TERMINAL REPORT

Training Workshop on Diagnostic of Begomovirus and Development of LAMP-PCR

Plant Biosecurity Division, Department of Agriculture Malaysia
Agricultural Biotechnology Division, MARDI,
Ministry of Agriculture and Food Industry
Serdang, Selