coffee processing, farming tools and equipment and their recent works in developing BCA, insect – rearing and others (Figs. 26 & 27).

Session IV. Detection of plant viruses using serological and molecular assay

Activities done under this session were performed during the second week of the training course. Basic concept on detecting RNA and DNA viruses using serological and molecular techniques were discussed in this session. Dr. Sri Hendrastuti Hidayat, Professor of Bogor Agricultural University explained the antibody-antigen interaction and various serological and molecular methods available for virus detection and identification (Fig. 28). These lectures were followed

by specific example *i.e.* case study of BBTV from the Philippine abaca and banana and the status and phylogenetic analyses of BBTV in Bali, Indonesia.

Under this session, 10 practical/lab activities dealing with virus nucleic acid extraction and detection using ELISA and PCR assay and gel electrophoretic analyses were conducted. Prior to laboratory sessions, results of mechanical inoculation were confirmed after 1 week of incubation. Mechanical inoculation of TMV on *Nicotiana glutinosa* showed local lesions (Fig. 29). Inoculated samples were collected for virus detection. At the same time, field collected samples were assessed (symptoms) and used for serological and molecular assays (Table 1).

A total of 10 different plant samples showing typical virus symptoms were collected from RM – CARES, CLSU, Nueva Ecija (Fig. 30).Indirect Enzyme – linked immunosorbent assay (I – ELISA) and dot blot immunoassay (DIBA) using nitrocellulose membrane (NCM) were conducted on both mechanically - inoculated and field collected samples (Figs. 31 – 33). Each sample was extracted for virus detection. As shown in the Table 2, mechanically – inoculated plants were positive to TMV using I-ELISA and DIBA. For the field collected samples only 5 out of 10 (50%) were found positive to CMV using I-ELISA including the *I. aquatica* which was firstly confirmed on mosaic sample in the Philippines (Table 3, Figs. 34 & 35).

In addition, simple demonstration on impregnating plant virus nucleic acid on FTA plant card was performed.

On the other hand, the persistent mode of transmission on Begomovirus and BBTV did not show early symptoms as expected due to their long incubation period. However, aphids inoculated on banana have multiplied and produced nymphs.

Nucleic acid extraction was conducted using CMV-, PRSV- and ZYMV-infected plant samples (Fig. 36). Results of their PCR products were analysed using gel electrophoresis (Fig. 37). Data on PCR assay were presented during the laboratory session (Fig. 38).

After the last laboratory activity, a complementary dinner courtesy of Bureau of Plant Industry (BPI) headed Atty. Paz Benavidez, OIC, Director was held at Isdaan, Bay, Laguna. The said dinner was also attended by officials and staff of Post Entry Plant Quarantine Station under BPI (Fig. 39).

Viewing of results of mechanically – and aphid-inoculated plants were done on the day before the post-evaluation test. BBTV – inoculated banana did not show any apparent symptom but aphids continue to multiply (Fig. 40).

Session V. Strategies of protecting crops from viruses

Disease management strategies such as cultural methods, attenuated virus, virus – free planting materials and deployment of resistant varieties were discussed on this session by Dr. KT Natsuaki and Ms. LM Dolores. Specific example on the significance of plant quarantine on potato viruses in Syria was emphasized to avoid the geographic distribution of infected planting materials. In the case of resistant varieties, specific examples on conventional and non-conventional methods of breeding for virus resistance were discussed. For the conventional method, screening protocol such as severity rating, phenotyping scale and screenhouse and field evaluations were included. While in the non-conventional way, genetic engineering is the common method.

Technical and organizational evaluations

The overall evaluation showed that majority of the participants achieved their expectations from the training workshop. This outstanding rating was reflected from the very satisfactory results of their post-evaluation test. Their level of judgement in assessing possible virus-infected crops using the symptomatology was increased from fair to good rating scale. Thirty percent (30%) got excellent level of confidence in performing the nucleic acid extraction from plant samples and 32% indicated good level of confidence (Table 4)

Additional post-evaluation questions were given to the participants (Fig. 41). Selected questions from each resource person were given to determine both the participants level of reasoning and computational ability and understanding. Most of the participants had good reasoning in dealing with virus – suspected crops. However, only 5 participants got the perfect scores, since most of them failed to do the computational problem.

Participants also suggested doing follow-up training on the following; (1) extraction of nucleic acid from FTA membrane, (2) DNA sequencing, (3) virus-vector identification, (4) primer design, (5) more field activities, (6) another methods of virus detection and (7) long-term training course.

The training team including the resource persons and logistics also received high percentage of overall rating of satisfaction from the participants (Table 5). Ratings have range from 4 (Good) and 5 (Excellent)

After the post-evaluation, certificates of appreciation and completion were given to resource persons and participants, respectively. Certificates were awarded by Dr. Soetikno S. Sastruotomo, Dr. Sri Hendrastuti Hidayat and Ms. Lolita M. Dolores (Figs. 42 – 47). Responses from the two participants were given by Ms. Preyapan Pongsapich (Thailand) and Mr. Tran Van Chien (Vietnam). Both of them expressed their special thanks to the organizers and the shared their happy moments and experiences during the training workshop.

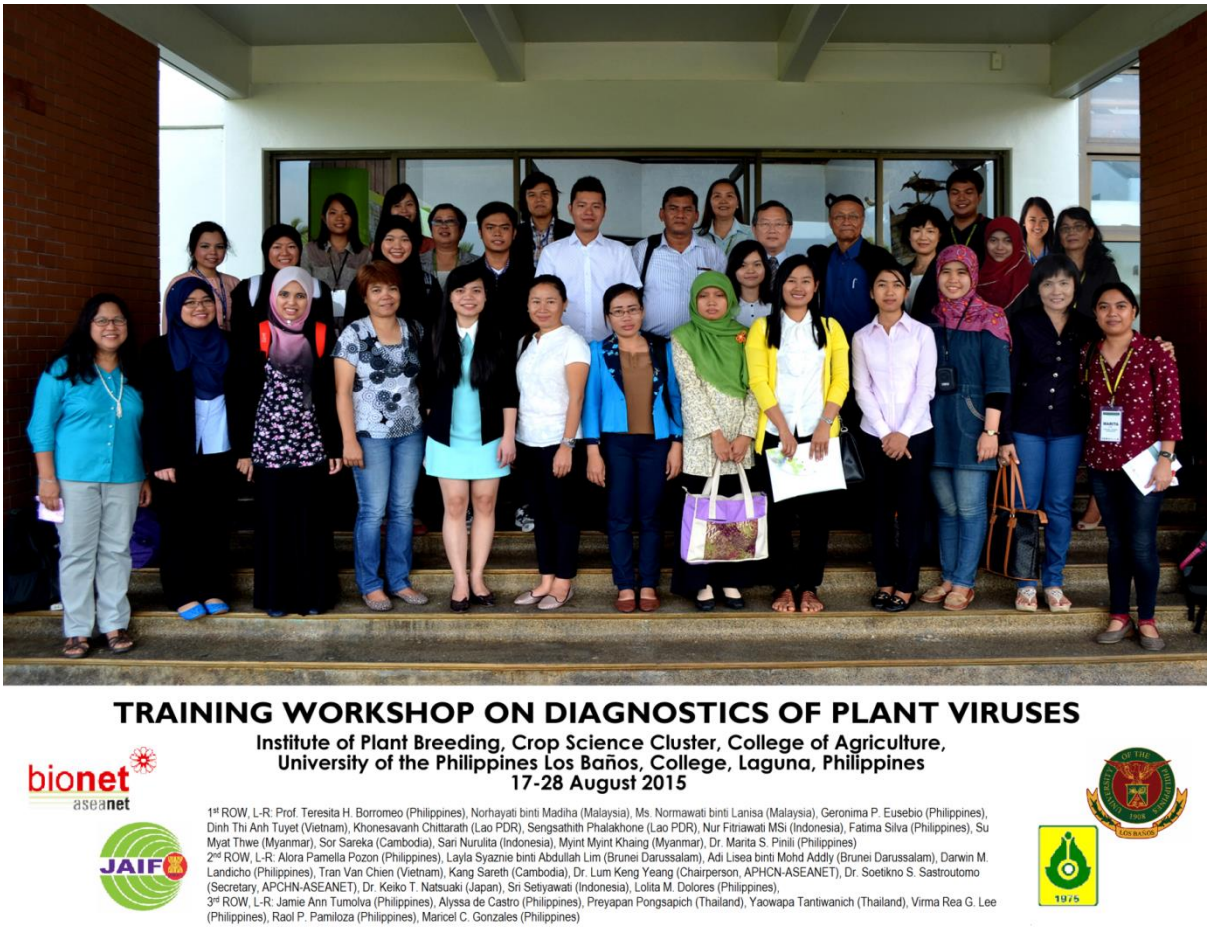


Fig. 1. Group photo during the Opening Ceremony of the training workshop at the Institute of Plant Breeding, UPLB on August 17, 2015.

Fig. 2. Welcome remarks and short message from Prof. Teresita H. Borromeo, OIC-CSC, UPLB (left) and Dr. Lum Keng Yeang, Chairperson of APHCN – ASEANET (right), respectively, during the Opening Ceremony at IPB, UPLB.

Fig 3. Ms. Lolita M. Dolores (left) explains the training overview, course objectives and outline and schedule of activities during the briefing on the training workshop. Dr. Marita S. Pinili (right) introduced the 19 participants from 9 countries belonging to the Southeast Asian region, resource persons and training team.

Fig. 4. Pre-evaluation test given during day 1 of the training workshop.

Fig. 5. Dr. Keiko T. Natsuaki, Professor in Tokyo University of Agriculture (Tokyo NODAI) delivered two lectures on discovery and basic classification of plant viruses during the day 1 of the training workshop at IPB Seminar Room on August 17, 2015.

Fig. 6. Country reports from representatives of each participating country. From top to bottom, Ms. Adi Lisea binti Mohd Addly (Department of Agriculture and Agrifood, Ministry of Industries and Primary Resources in Brunei Darussalam), Ms. Sor Sareka (General Directorate of Agriculture, Ministry of Agriculture, Forestry and Fisheries (MAFF) in Cambodia) and Ms. Norhayati binti Madiha (Plant Biosecurity Division, Department of Agriculture in Malaysia).

Fig. 7. Reception dinner held at Kamayan Restaurant in Bay, Laguna on Ausgust 17, 2015. Traditional Filipino food was served during the dinner and guests were serenade by quartet band with special song number from Mr. Darwin Landicho from the Philippines (lower leftmost photo).

Fig. 8. Lecture on virus diseases of cucurbits and vegetable crops in the Philippines presented by Ms. LM Dolores during day 2 of the training workshop.

Fig. 9. Instructions and some pointers regarding the activity were given prior to the actual performance in the laboratory.

Fig. 10. Preparation of stock solutions and buffers were conducted during day 2 of the training workshop. Particpants familiarized themselves on basic chemical components, calculation of molarity (M) and weighing of reagents.

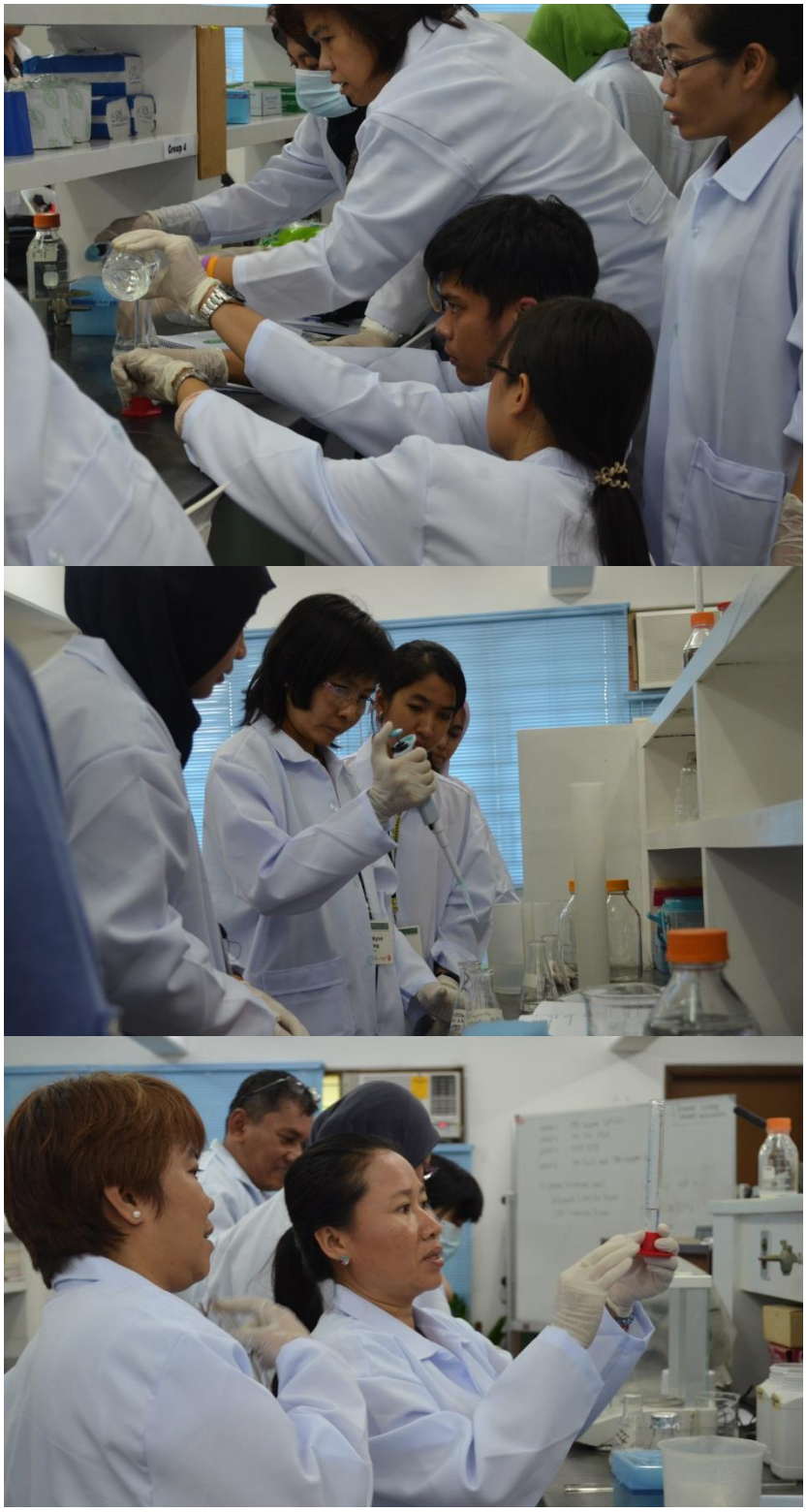


Fig. 11. Each group was assigned to prepare their respective stock solutions and extraction buffer. Measuring of liquids and precise pipetting of small volume of solutions were performed.

Fig.12. Day 3 of the training workshop was held in IPB seminar room with active discussions on issues on updating published virus diseases and accessing database for plant quarantine purposes.

Fig. 13. In the laboratory session of day 3, participants individually performed non-persistent mode of virus transmission by inoculating aphids, *Aphis gossypii* into different host plants.

Fig.14. Mechanical inoculation of TMV and ZYMV was conducted by gentle rubbing of the inoculum to the indicator plants.

Fig.15. Transmission on Begomovirus using whitefly, *Bemisia tabaci* (upper). The set-up was done under the screen cage to avoid escape of the insect-vectors. All mechanically – and insect – inoculated plants were all kept under screen house condition for symptom development.

Fig. 16. Transmission of *Banana bunchy top virus* using aphids, *Pentalonia nigronervosa* on banana cv. 'Lakatan' was conducted during day 4 of the laboratory session (upper). Discussion on the efficiency of virus transmission was done after performing the lab exercise (lower).

Fig.17. After the day 4 activity, a certificate of appreciation was awarded to Dr. KT Natsuaki for being the resource person during the first week of the training workshop (upper). This was followed by a group photo at the entrance gate of IPB-UPLB.

Day 1 of Field tour in Southern Luzon, August 21, 2015

Fig.18. Mr. Toto Faner, owner of the cut flower farm in Silang, Cavite explains the production of Chrysanthemum and how to manage pests and diseases.

Fig.19. Participants observing Gerbera plants for possible virus disease symptoms.

Fig.20. Group photo after the field tour at Flower Farm in Silang, Cavite.

Fig. 21. Field tour at Gourmet Farms, Silang, Cavite, one of the first organic farms in the Philippines.

Fig. 22. Quick tour to lettuce and herbs field in Gourmet Farms (upper left). Organically – grown vegetables and other products were also served during lunch time.

Day 2 of Field Tour, Central Luzon, August 22, 2015

Fig. 23. Visit to Ramon Magsaysay Centre for Agricultural Resources and Environment Studies (RM-CARES) in Central Luzon State University, Muñoz, Nueva Ecija. A short orientation on mission, vision and activities of RM-CARES were presented by Dr. Jonathan L. Galindez, Deputy Director of RM-CARES.

Fig. 24. Symptom observation and sampling of organically-grown crops in RM-CARES for virus detection.

Fig. 25. Warm reception from the Philippine Centre for Postharvest Development and Mechanization (PhilMech), Muñoz, Nueva Ecija headed by Dr. Dionisio G. Alvindia, Supervising Science Research Specialist (lower leftmost) and Dr. Rodolfo P. Estigoy, Chief, Applied Communication Division (lower middle).

Fig. 26. Tour and demo on processing and farm machineries inside PhilMech facilities.

Fig. 27. Laboratory tour in PhilMech.

Fig. 28. Lectures on serological and molecular detections of plant viruses were presented by Dr. Sri Hendrastuti Hidayat, Professor in Bogor Agricultural University during week 2 of the training workshop.

Fig. 29. In the afternoon session of day 7 of the training workshop, mechanically - inoculated plants were visited for symptom development and samples were collected for serological detection.

Fig. 30. Some of the field collected samples from RM-CARES, CLSU, Muñoz, Nueva Ecija showing vein clearing, mosaic and crinkling of leaves. *Ipomoea aquatic, Abelmoschus esculentus, Luffa, Chilli, Serpentina* sp. and *Zinnia elegans* (from left clockwise).

Fig. 31. Extraction of plant samples and loading of extracts into ELISA plate for incubation.

Fig. 32. Preparation of samples and reagents for Dot Blot Immunoassay (DIBA).

Fig. 33. Washing of ELISA plates and application of enzyme-conjugate for the Indirect ELISA were done during day 8 of the training workshop. Laboratory lecture was also conducted by Dr. Hidayat during the incubation period of serological assay.

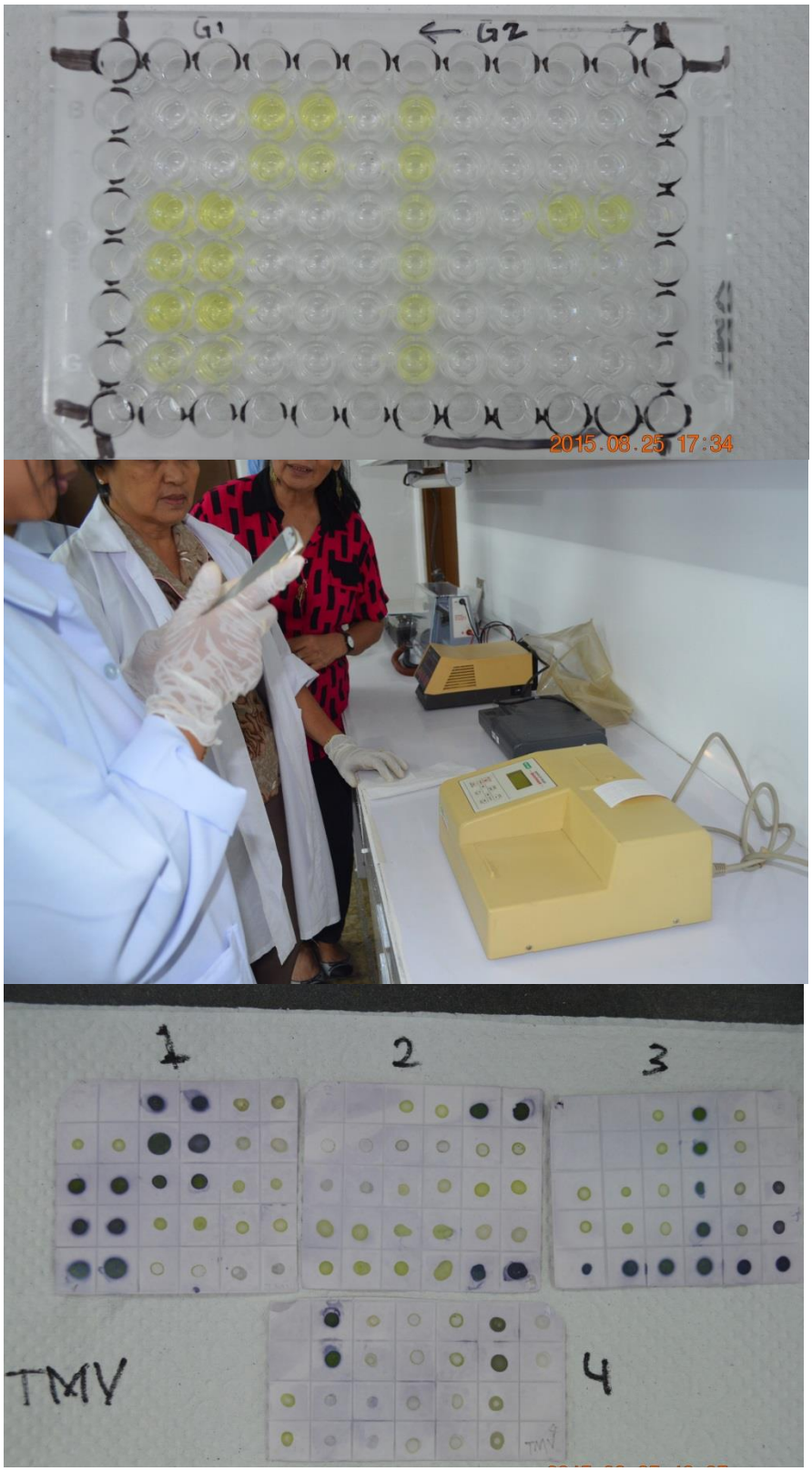


Fig. 34. Color observation of ELISA results (upper) and reading of the absorbance value using ELISA reader (middle). Visual observation on DIBA was also performed during day 8 of laboratory exercise (lower).

Fig. 35. RNA extraction was performed during day 9 of the laboratory session.

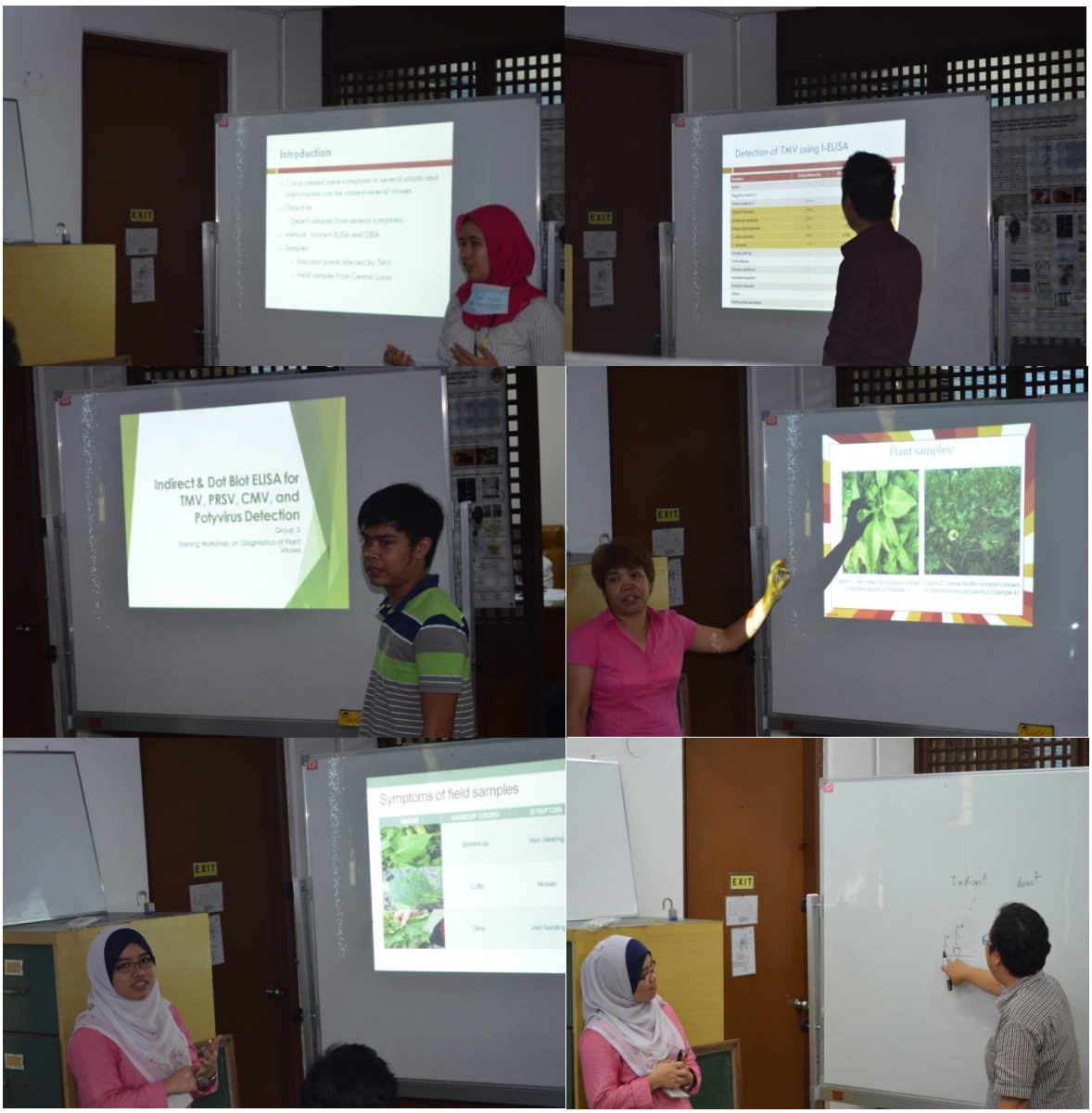


Fig. 36. I-ELISA and DIBA results were presented by each group and were discussed to assess the sensitivity of serological assays, possible errors encountered and novel findings from field collected and artificially-inoculated samples.

Fig. 37. Performing PCR assay on CMV -, PRSV – and ZYMV – infected samples and (upper) viewing of gel using GelDoc (below).

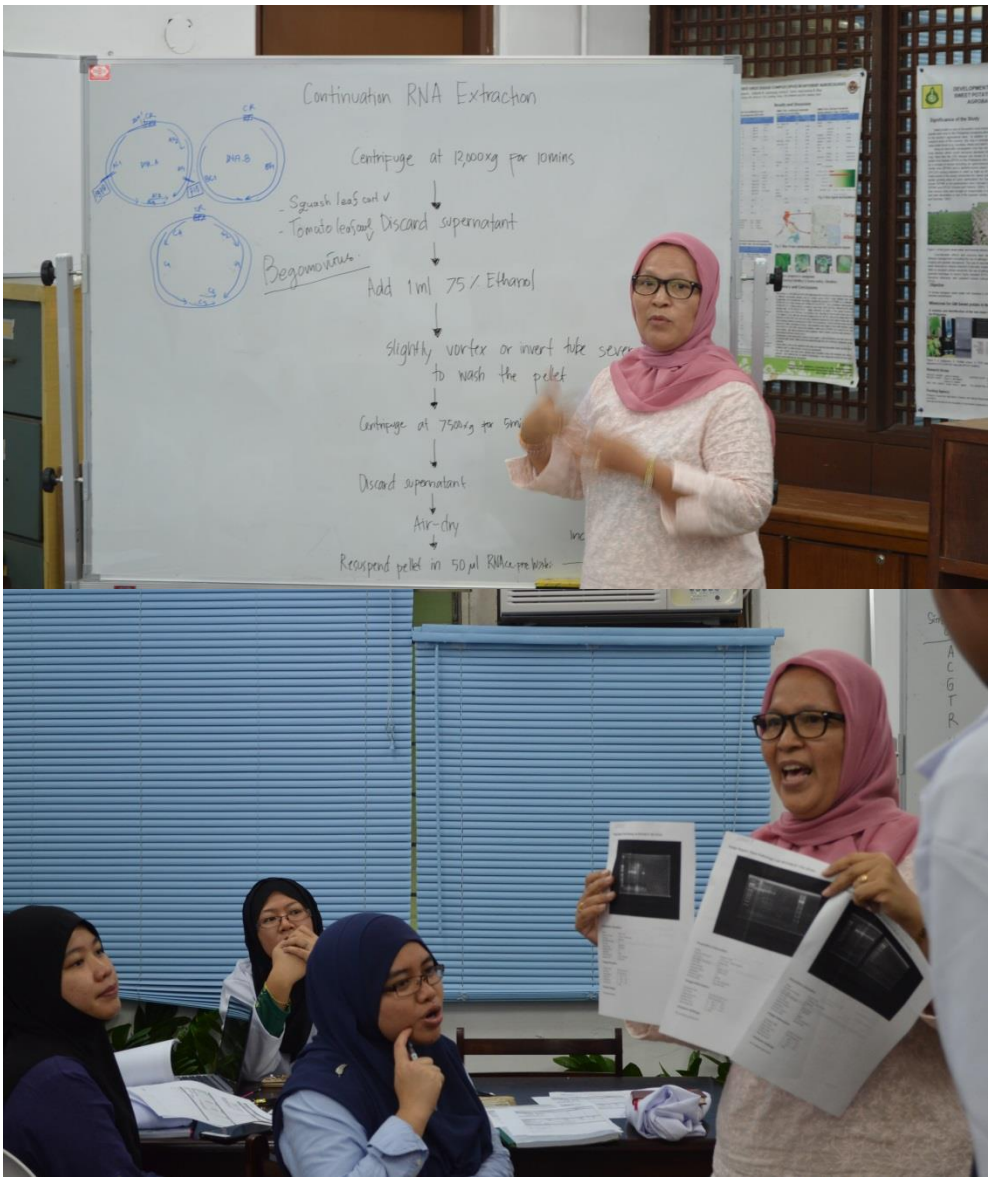


Fig. 38. Presentation and discussion of PCR assay results with Dr. Hidayat.

Fig. 39. After the tedious laboratory activities, a complementary dinner courtesy of Bureau of Plant Industry headed by Atty. Paz Benavidez (lower middle) was held at Isdaan Restaurant located in Bay, Laguna.

Fig. 40. Viewing of results of mechanically – and aphid – inoculated plants in the screen house. Most of the mechanically-inoculated plants showed distinct local lesions (upper), whereas no apparent bunchy top symptom was observed from BBTV-inoculated banana instead continous multiplication of aphids, *P. nigronervosa* (lower).

Fig. 41. Post-evaluation exam was given prior to the Closing Ceremony on August 28, 2015 at IPB seminar room.

Fig. 42. Awarding of Certificate of Appreciation to Dr. Sri Hendrastuti Hidayat for being the resource person during the second week of the training workshop.

Fig. 43. Awarding of Certificate of Completion to participants; Ms. Adi Lisea binti Mohd Addly and Ms. Layla Syaznie binti Abdullah Lim (Brunei Darussalam), Ms. Sor Sareka and Mr. Kang Sareth (Cambodia) and Ms. Sri Setiyawati, Ms. Nur Fitriawati and Ms. Sari Nurulita (Indonesia).

Fig. 44. Awarding of Certificate of Completion to participants; Ms. Khonesavanh Chittarath and Ms. Sengsathith Phalakhone (Lao PDR), Ms. Norhayati binti Madiha and Ms. Normawati binti Lanisa (Malaysia), Ms. Myint Myint Khaing and Ms. Su Myat Thwe (Myanmar).

Fig. 45. Awarding of Certificate of Completion to the participants; Ms. Geronima Eusebio and Mr. Darwin Landicho (Philippines), Ms. Prepyapan Pongsapich and Ms. Yaowapa Tantiwanich (Thailand), Ms. Dinh Thi Anh Tuyet and Mr. Tran Van Chien (Vietnam).

Fig. 46. Awarding of Certificate of Appreciation to Dr. Marita S. Pinili, Regional Training Coordinator and Collaborator (upper) and to Ms. Maricel C. Gonzales, Training Secretary (lower).

Fig. 47. Awarding of Certificate of Appreciation to members of the training team, Mr. Raol P. Pamiloza (upper) and Mr. Yron M. Retuta (lower).

Fig. 48. Responses from the two participants were given by Ms. Preyapan Pongsapich (Thailand) and Mr. Tran Van Chien (Vietnam).

Table 1. Symptom description of the samples collected from RM-CARES, CLSU, Muñoz, Nueva Ecija.

Plants	Symptoms
Abelmoschus esculentus	Mild yellow mottle, vein clearing, vein banding
Allium cepa	Asymptomatic, mild stripes
Capsicum annum	Mosaic, mottle, malformation of leaf, leaf curl
Ipomoea aquatica	Vein clearing, crinkling, vein yellowing
Luffa sp.	Mosaic, yellow spots
Serpentina sp.	Yellow mottle
Ocimum bacilicum	Mosaic
Pandanus amaryllifolius	Mosaic
Zinia elegans	Mosaic
Vigna unguiculata	Mosaic, leaf curl, mild mottle

Table 2. Results of CMV, TMV and PRSV detection using I-ELISA and DIBA on artificially – inoculated samples.

Sample	I-ELISa			DIBA		
	CMV	TMV	PRSV	CMV	TMV	PRSV
Carica papaya	N	N	P	N	N	P
Chenopodium amaranticolor	N	P	N	N	P	N
C. quinoa	N	P	N	N	P	N
C. murale	N	P	N	N	P	N
Cucumis sativus	N	N	N	N	N	N
Gomphrena globosa	N	P	N	N	P	N
Datura metel	N	N	N	N	N	N
Nicotiana glutinosa	P	N	N	P	N	N
N.benthamiana	*	*	*	*	*	*
N.tabacum cv. 'Santi'	P	P	N	P	P	N
Physalis floridana	N/P	P	N	N	P	N
Positive control	P	P	P	P	P	P
Negative control	N	N	N	N	N	N
Buffer	N	N	N	N	N	N

P – positive (+,++ or +++) with absorbance value of 2 times the negative control for ELISA and blue color reaction on DIBA.
N – negative
**No data*

Table 3. Results of CMV, TMV and PRSV detection using I-ELISA and DIBA on field collected samples from RM-CARES, CLSU, Muñoz, Nueva Ecija.

Sample	I-ELISa			DIBA		
	CMV	TMV	PRSV	CMV	TMV	PRSV
Allium cepa	P	N	N	N	N	N
Abelmoschus esculentus	N	N	N	N	N	N
Capsicum annum	P	N	N	N	N	N
Ipomoea aquatica	P	N	N	N/P	N	N
Luffa sp.	N	N	P	N	N	N
Ocimum basilicum	N	N	N	N	N	N
Pandanus amaryllifolius	N	N	N	N	N	N
Serpentina sp.	P	N	N	N	N	N
Vigna unguiculata	N	N	N	N	N	N
Zinia elegans	P	N	N	P	N	N
Positive control	P	P	P	P	P	P
Negative control	N	N	N	N	N	N
Buffer	N	N	N	N	N	N

P – positive (+,++ or +++) with absorbance value of 2 times the negative control for ELISA and blue color reaction on DIBA.
N – negative

Table 4. Results (in percentage) of the technical evaluation test.

Questions	Pre-evaluation	Post-evaluation
Name three (3) diseases of plants caused by viruses		
3 correct answers	42.11	36.84
2 correct answers	15.79	10.52
1 correct answer	0.00	5.26
No correct answer	10.53	0.00
No answer	0.00	0.00
Answered causal organism instead of disease	31.58	47.37
Give three (3) examples of virus symptoms in plants		
3 correct answers	89.47	94.74
2 correct answers	5.26	5.26
1 correct answer	0.00	0.00
No correct answer	0.00	0.00
No answer	5.26	0.00
How confident are you that you could identify virus-infected crops in the field?		
Not Confident	15.79	10.52
Fairly Confident	57.89	52.63
Confident	26.32	36.84
How confident are you that you could give advice that plant samples sent to you are infected with virus(es)?		
Not Confident	21.05	5.26
Fairly Confident	52.63	52.63
Confident	26.32	36.84
No Answer	0.00	5.26
How confident are you that you could reject plant samples or planting materials that might be infected with virus(es)?		
Not Confident	15.79	21.05
Fairly Confident	57.89	42.10
Confident	26.32	31.58
No Answer	0.00	5.26
Do you know how to identify potential insect-vector(s) of plant viruses?		
No idea how	15.79	5.26
Have some idea	52.63	15.79
Good idea how	21.05	63.16
Know well how to get help	10.53	10.53
No answer	0.00	5.26

Table 4. Results (in percentage) of the technical evaluation test…*continued*

Questions	Pre-evaluation	Post-evaluation
Do you think you can demonstrate how to conduct ELISA to your colleagues?		
No	36.84	0.00
Yes, but need some help	36.84	36.84
Strongly yes	26.32	63.16
Which one of them is not a plant virus vector?		
Correct answer	63.16	94.74
Incorrect answer	36.84	5.26
What is the first discovered plant virus?		
Correct answer	78.95	94.74
Incorrect answer	21.05	5.26
Name two (2) viruses that are transmitted by whiteflies		
2 correct answers	15.79	73.68
1 correct answer	26.32	21.05
No correct answer	47.37	5.26
No answer	10.53	0.00
Name two (2) viruses that are transmitted by aphids		
2 correct answers	31.58	63.16
1 correct answer	47.37	36.84
No correct answer	10.53	0.00
No answer	10.53	0.00
ELISA and PCR are two methods commonly used for virus detection with differences in their target. What is the target component of the virus for each method?		
A. ELISA		
Correct answer	63.16	78.95
Incorrect answer	21.05	15.79
No answer	15.79	5.26
B. PCR		
Correct answer	73.68	73.68
Incorrect answer	10.53	15.79
No answer	15.79	10.53

Table 4. Results (in percentage) of the technical evaluation test…*continued*

Questions	Pre-evaluation	Post-evaluation
Specificity of detection method is determined by certain component in the reaction. Give the specific components for each method.		
A. ELISA		
Correct answer	57.89	73.68
Incorrect answer	26.32	21.05
No answer	15.79	5.26
B. PCR		
Correct answer	47.37	78.95
Incorrect answer	36.84	5.26
No answer	15.79	15.79
Consideration(s) in choosing a method for virus detection		
A. ELISA		
Correct answer	42.11	63.16
Incorrect answer	21.05	5.26
No answer	36.84	31.58
B. PCR		
Correct answer	42.11	63.16
Incorrect answer	21.05	15.79
No answer	36.84	21.05
How would you rate your knowledge on plant virus disease?		
A. Symptomatology		
Poor	5.26	0.00
Fair	36.84	10.53
Average	26.32	36.84
Good	26.32	47.37
Excellent	5.26	5.26
B. Possible virus species		
Poor	31.58	0.00
Fair	42.11	21.05
Average	21.05	52.63
Good	5.26	26.32
Excellent	0.00	0.00
C. Mode of virus transmission		
Poor	10.53	0.00
Fair	52.63	15.79
Average	21.05	26.32
Good	15.79	31.58
Excellent	0.00	15.79
No answer	0.00	10.53

Table 4. Results (in percentage) of the technical evaluation test…*continued*

Questions	Pre-evaluation	Post-evaluation
D. Ability to identify insect and other vector(s)		
Poor	42.11	5.26
Fair	15.79	5.26
Average	31.58	47.37
Good	10.53	31.58
Excellent	0.00	10.53
E. How to extract virus nucleic acid from samples collected in the field.		
Poor	26.32	0.00
Fair	15.79	5.26
Average	21.05	26.32
Good	21.05	31.58
Excellent	15.79	36.84

Table 5. Post-evaluation summary

1. Lecture Sessions

RATING SCALE	PERCENTAGE
5 – Excellent	58.0
4 – Good	38.5
3 – Average	2.8
2 – Fair	0.7
1 - Poor	0

2. Laboratory Sessions

RATING SCALE	PERCENTAGE
5 – Excellent	68.1
4 – Good	25.7
3 – Average	5.6
2 – Fair	0.7
1 - Poor	0

3. Main/Principal Speaker(s) and Facilitator(s) Evaluation

3.1 Dr. Keiko T. Natsuaki

RATING SCALE	PERCENTAGE
5 – Excellent	90.2
4 – Good	9.7
3 – Average	0
2 – Fair	0
1 - Poor	0

3.2 Dr. Sri Hendrastuti Hidayat

RATING SCALE	PERCENTAGE
5 – Excellent	76.7
4 – Good	21.1
3 – Average	2.3
2 – Fair	0
1 - Poor	0

3.3 Dr. Marita S. Pinili

RATING SCALE	PERCENTAGE
5 – Excellent	85.7
4 – Good	13.5
3 – Average	0.8
2 – Fair	0
1 - Poor	0

3.4 Ms Lolita M. Dolores

RATING SCALE	PERCENTAGE
5 – Excellent	61.7
4 – Good	32.3
3 – Average	6.0
2 – Fair	0
1 - Poor	0

4. This activity might be more useful if:
    - the number of members per group will be reduced
    - geared or appropriate in a quarantine laboratory set-up
    - there is additional time for the laboratory sessions
    - there is a lecture on sequence analysis
    - there is further emphasis on rice viruses which are more important and relevant to other countries
    - the lecture notes are given from the start of the workshop
    - added time will be given to practical and field survey
    - focus only in some specific viruses then how to identify and characterize those viruses

5. General arrangements – logistics, field trip, etc.
    - It would be better to conduct the training during summer.
    - Accommodations should be arranged near the campus or training venue.
    - Comment on how the drivers drove the participants to destinations.
    - Long travel and limited time to collect samples.

6. Recommendations to future trainings re: logistical arrangements.
    - Hotel should be near the campus or training venue.
    - All participants to be accommodated in one place.
    - More practical work.
    - Slow or detailed explanations for participants who has little knowledge on the subject.
    - Better time management during field trip (short travel time and more time for sample collection).
    - Better internet connection.

Overall Rating

	5 - Excellent	4 - Good	3 - Satisfactory	2 - Unsatisfactory	1 – Poor
Accommodation	5.9%	70.6%	17.7%	0	5.9%
Workshop Venue/Training Facilities	21.1%	79.0%	0	0	0
Travel arrangements	31.6%	57.9%	10.5%	0	0
Field trip	26.3%	52.6%	15.8%	5.3%	0
Food/refreshments	5.3%	52.6%	31.6%	5.3%	0

7. Other comments

- Group discussions were informative.
- Learned from experiences of other plant quarantine officers.
- Include other detection methods like IC-PCR, qPCR, etc.
- Difficult to find transportation from the hotel.
- More activities on identifying virus symptoms in the field.
- Concerns on the “Halal” food provided
    1. Absence of certification (simple statement card placed near the food stating it is “halal” with evidence e.g. halal certification; from where the food were bought)
    2. Waiters’ knowledge on which food is halal and which is not

IV. Recommendations

To further strengthen the capability of participants in the field of advance plant virology, the technical team recommends the following training course program for future implementation;

1. Storage and transporting virus nucleic acid using FTA plant card and its method of DNA/RNA extraction and detection
2. Primer design (specific and degenerate primers)
3. DNA sequencing protocol and sequence analysis
4. Methods of detection of plant viruses from viruliferous insect-vectors
5. Development of monoclonal and polyclonal antibodies using conventional and *E. coli* techniques
6. Development of one-step kit for virus detection Ex. Rapid immunofilter paper assay (RIPA)

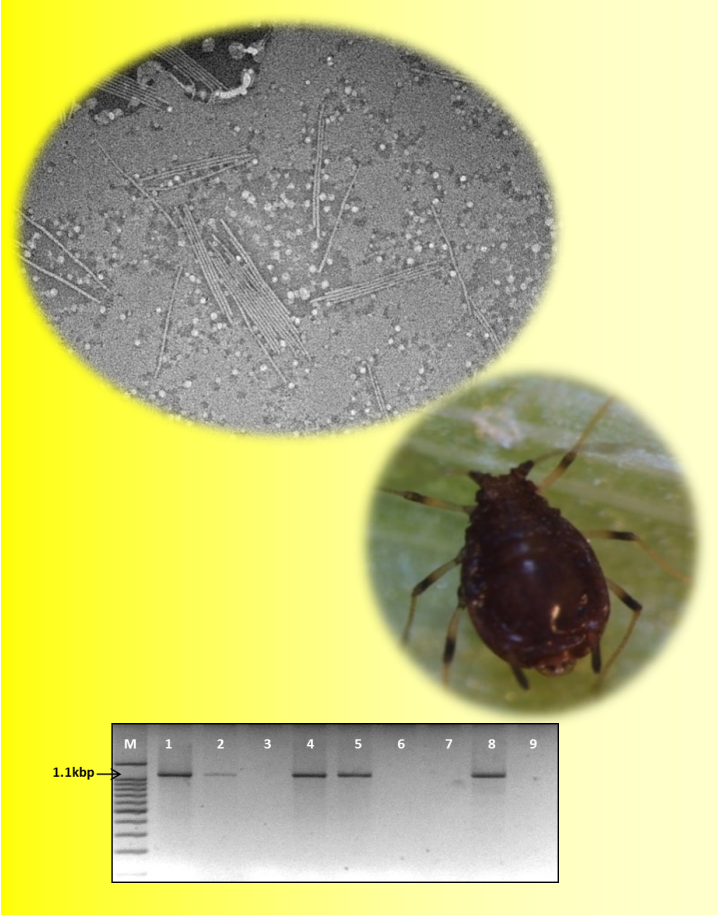
The following names of participants are highly recommended by the technical team for the advance training course program in plant virology. The selection is based on their written pre- and post- evaluation tests and actual performances during the 2-week training workshop and supported by their academic and technical background.

1. Ms. Preyapan Pongsapich (Thailand)
2. Ms. Norhayati binti Madiha (Malaysia)
3. Ms. Layla Syaznie binti Abdullah Lim (Brunei)
4. Mr. Tran Van Chien (Vietnam)
5. Ms. Sari Nurulita (Indonesia)
6. Mr. Darwin M. Landicho (Philippines)

The successful training workshop held at IPB, UPLB on August 28, 2015.

DIAGNOSTICS OF PLANT VIRUSES

TRAINING MANUAL



Training Workshop on Diagnostics of Plant Viruses

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DIAGNOSTICS OF PLANT VIRUSES

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SAMPLE COLLECTION

Materials:

- Disposable gloves
- Scissors
- Collection plastics
- Newspaper/Paper towels
- Marker
- Masking tape/Rubber bands
- Cotton
- Tissue paper
- 70% alcohol

Procedure:

1. Cut sample tissue from the plant with clean scissors.

   Note: Wipe clean scissors with 70% alcohol in every collection of sample.

2. Put in collection plastic with wet cotton. Tie a knot to close or use a rubber band. Another way is to wrap the sample in newspaper or paper towel then secure using a masking tape.

   Note: Properly label each sample. Don't forget to include the place and date of collection.

3. Fill-up sampling details in the collection form.

4. Extract the DNA or RNA immediately after collection or store samples in a refrigerator then process it the next day.

   Note: RNA can be easily degraded, that is why it is important to immediately process the samples. Also some plant tissues oxidized quickly like banana leaves. However, some plant samples can be stored in silica gel like tomato, squash and pepper leaves.

LIST OF PLANT SAMPLES

Sample No.	Crop	Place of Collection	Farmer's or Farm's Name	Variety	Age of Plant (days)	Farm Size	Symptoms	Estimated %Infection	Date of Collection
1	Squash	Tayug, Pangasinan	DA Paraiso	Suprema	120	500 sq.m.	Leaf curling, moscaic	30	Feb 10, 2015

INDIRECT ELISA

Materials:

- Blade or scissors
- Collection plastic (4x6in)
- Paper towels
- Wash Bottle
- Pipettes and Tips
- Disposable gloves

Equipment:

- ELISA Reader
- Weighing balance
- Incubator (Set at 37 °C)

Buffers and Solutions:

- PBS Buffer (pH7.4)
- Coating Buffer (pH9.6)
- Blocking Buffer
- Antibody Buffer
- Washing Buffer (PBS-T)
- Substrate Buffer (pH 9.8)
- Virus-specific antibody
- Goat anti-rabbit enzyme conjugate (GARAP)
- p-ntrophenylphosphatae (pNPP)
- 3M NaOH (Stop solution)

Procedure:

1. Grind leaf samples in coating buffer in a dilution of 1:10.
2. Load 200 µl plant sap per well of the ELISA plate.
3. Incubate overnight at 4 °C.
4. After incubation, remove the plant sap from the wells. Fill the wells with washing buffer; let it stand for 5 min then empty the plates. Repeat 3 times.
5. Add 300 µl blocking solution to each well and incubate at room temperature for 1 hr.
6. Repeat step 4 (Washing).
7. Add 100 µl virus-specific antibody with a dilution of 1:200 in antibody buffer to each well.
8. Incubate at 37 °C for 2-3 hrs.
9. Repeat washing as in step 4.
10. Add 100 µl goat anti-rabbit enzyme conjugate (GARAP) with a dilution of 1:1000 in antibody buffer.
11. Incubate at 37 °C for 2-3 hrs.
12. Repeat washing step.
13. Add 100 µl of p-nitrophenylphosphatase (pNPP) in substrate buffer to each well.
14. Incubate at room temperature for 30-60 min. Observe color reaction.
15. Stop reaction by adding 50 µl 3M NaOH.
16. Assess results by:
    - Visual observation
    - Absorbance Reading at 405 nm using an ELISA Reader.

DOT-BLOT ELISA

Materials:

- Blade or scissors
- Collection plastic (4x6in)
- Paper towels
- Wash Bottle
- Pipettes and Tips
- Disposable gloves

Equipment:

- Weighing balance

Buffers and Solutions:

- Nitrocellulose membrane (45 µm)
- TBS Buffer (pH8.0)
- Blocking Buffer
- Washing Buffer (TBS-Tween 20)
- Substrate Buffer (pH 8.0)
- Virus-specific antibody
- Goat anti-rabbit enzyme conjugate (GARAP)
- Nitro Blue Tetrazolium (NBT)
- 5-bromo-4cholro-3-indolyl phosphate (BCIP)
- 1.5% Sodium hypochlorite (Stop solution)

Procedure:

1. Gently draw a grid pattern on the nitrocellulose membrane (NCM) with a pencil and ruler. Dip membrane onto PBS buffer then air-dry before using.
2. Grind samples at a ratio of 1:20 (w/v) in TBS buffer.
3. Drop 2-3 µl plant sap onto the membrane. Incubate overnight at room temperature.
4. Evenly cover the membrane with blocking buffer in a plastic container with cover then incubate at room temperature for 1 hr.
5. Remove blocking buffer. Dry container with clean paper towel.
6. Add 1:200 (v/v) of virus specific antibody in TBS buffer.
7. Incubate at room temperature for 1-2 hr.
8. After incubation, remove antibody and buffer then rinse 3 times with TBS-T with 10 min interval.
9. Add 1:1000 (v/v) goat anti-rabbit enzyme conjugate (GARAP) in blocking buffer.
10. Incubate at room temperature for 1-2 hr.
11. Remove conjugate buffer. Incubate in TBS buffer at room temperature for 30 min.
12. Remove buffer. Soak membrane in 5 ml substrate buffer with 33 µl NBT and 16.5 µl BCIP.
13. Observe color reaction. After color reaction (purple or blue) developed in the positive check, pour off the substrate buffer.
14. Add 1.5 % sodium hypochlorite and incubate at room temperature for 5 min to eliminate color background and stop the reaction.

COMPOUND ELISA
(BBTV Detection)

Materials:

- Blade or scissors
- Collection plastic (4x6in)
- Paper towels
- Wash Bottle
- Pipettes and Tips
- Disposable gloves

Buffers and Solutions:

- General Extract Buffer (GEB 1X)
- Carbonate Coating Buffer (1X)
- PBST Buffer (Wash Buffer) (1X)
- ECI Buffer (1X)
- PNP Buffer (1X)

Equipment:

- ELISA Reader
- Weighing balance

Procedure:

1. Prepare capture antibody.

   Note: All antibodies and enzyme conjugates should be prepared in a container made of a material such as polyethylene or glass that does not readily bind antibodies. Do not use polysterene, polypropylene or polycarbonate

   Prepare the volume of carbonate coating buffer needed for the test. *Example: If the dilution given on the bottle of concentrated capture antibody is 1:200, and you are preparing 10 ml of the capture antibody solution, you should mix 10 ml of carbonate coating buffer with 50 µl of concentrated capture antibody. Mix the prepared antibody solution thoroughly and use immediately.*

2. Coat plate. Pipette 100 µl of the prepared capture antibody solution into each well.

3. Incubate plate. Cover the plate with cling plastic wrap and incubate in a humid box for 4 hrs at room temperature or overnight in the refrigerator (4°C). *Do not store coated plates longer than 24 hrs.*

4. Wash plate. Empty the wells into a sink or container. Fill the test wells completely with 1X PBST, and then quickly empty them again. Repeat 2 more times.

   Hold the plate upside down and tap firmly on folded paper towel to remove excess liquid or if you have an automated plate washer, calibrate the washer and wash the plate 3x.

5. Grind and dilute the samples. Select samples showing symptoms. Young leaf tissue is recommended. Seed, stem and other tissue can also be tested depending on the crop.

   Grind plant tissue in General Extract Buffer (GEB) at 1:10 ratio (*tissue weight in g: buffer volume in ml*). You can use mortar and pestle or any grinding devices. Be sure to wash and rinse the grinding device thoroughly between samples.

6. Dispense the sample. Following the loading diagram (see sample diagram), dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into positive control wells, and dispense 100 µl of sample extraction buffer into buffer wells.

7. Incubate plate. Set the plate, cover it with cling wrap and incubate in humid box for 2 hrs at room temperature or overnight in the refrigerator (4°C).

8. Prepare enzyme conjugate.

   Note: always prepare enzyme conjugate within 10 min before use. Bottles of alkaline phosphatase enzyme conjugate and detection antibody are supplied as a concentrate and must be diluted with ECI buffer before use. Please follow the recommended dilution stated in the product.

   Example: If the dilution given on the bottles of concentrated detection antibody (A) and alkaline phosphatase enzyme (B) conjugate is 1:200, and you are preparing 10 ml of enzyme conjugate solution, you should first dispense 10 ml of ECI buffer. Then, add 50 µl of A and 50 µl of B to the ECI buffer.

   After adding the reagents from A and B, mix thoroughly the enzyme conjugate solution well.

9. Wash plate. When sample incubate is complete, wash the plate. Use the quick flipping motion to dump the wells into a sink without mixing the contents. Fill all the test wells completely with 1X PBST, and then quickly empty them. Repeat for 2 more times. After washing tap the plate firmly on folded paper towel to remove all droplets of wash buffer.

   Note: It is important to inspect the test wells. It should be free from any plant tissue and dirt. If plant tissue is present repeat the wash step and tap firmly. Avoid touching the bottom of the plate.

10. Add enzyme conjugate. Dispense 100 µl of prepared enzyme conjugate solution per well.

11. Incubate the plate. Cover the plate with cling wrap and incubate in humid box for 2 hrs at room temperature.

12. Prepare PNP solution. Each PNP tablet will make 5 ml of PNP solution, at a concentration of 1 mg/ml about enough for five 8-well strips. About 15 min before the end of incubation step, measure 5 ml of room temperature 1X PNP buffer for each tablet you will be using. Then without touching the tablets, add the PNP tablets to the buffer.

    Note: Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.

13. Wash plate. Follow the same wash procedure previously described.

14. Add PNP substrate. Dispense 100 µl of PNP substrate into each testwell.

15. Incubate plate. Cover the plate with cling wrap and incubate in humid box for 60 min at room temperature. Plates should be protected from direct or intense light.

16. Evaluate results.

    Qualitative interpretation. Examine each well by eye, or measure on a plate reader at 405 nm. Air bubbles should be removed; it can alter results at the time of reading.

    Wells in which color develops indicate positive results. Wells in which there is no significant color development indicate negative result. Test results are valid only if positive control wells give a positive result and buffer wells remain colorless.

    Results may be interpreted after more than 60 min of incubation as long as negative wells remain virtually clear.

    Quantitative interpretation. Appropriate controls should be included for reference, since ELISA values may differ in different microtiter plates due to possible plate-to-plate variation in sensitivity. Overlapping range of specific and non-specific reaction values causes difficulty in interpretation. In this case, it is necessary to include large number of known healthy control samples and determine statistically a threshold level for infection. *To establish thresholds, several authors have used the mean value for healthy controls plus three times their standard deviation ($\bar{x}$ + 3SD).*

    Alternatively, values more than twice those healthy controls have been considered infected.

TOTAL PLANT DNA EXTRACTION

(Dellaporta Miniprep)

Materials:

- Blade or scissors
- Mortar and pestle (sterilized)
- 1.5 mL Eppendorf tubes
- Pipettes and Tips
- Face mask
- Disposable gloves
- Paper towels

Equipment:

- Fume hood
- Water bath or Dri-bath incubator
- Centrifuge
- Vortex
- Weighing balance

Buffers and Solutions:

- Nitrocellulose membrane (45 µm)
- Dellaporta extraction buffer
- 20% Sodium Dodecyl Sulfate (SDS)
- 5M Potassium acetate (KAC)
- Isopropanol
- 80% ethanol
- Sterile distilled water or TE buffer

Procedure:

1. Collect 2 leaf discs about 0.9cm in diameter.
2. Homogenize in 500 µl Dellaporta extraction buffer using a mortar and pestle.
3. Transfer in a 1.5 ml microfuge tube then add 33 µl 20% SDS. Gently invert tube to mix the solution.
4. Incubate at 65°C for 10min.
5. Add 160 µl 5M KAC and mix by gently inverting the tube.
6. Centrifuge at 13,000 rpm for 10 min.
7. Transfer supernatant into a new 1.5 ml microfuge tube, avoiding the plant tissue debris.
8. Repeat centrifugation. Transfer 500µl supernatant into a new tube.
9. Add 0.5 volume (250 µl) isopropanol and invert tube gently.
10. Centrifuge at 13,000 rpm for 10min.
11. Carefully discard supernatant
    Note: Make sure that the pellet does not become aspirated. It may be necessary to leave some (about 15 µl) supernatant behind. The following step will delete any problems it may cause.
12. Add 500 µl 80% ethanol and centrifuge at 13,000 rpm for 5min.
13. Carefully discard as much supernatant as possible.
14. Air-dry for 1hr or speed-vacuum for 5min.
15. Resuspend pellet in 500 µl sterile distilled water or DEPC-treated water.

CTAB DNA EXTRACTION

(For Banana Samples)

Materials:

- Blade or scissors
- Mortar and pestle (sterilized)
- 1.5 mL Eppendorf tubes
- Pipettes and Tips
- Face mask
- Disposable gloves
- Paper towels

Buffers and Solutions:

- CTAB extraction buffer
- 2-mercaptoethanol
- Phenol:Chloroform:Isoamyl (PCI) 25:24:1
- 95% ethanol
- 70% ethanol
- Sterile distilled water or TE buffer

Equipment:

- Fume hood
- Water bath or Dri-bath incubator
- Centrifuge
- Vortex
- Weighing balance

Procedure:

1. Clean working station and pipettors with 70% alcohol.
2. Cut 0.5 g leaf sample using sterile blade or clean scissors.
3. Homogenize leaf sample using a sterile mortar and pestle with 4 mL CTAB extraction buffer and 8 µl 2-mercaptoethanol.
4. Transfer 500 µL leaf extract in a sterile 1.5 mL sterile microfuge tube.
5. Incubate in a water bath at 60 °C for 1 hr. Gently swirl tubes every 15 min.
6. After incubation, add 400 µL Phenol:Chloroform:isoamyl (PCI 25:24:1). Mix by vortexing for 15 sec.
7. Centrifuge at 10,000 rpm for 5 min.
8. Collect 500 µL of the upper phase using a wide-bore tip and transfer it to a new 1.5 mL microfuge tube.
9. Precipitate the DNA by adding 2 volumes of ice-cold 95% ethanol. Mix well by gently inverting the tube. Incubate in the freezer for 1 hr or overnight.
10. Centrifuge at 10,000 rpm for 15 min.
11. Discard the supernatant. Wash the pellet with 500 µL ice-cold 70% ethanol and gently invert the tube for 3-5 min.
12. Short spin then discard the ethanol. Air-dry the pellet for 2-3 min at room temperature in the laminar flow or until the wall of the tube is already dry.
13. Resuspend the pellet in 100 uL TE buffer or DNAse-free water.
14. Store at -20°C.

RNA EXTRACTION

Materials:

- Blade or scissors
- Mortar and pestle (sterilized)
- 1.5 mL Eppendorf tubes
- Pipettes and Tips
- Face mask
- Disposable gloves
- Paper towels

Buffers and Solutions:

- Trizol® Reagent (Invitrogen)
- Chloroform
- Isopropanol
- 75% ethanol
- RNAse-free water or TE buffer
- RNAse Away
- 70% alcohol

Equipment:

- Fume hood
- Water bath or Dri-bath incubator
- Refrigerated centrifuge
- Vortex

Procedure:

1. Clean working station and pipettors with 70% alcohol or RNAse Away.
2. Homogenize about 100 mg leaf sample in 1 ml Trizol® Reagent using an ice-cold mortar and pestle.
3. Incubate at room temperature for 5 min.
4. Centrifuge at 12,000xg for 10 min at 8°C.
5. Transfer the supernatant using a wide-bore tip into a new 1.5 ml microfuge tube then add 200 µl chloroform.
6. Shake vigorously by hand for 15 sec.
7. Incubate at room temperature for 3 min then centrifuge at 12,000xg for 10 min at 8°C.
8. Collect the aqueous phase into a new 1.5 ml microfuge tube then add 500 µl ice-cold isopropanol.
9. Incubate at -20 °C for 2 hrs or overnight if precipitate fails to form.
10. Discard supernatant and wash pellet with ice-cold 75 % ethanol.
11. Centrifuge at 7,500xg for 5 min at 8°C.
12. Discard ethanol then air-dry pellet for 10 min in a laminar flowhood.
13. Resuspend pellet in 50 µl RNAse-free water or TE buffer.
14. Incubate at 55 °C for 10 min before storing at -20 °C or in a biofreezer (-80 °C).

GEL ELECTROPHORESIS

Materials:

- Casting tray
- Gel comb
- Pipettes and Tips
- Disposable gloves

Buffers and Solutions

- 0.5X TBE
- Agarose
- GelRed
- 1 KB plus DNA Ladder

Equipment:

- Gel electrophoresis system
- Weighing balance
- Microwave oven

Procedure:

1. Prepare 1.2% agarose gel in 0.5X TBE buffer.
2. Completely dissolve the agarose in the buffer using a microwave.
3. Let the solution to slightly cool down (~5 min).
4. Pour the solution slowly into the casting tray with the gel comb in place. Avoid forming any bubbles.
5. Let the agarose gel to solidify (~30 min) then carefully remove the gel comb.
6. Place the solidified agarose gel into the electrophoresis unit. Fill in with 0.5X TBE buffer until the gel is fully submerged.
7. Pipette several 2 µl loading dye (depending on the number of samples) in a piece of parafilm.
8. Mix 5 µl PCR product into the dye by carefully pipetting the solution in and out of the tip. Avoid forming of bubbles.
9. Load the sample mixture into each well of the gel. Avoid spilling over the sides of the wells to prevent contamination.
   Note: Start loading samples on the second well.
10. Lastly, load 2 µl DNA ladder on the first well.
11. Run samples for 30-45 min or until the dye has migrated about 75-80% of the gel.
12. After the run, stain the gel by submerging it in the GelRed solution for about 15-30 min.
13. Visualize the amplified DNA bands using a gel documentation system.

GEL DOCUMENTATON AND ANALYSIS OF THE RESULTS

Materials:

- Disposable gloves
- Kimwipes
- Paper towel or tissue

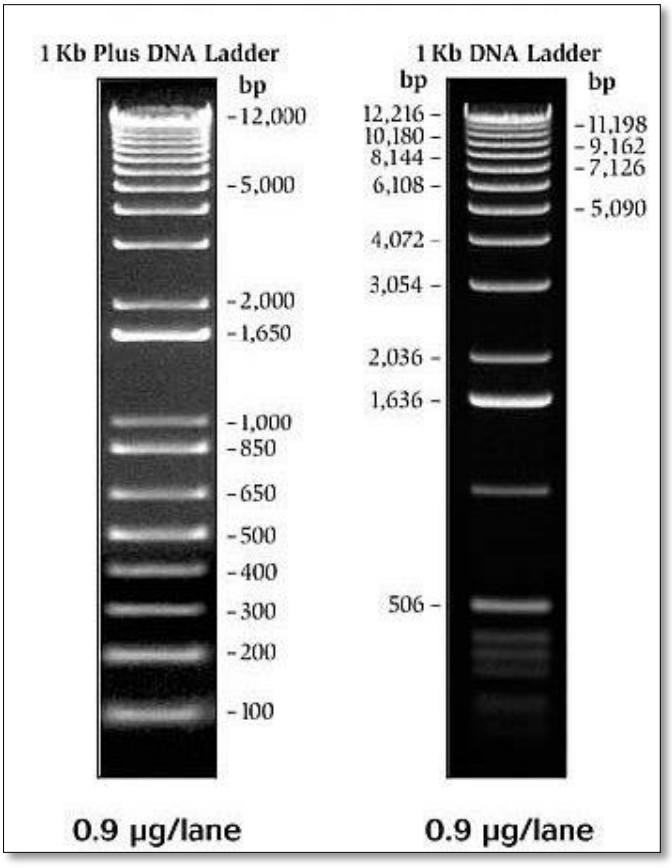
Equipment:

- GelDoc
- Computer

Procedure:

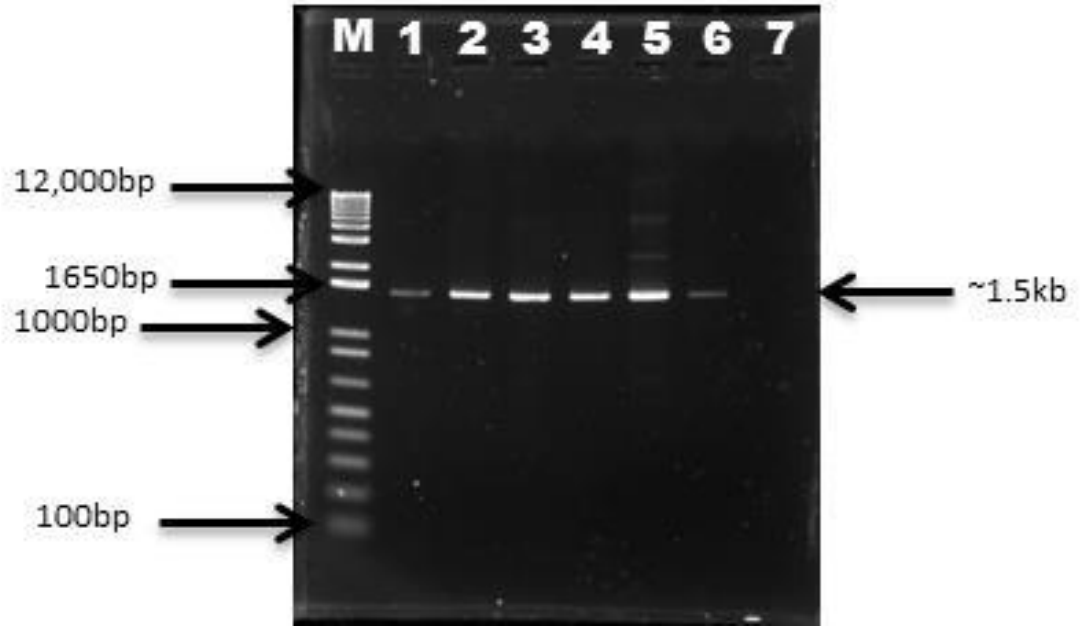
1. After staining the gel, the PCR result can be viewed using a gel documentation system or GelDoc.

2. Analyze the result using the DNA ladder.



Example of DNA ladders (Invitrogen)

3. If the PCR run is successful, the PCR product is within the expected DNA sequence size.



Agarose gel showing the expected band size of around 1.5kb after PCR amplification.

4. The presence of the expected band, confirms the presence of the virus.

   Note: It is important that in every gel electrophoresis for PCR analysis, ***DO NOT FORGET TO INCLUDE THE DNA LADDER.***

POLYMERASE CHAIN REACTION (PCR)

Materials:

- 0.2ml PCR tubes
- 1.5 ml microfuge tubes
- 70% ethanol
- Pipettes and Tips
- Disposable gloves
- Paper towel or tissue

Equipment:

- PCR machine
- Centrifuge

Buffers and Solutions

- 10X PCR buffer
- 50 mM $MgCl_2$
- 10 mM dNTPs
- 10 µM forward and reverse primers
- RNAse-free water
- Taq Polymerase
- Template DNA

Procedure:

1. Before starting:
    a. Calculate the amount of the PCR reagents needed according to the number of samples to be tested. Give allowance for pipetting error.
    b. Clean working bench, pipettors and microfuge rack with 70% ethanol.
    c. Thaw reagents (PCR buffer, $MgCl_2$, dNTPs, forward and reverse primers, RNAse-free water and DNA samples) on ice.
    d. Flick tubes to mix its content then short spin.
    e. Prepare and label 0.2ml PCR tubes.
2. Prepare a cocktail mix. Add all the PCR reagents in appropriate amounts in a 1.5 ml microfuge tube.
3. Lastly add the Taq polymerase to the cocktail.
4. Gently flick the tube to mix the reagents then short spin the tube using a centrifuge.
5. Dispense the PCR mix on each labeled 0.2 ml PCR tube. Add the DNA template then gently mix the contents.
6. Place tubes in the thermal cycler then begin run with the appropriate PCR program.

PCR Troubleshooting

CAUSE	SOLUTION
Problem: Faint bands or no PCR product	
a. Too little DNA template in the reaction	Increase the amount of the DNA template.
b. Damaged or degraded DNA template	Assure the purity and integrity of the DNA template. Be careful in handling DNA. Prevent freeze-thawing of the DNA samples by preparing aliquots.
c. Insufficient *Taq* Polymerase	Increase the DNA polymerase concentration in increments of 0.5 units per 100 µL of reaction.
d. Insufficient number of cycles	Increase cycle number by 5 to 10 cycles.
e. Presence of PCR inhibitors	Re-purify DNA samples.
f. To low $MgCl_2$ concentration	Increase magnesium concentration in increments of 0.1mM.
g. Too long or too short denaturation time.	Adjust denaturation time in increments of 5sec.
h. Too high annealing temperature	Lower annealing temperature in increments of 2°C. Compute the melting temperature (T_m) of the primers. The annealing temperature should be 5°C less to the primer T_m.
i. The primer extension period is too short.	Increase extension time in increments of 1 minute.
Multiple bands or smearing	
a. Too much DNA template	Decrease the amount of template DNA
b. Too low annealing temperature	Increase annealing temperature in increments of 2°C.
c. Too high concentration of *Taq* Pol	Decrease enzyme concentration in increments of 0.5 units per 100-µL reaction.
d. Magnesium concentration is too high.	Decrease the magnesium concentration in increments of 0.1mM.
e. Denaturation time is too short or too low	Increase the denaturation time in increments of 5sec. and temperature by 1°C.
f. Too many cycles	Reduce the cycle number by 5 to 10 cycle.
g. Extension time is too long	Reduce the extension time in increments of 1min.
h. Review primer design and composition.	Design new primers.

PCR Cocktail & Thermal Profile Using Degenerate Primers for *Begomoviruses*

1. PCR Cocktail

PCR Cocktail Preparation			
Components	**Stock Concentration**	**Final Concentration**	**1x**
DEPC-treated Water			17.65
PCR Buffer	10x	1x	2.5
$MgCl_2$	50mM	2.5 mM	1.25
dNTPS	10mM	0.2 mM	0.5
Primer F	10µM	0.5 µM	0.5
Primer R	10µM	0.5 µM	0.5
Taq	5U/µl	0.06	0.1
DNA			2
Total Reaction Volume			25 µl
Aliquot 23µl to each DNA tube			

2. PCR Profile

Steps	Temperature (°C)	Time	Number of Cycles
Denaturation	94	1 min	
Annealing	57	2 min	30x
Elongation	72	2 min	
Final Extension	72	10 min	1
Hold	16	∞	-

3. Primer Sequence (amplifies the fragment of DNA-A including the 5' end of CI, IR, V2 and the 5' end of the coat protein (CP), Tsai etal., 2011)

Primer Name	Sequence	Amplicon Size (bp)
PAL1v1978B	GCATCTGCAGGCCCACATBGTYTTHCCNGT	~1.5 Kb
PAR1c715H	GATTTCTGCAGTTDATRTTHTCRTCCATCCA	

PCR Cocktail & Thermal Profile for *Banana bunchy top virus* (BBTV)

1. PCR Cocktail

PCR Cocktail Preparation			
Components	**Stock Concentration**	**Final Concentration**	**1x**
DEPC-treated Water			17.65
PCR Buffer	10x	1x	2.5
$MgCl_2$	50mM	2.5 mM	1.25
dNTPs	10mM	0.2 mM	0.5
Primer F	10µM	0.5 µM	0.5
Primer R	10µM	0.5 µM	0.5
Taq	5U/µl	0.06	0.1
DNA			2
Total Reaction Volume			25 µl
Aliquot 23µl to each DNA tube			

2. PCR Profile

Steps	Temperature (°C)	Time	Number of Cycles
Initial Denaturation	94	4min	1
Denaturation	94	1 min	29x
Annealing	61	1 min	
Elongation	72	2 min	
Final Extension	72	10 min	1
Hold	16	∞	-

3. Primer Sequence (amplifies the BBTV-R genome)

Primer Name	Sequence	Amplicon Size (bp)
D11	GGAAGAAGCCTCTCATCTGCTTCAGACARC	~1.1 Kb
D12	TTCCCAGGCGCACACCTTGAGAAACGAAAG	

RT-PCR/PCR Cocktail & Thermal Profile Using Degenerate Primers for *Potyviruses*

1. RT-PCR Cocktail

PCR Cocktail Preparation			
Components	**Stock Concentration**	**Final Concentration**	**1X**
DEPC-treated Water			2.2
Reaction Mix	2X	1X	5
Primer F	10µM	0.5 µM	0.2
Primer R	10µM	0.5 µM	0.2
SuperScript III RT			0.4
RNA			2
Total Reaction Volume			10 µl
Aliquot 8 µl to each DNA tube			

2. PCR Profile

Steps	Temperature (°C)	Time	Number of Cycles
cDNA Synthesis	55	30 min	1
Initial Denaturation	94	2 min	1
Denaturation	94	15 sec	
Annealing	55	30 sec	40x
Elongation	68	1 min	
Final Extension	68	5 min	1
Hold	16	∞	-

3. Primer Sequence (amplifies the 3' terminal portion of the genomes of various *potyviruses*, Gibbs and Mackenzie, 1997)

Primer Name	Sequence	Amplicon Size (bp)
Potyvirid 1	CACGGATCCCGGG(T)17VGC	~1.6 Kb
Potyvirid 2	ACCACAGGATCCGGBAAYAAYAGYGGDCARCC	

RT-PCR/PCR Cocktail and Thermal Profile for *Papaya ringspot virus* (PRSV)

1. RT-PCR Cocktail

PCR Cocktail Preparation			
Components	**Stock Concentration**	**Final Concentration**	**1X**
DEPC-treated Water			2.2
Reaction Mix	2X	1X	5
Primer F	10µM	0.5 µM	0.2
Primer R	10µM	0.5 µM	0.2
SuperScript III RT			0.4
RNA			2
Total Reaction Volume			10 µl
Aliquot 8 µl to each DNA tube			

2. PCR Profile

Steps	Temperature (°C)	Time	Number of Cycles
cDNA Synthesis	55	30 min	1
Initial Denaturation	94	2 min	1
Denaturation	94	15 sec	
Annealing	58	30 sec	40x
Elongation	68	1 min	
Final Extension	68	5 min	1
Hold	16	∞	-

3. Primer Sequence (amplifies the PRSV-CP gene, Bateson etal., 1994)

Primer Name	Sequence	Amplicon Size (bp)
MB11	GGATCCATGTCCAAAAATGAAGCTGTGGATGCT	~900 bp
MB12	TCAATTGGCGCATACCCAGGAGAGT	

MECHANICAL INOCULATION

Materials:

- Mortar and pestle
- Wash bottle
- Carborundum (500mesh)/Celite
- Disposable gloves
- Labels and water resistant marker
- Detergent soap

Buffers and Solutions

- 0.01M Phosphate Buffer pH 7.2
- Sodium sulphite (2%)

Procedure:

1. Arrange plants to be inoculated.
2. Homogenize infected leaf sample in 0.01M Phosphate buffer at 1:10 dilution using a mortar and pestle. Add 2% Sodium sulphite.
3. Add a small amount of celite (0.5-1% w/v) onto the inoculum or spread small amount of carborundum into the leaf to be inoculated.
4. Moisten two fingers with the inoculum then gently rub onto the first and second fully expanded leaves while supporting it with the other hand.
5. Rinse inoculated plants with tap water within 2-5 min.
6. Observe test plants for symptoms at least twice a week for a month.
7. Label with date of inoculation and name of virus inoculation.

Note: To prevent cross contamination, change or wash thoroughly gloves between samples. Also separate test plants of different samples.

Optimum stage of most common test plant species.

Test plant species	Number of leaves	Remarks
Abelmoschus esculentus	1-2	
Capsicum annuum	2-3	
Chenopodium amaranticolor	3-4	for local symptoms only
Chenopodium quinoa	3-4	for local symptoms only
Cucumis sativus	2 cotyledons	remove leaves, except top leaf
Cucurbita maxima	2 cotyledons	
Datura metel	2-3	
Gomphrena globosa	about 6	
Gossypium herbaceum	1-2	
Nicotiana benthamiana	3-4	
Nicotaina glutinosa	3-4	
Nicotiana tabacum 'Xanthii'	1-2	
Solanum lycopersicum 'Money-maker'	1-2	
Solanum melongena	1-2	
Vigna unguiculata	2 cotyledon	

Source: www.q-bank.eu

INSECT TRANSMISSION OF VIRUS

I. Non - Persistent Mode of Transmission

Materials:

- Insect vector, *Aphis gossypii*
- Test plants
- Symptomatic plants/Inoculum
- Camel hair brush
- Close container with screen window

Procedure:

1. Starve the non-viruliferous insect vectors for 30 minutes before virus acquisition.

2. Place the starved insect vectors to symptomatic host plant for 15-20 minutes to acquire virus.

3. Transfer the viruliferous insect vectors (15-20 aphids) to the uninfected/clean test plant and allow the insects to feed and transmit the virus for 1-2 hours.

4. Manually remove or eliminate the insects by spraying insecticide.

5. Transfer the test plants to insect-free/insect-proof cages.

6. After 1-2 months, check the test plants for virus infection symptom/s and collect samples for ELISA and/or PCR tests.

INSECT TRANSMISSION OF VIRUS

IIa. Persistent Mode of Transmission (*Banana bunchy top virus*)

Materials:

- Insect vector, *Pentalonia nigronervosa*
- Test plant, 1-mo old banana seedling
- Symptomatic plants/Inoculum
- Camel hair brush
- Close container with screen window

Procedure:

1. Starve the non-viruliferous insect vectors for 30 minutes before virus acquisition.

2. Place the starved insect vectors to symptomatic host plant for 24 hours to acquire virus.

3. Transfer the viruliferous insect vectors (15-20 aphids) to the uninfected/clean test plant and allow the insects to feed and transmit the virus for 24 hours.

4. Manually remove or eliminate the insects by spraying insecticide.

5. Transfer the test plants to insect-free/insect-proof cages.

6. After 1-2 months, check the test plants for virus infection symptom/s and collect samples for ELISA and/or PCR tests.

Note: In the case of Banana bunchy top virus, symptom expression may take (up to 3 months) depending on the susceptibility of the host plant.

INSECT TRANSMISSION OF VIRUS

IIb. Persistent Circulative Mode of Transmission (*Tomato leaf curl virus*)

Materials:

- Aviruliferous whiteflies, *Bemisia tabaci*
- ToLCV infected tomato (source of inoculum)
- Tomato seedlings (healthy)
- Aspirator
- Screen cages

Procedure:

1. Collect/aspirate whiteflies and allow having access on ToLCV infected tomato plant for 24-48 hours.

2. Remove whiteflies from the source and transfer 10-20 whiteflies to healthy tomato seedling inside insect proof screen cages.

3. Allow the viruliferous whiteflies to feed on test plants for 48 hours then remove or spray insects with insecticide.

4. Observe the inoculated plants for virus symptoms (leaf curl) after 3-4 weeks until 2 months and collect samples for PCR.

BUFFERS AND SOLUTIONS

Indirect ELISA

PBS Buffer (pH7.4)

Sodium Chloride (NaCl)	8 g
Monobasic Potassium Phosphate (KH_2PO_4)	0.2 g
di-Sodium hydrogen Orthophosphate ($Na_2HPO_4x12H_2O$)	1.44 g
Potassium Chloride (KCl)	0.2 g
Distilled water	1000 ml

Coating Buffer (pH9.6)

Sodium Carbonate (Na_2CO_3)	1.5 g
Sodium Hydrogen Carbonate ($NaHCO_3$)	2.93 g
Distilled water	1000 ml

Washing Buffer (PBS-T)

PBS Buffer	1000 ml
Tween 20	0.05%

Blocking Buffer (must be freshly prepared)

PBS Buffer	500 ml
BSA or skim milk	1%

Antibody Buffer (must be freshly prepared)

PBS Buffer	100 ml
Egg albumin	0.2%

Substrate Buffer (pH 9.8)

Diethanolamine	97 ml
Distilled water	800 ml

Adjust pH to 9.8 then volume to 1000 ml.

Dot Blot ELISA

TBS Buffer (pH8.0)

Tris	6.057 g
Distilled water	800 ml
Adjust pH to 8.0.	
NaCl	8.766 g
Volume to 1000 ml.	

Washing Buffer (TBS-Tween 20)

TBS Buffer	1000 ml
Tween 20	0.5 ml

Blocking Buffer

Skim Milk	3 g
Glycine	2 g
TBS-Tween 20	100 ml

Substrate Buffer (pH 8.0)

Tris	1.214 g
Dissolve in 80 ml distilled water then adjust pH to 8.0.	
NaCl	0.5844 g
$MgCl_2x6H_2O$	0.102 g
Volume up to 100 ml.	

Nitro Blue Tetrazolium (NBT) Solution

Nitro Blue tetrazolium chloride	0.5 g
Dimethylformamide (70%)	10 ml
Store at 4°C.	

5-bromo-4-chloro-3-indolyl phosphate (BCIP)

Disodium salt BCIP	0.5 g
Dimethylformamide (100%)	10 ml

Compound ELISA

General Extract Buffer (GEB 1X)

Dissolve in 1000 ml of 1X PBST:

Sodium sulfite (anhydrous)	1.3g
Polyvinylpyrrolidone (PVP) MW 24-40,000	20g
Sodium azide	0.2g
Powdered egg (chicken) albumin, Grade II	2g
Tween-20	20g

Adjust pH to 7.4. Store at 4°C

Carbonate Coating Buffer (1X)

Dissolve in distilled water to 1000 ml

Sodium carbonate (anhydrous)	1.59g
Sodium bicarbonate	2.93g
Sodium azide	0.2g

Adjust pH to 9.6. Store at 4°C

PBST Buffer (Wash Buffer) (1X)

Dissolve in distilled water to 1000 ml:

Sodium chloride	8g
Sodium phosphate, dibasic (anhydrous)	1.15g
Potassium phosphate, monobasic (anhydrous)	0.2g
Potassium chloride	0.2g
Tween-20	0.5g

Adjust pH to 7.4

ECI Buffer (1X)

Add to 1000 ml 1X PBST:

Bovine serum albumin (BSA)	2g
Polyvinylpyrrolidone (PVP) MW 24-40,000	20g
Sodium azide	0.2g

Adjust pH to 7.4 Store at 4°C

PNP Buffer (1X)

Dissolve in 800 ml distilled water:

Magnesium chloride hexahydrate	0.1g
Sodium azide	0.2g
Diethanolamine	97g

Adjust pH to 9.8 with hydrochloric acid.

Adjust final volume to 1000 ml with distilled water. Store at 4°C

Total DNA Extraction

STOCK SOLUTIONS

1 M Tris-Base

Tris-Base	60.55 g
Distilled water	500 ml

0.5 M EDTA

EDTA	18.61 g
Distilled water	100 ml

5 M NaCl

Sodium chloride (NaCl)	29.22 g
Distilled water	100 ml

Dellaporta Extraction Buffer

Stock Concentration	Final Concentration	100 ml	500 ml
1M Tris-Base pH 8.0	100 mM	10 ml	50 ml
0.5M EDTA	8.5 mM	1.7 ml	8.5 ml
5 M NaCl	500 mM	10 ml	50 ml
Distilled Water	-	78.3 ml	391.5 ml
Sterilize			
2-mercaptoethanol	10 mM	78 µl	391 µl

5M Potassium Acetate (KAC)

KAC	49.07 g
Distilled water	100 ml

Sterilize

20 % SDS

Sodium dodecyl sulphate (SDS)	10 g
Distilled water	100 ml

Sterilize

CTAB Extraction Buffer

Stock Concentration	Final Concentration	100 ml	500 ml
1M Tris-HCl pH 8.0	0.1 M	10 ml	50 ml
0.5M EDTA	0.02 M	4 ml	20 ml
5 M NaCl	1.4 M	28 ml	140 ml
CTAB	2% (w/v)	2 g	10 g
Distilled Water	-	58 ml	290 ml

Sterilize

TE Buffer

Stock Concentration	Final Concentration	100 ml	1 L
1M Tris-HCl pH 7.5	10 mM	1 ml	10 ml
0.5M EDTA pH 8.0	1 mM	200 µl	2 ml
Distilled Water	-	98.8 ml	988 ml

Sterilize.

Gel Electrophoresis

10x TBE Buffer

Tris Base	54.5 g
Boric Acid	27.2 g
0.5 M EDTA	20 ml
Distilled water	480 ml

Note: TAE can also be used.

0.5x TBE Buffer

10x TBE buffer	50 ml
Distilled water	950 ml

10X Loading Dye

Sucrose	6.5 g
1M Tris-HCl pH 7.5	100 µl
0.5M EDTA	200 µl
Bromophenol Blue	0.03 g
Distilled water	9.7 ml

6X Loading Dye

10X Loading dye	600 µl
Distilled water	400 µl

1Kb plus DNA Ladder (0.1 µg/ml)

DNA Marker (1 µg/ml)	50 µl
6X Loading Dye	85 µl
Sterile distilled water	365 µl

1.2% Gel

Agarose	0.48 g
0.5X TBE buffer	40 ml

Melt agarose using a microwave.

GelRed Solution

5M NaCl*	2 ml
GelRed	30 µl
Distilled water	98 ml

*optional

**or add 4 µl GelRed directly to the melted agarose gel (40 ml)

Note: Avoid exposing the solution in light as GelRed is light sensitive.

PCR

10mM dNTPs

100mM ATP	10 µl
100mM TTP	10 µl
100 mM GTP	10 µl
100 mM CTP	10 µl
RNAse-free water	60 µl

Reconstitution of primers

1. Centrifuge tubes for a few seconds then add the appropriate volume of TE buffer.
2. Rehydrate for 2 min then vortex for 15 sec.
3. Short spin to collect contents in the bottom. Store at -20 °C.

Mechanical Inoculation

Phosphate Buffer

Solution A KH_2PO_4	1.36 g
Distilled water	1000 ml
Solution B $Na_2HPO_4 \times 2H_2O$	1.78 g
Distilled water	1000 ml

0.01M Phosphate Buffer, pH 7.2

Solution A	51 ml
Solution B	49 ml

Phospahte Buffer (10X)

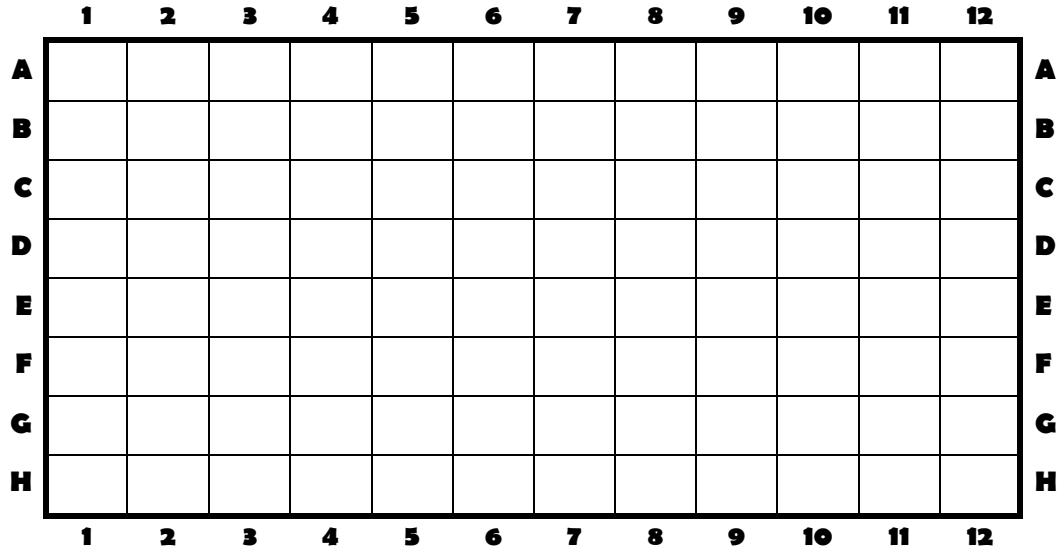
KH_2PO_4	2.72 g
$Na_2HPO_4 \times 2H_2O$	14.2 g
Distilled water	800 ml
Adjust pH to 7.2 then volume to 1000 ml	

0.01M Phosphate Buffer, pH 7.2

Phosphate buffer (10X)	100 ml
Distilled water	900 ml

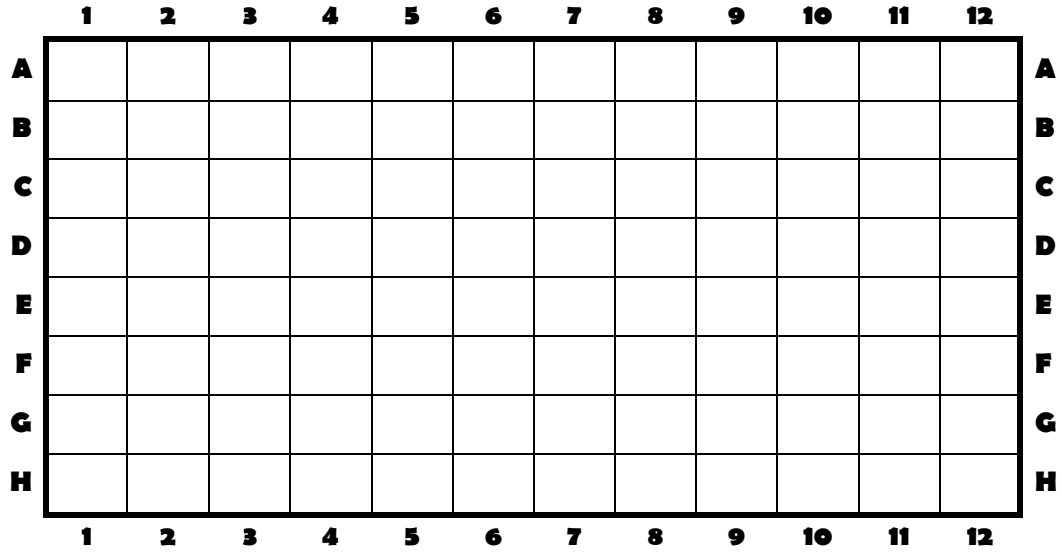
ELISA Form

Type of ELISA: _____ Date: _____
Antibody: _____ Plate Number: _____
Crop: _____ Performed by: _____



REMARKS: _____

Type of ELISA: _____ Date: _____
Antibody: _____ Plate Number: _____
Crop: _____ Performed by: _____



REMARKS: _____

PCR Form for Begomoviruses

PCR Experimental Set-up for Begomoviruses				
Date:	Ref. Expt. #:		Ref. Gel #:	
Title/Description:			Thermalcycler:	
Time Start:	Time End:		Total Run Time:	
Test Parameters				
PCR Profile:			**DNA Samples:**	
Denaturation 94°C 1 min Annealing 57°C 2 min Elongation 72°C 2 min Final Extension 72°C 10 min		30x		
Primer Pair: PAL1v1978B/PAR1c715H				
Expected Product Size: ~1.5kb				
Target Gene(s): partial DNA-A				

PCR Cocktail Preparation				
Components	**[Stock]**	**[Final]**	**1x=25µl**	
DEPC Water			17.65	
PCR Buffer	10x	1x	2.5	
MgCl2	50mM	2.5mM	1.25	
dNTPS	10mM	0.2mM	0.5	
Primer F	10µM	0.2µM	0.5	
Primer R	10µM	0.2µM	0.5	
Taq			0.1	
DNA			2	
Total Reaction Volume			25	
Aliquot **23µl** to each DNA tube				

Gel Electrophoresis		**Notes/Remarks:**
%Gel:	Voltage:	
Run Time:	Staining Time:	

Documentation
Set-up by:

PCR Form for BBTV

PCR Experimental Set-up for BBTV				
Date:	Ref. Expt. #:		Ref. Gel #:	
Title/Description:			Thermalcycler:	
Time Start:	Time End:		Total Run Time:	
Test Parameters				
PCR Profile:		**DNA Samples:**		
Initial Denaturation 94°C 4 min				
Denaturation 94°C 1 min ┐				
Annealing 61°C 1 min ├ 29x				
Elongation 72°C 2 min ┘				
Final Extension 72°C 10 min				
Primer Pair: D11/D12				
Expected Product Size: ~1.1kb				
Target Gene(s): DNA-R				
PCR Cocktail Preparation				
Components	**[Stock]**	**[Final]**	**1x=25µl**	
DEPC Water			17.65	
PCR Buffer	10x	1x	2.5	
MgCl2	50mM	2.5mM	1.25	
dNTPS	10mM	0.2mM	0.5	
Primer F	10µM	0.2µM	0.5	
Primer R	10µM	0.2µM	0.5	
Taq			0.1	
DNA			2	
Total Reaction Volume			25	
Aliquot **23µl** to each DNA tube				
Gel Electrophoresis		**Notes/Remarks:**		
%Gel:	Voltage:			
Run Time:	Staining Time:			
Documentation				
Set-up by:				

RT-PCR Form for Potyviruses

PCR Experimental Set-up for Potyviruses				
Date:	Ref. Expt. #:		Ref. Gel #:	
Title/Description:			Thermalcycler:	
Time Start:	Time End:		Total Run Time:	
Test Parameters				
PCR Profile:		**DNA Samples:**		
cDNA Synthesis	55°C 30min			
Initial Denaturation	94°C 2 min			
Denaturation	94°C 15 sec			
Annealing	55°C 30sec			
Elongation	68°C 1 min			
	40x			
Final Extension	68°C 5 min			
Primer Pair: Potyvirid1/Potyvirid2				
Expected Product Size: ~1.6kb				
Target Gene(s): 3' terminal portion of the genome				
PCR Cocktail Preparation				
Components	**[Stock]**	**[Final]**	**1x=10µl**	
DEPC Water			2.2	
Reaction mix	2x	1x	5	
Primer F	10µM	0.5µM	0.2	
Primer R	10µM	0.5µM	0.2	
Superscript III RT			0.4	
RNA			2	
Total Reaction Volume			10	
Aliquot **8µl** to each DNA tube				
Gel Electrophoresis		**Notes/Remarks:**		
%Gel:	Voltage:			
Run Time:	Staining Time:			
Documentation				
Set-up by:				

RT-PCR Form for PRSV

PCR Experimental Set-up for PRSV				
Date:	Ref. Expt. #:		Ref. Gel #:	
Title/Description:			Thermalcycler:	
Time Start:	Time End:		Total Run Time:	
Test Parameters				
PCR Profile:		**DNA Samples:**		
cDNA Synthesis	55°C 30min			
Initial Denaturation	94°C 2 min			
Denaturation	94°C 15 sec			
Annealing	58°C 30sec			
Elongation	68°C 1 min			
Final Extension	68°C 5 min			
Primer Pair: MB11/MB12				
Expected Product Size: ~900 bp				
Target Gene(s): CP gene				

(40x)

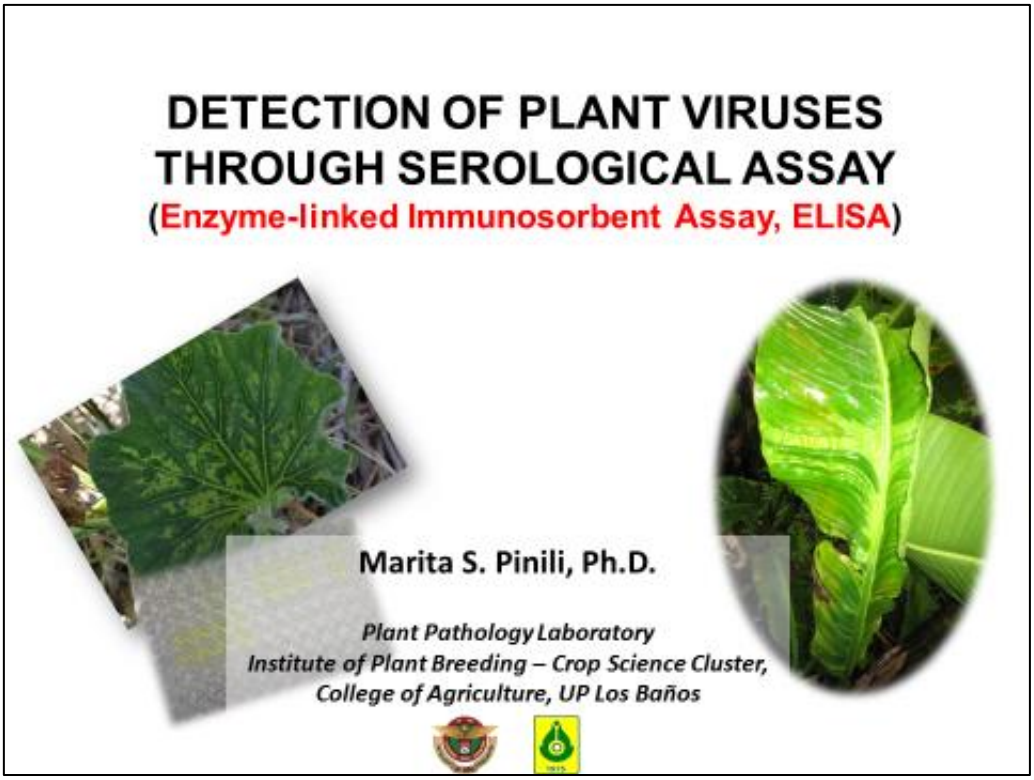
PCR Cocktail Preparation				
Components	**[Stock]**	**[Final]**	**1x=10µl**	
DEPC Water			2.2	
Reaction mix	2x	1x	5	
Primer F	10µM	0.5µM	0.2	
Primer R	10µM	0.5µM	0.2	
Superscript III RT			0.4	
RNA			2	
Total Reaction Volume			10	
Aliquot **8µl** to each DNA tube				

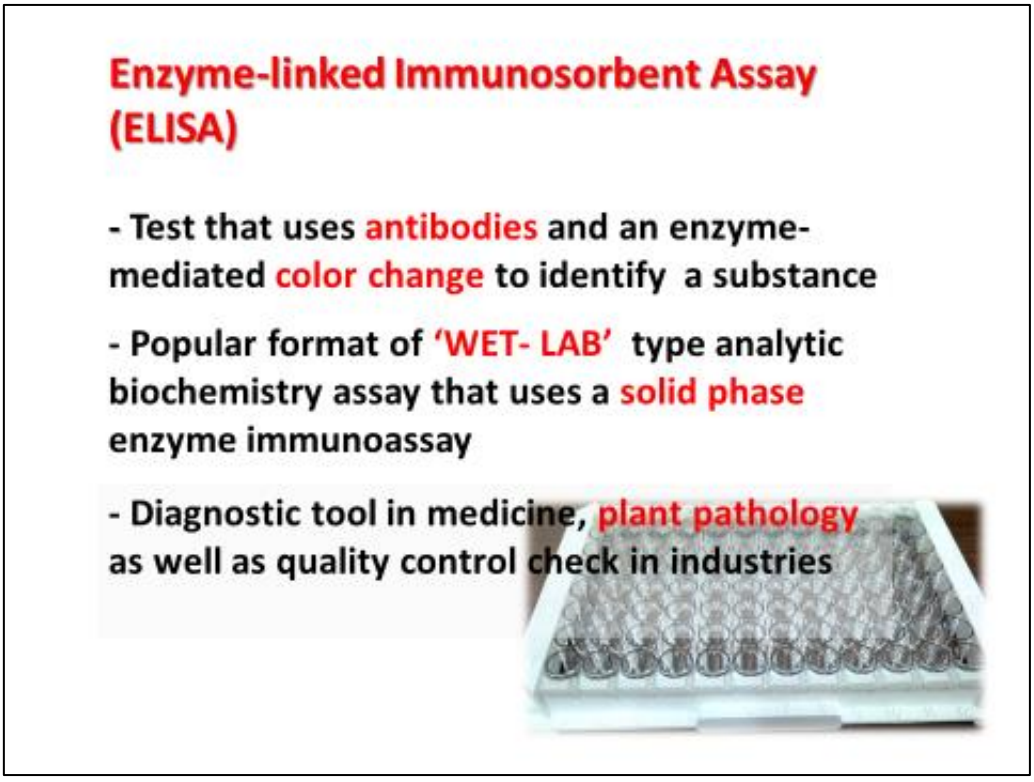
Gel Electrophoresis		**Notes/Remarks:**
%Gel:	Voltage:	
Run Time:	Staining Time:	

Documentation

Set-up by:

DETECTION OF PLANT VIRUSES THROUGH SEROLOGICAL ASSAY (Enzyme-linked Immunosorbent Assay, ELISA)





DETECTION OF PLANT VIRUSES THROUGH SEROLOGICAL ASSAY (Enzyme-linked Immunosorbent Assay, ELISA)

Enzyme-linked Immunosorbent Assay (ELISA)

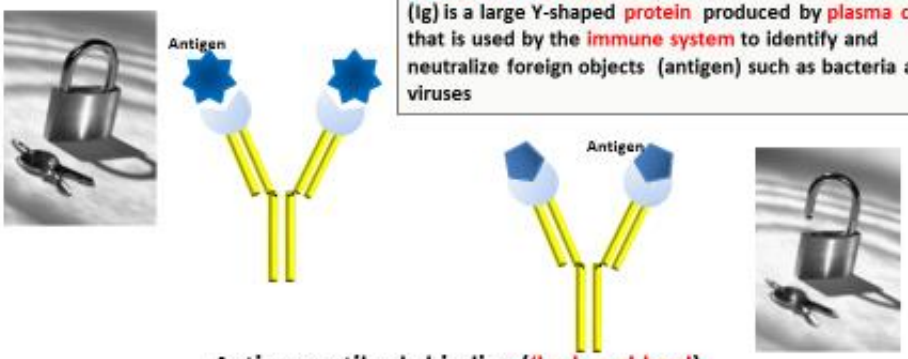
1960- Radioimmunoassay (RIA) first described by Yalow and Berson

1971 - Peter Perlman and Eva Engvall at Stockholm University, Sweden and Anton Schuurs and Bauke van Weemen in the Netherlands independently published papers that synthesized knowledge into methods to perform ELISA

Principles of ELISA

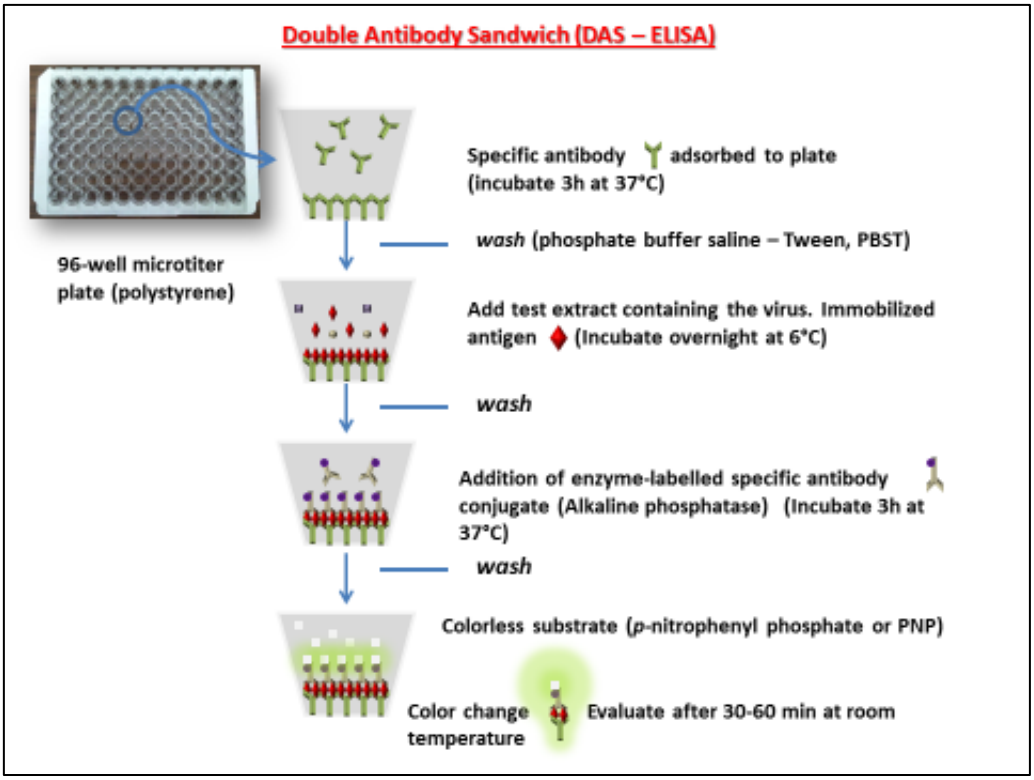
Antigen or antibody generator – Any substance which provokes an adaptive immune response. Ex. plant viruses

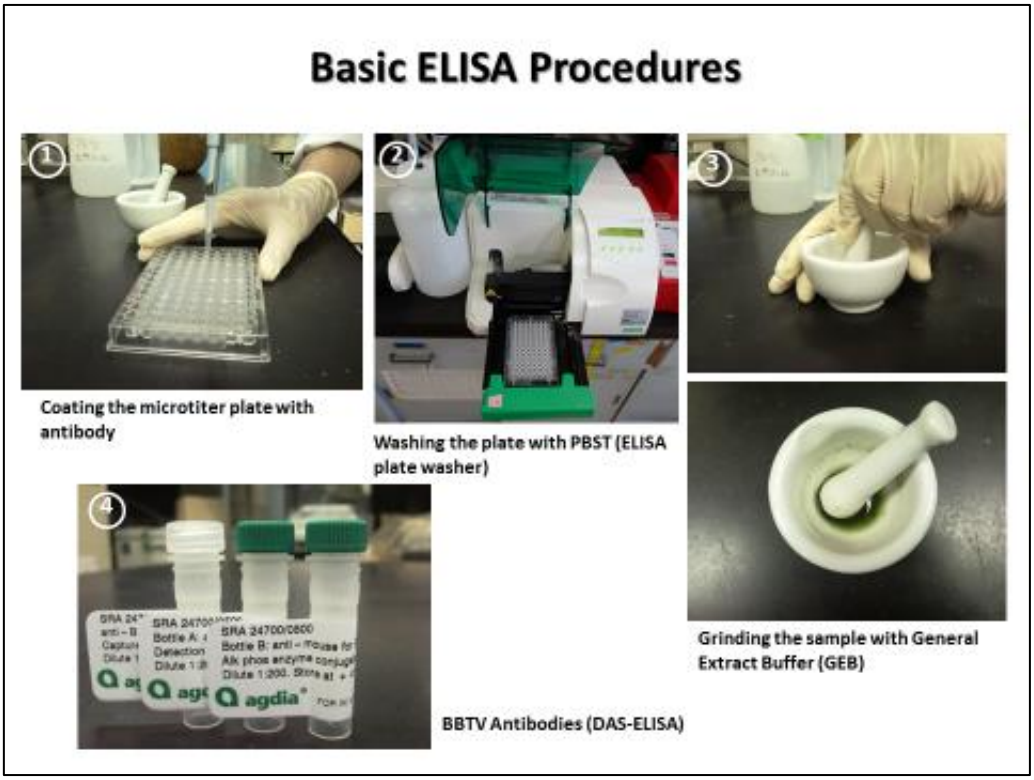
Antibody /Antiserum – also known as immunoglobulin (Ig) is a large Y-shaped protein produced by plasma cells that is used by the immune system to identify and neutralize foreign objects (antigen) such as bacteria and viruses



Antigen-antibody binding ('lock and key')

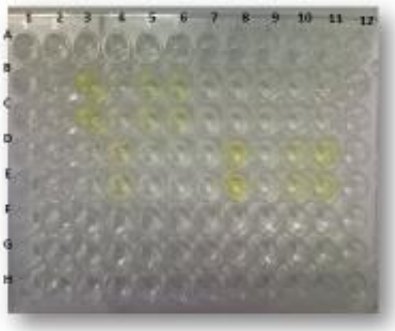
DETECTION OF PLANT VIRUSES THROUGH SEROLOGICAL ASSAY (Enzyme-linked Immunosorbent Assay, ELISA)





DETECTION OF PLANT VIRUSES THROUGH SEROLOGICAL ASSAY (Enzyme-linked Immunosorbent Assay, ELISA)

Basic ELISA Procedures



96-well polystyrene microtiter plate (yellow color indicates presence of BBTV on abaca samples)

Spectrophotometer

Advantages of DAS-ELISA

1. Extreme sensitivity
2. Applicability to large number of samples
3. Economy in use of high cost antisera
4. Semi-automatable
5. Quantitative
6. Independent of virus morphology
7. Independent of virus concentration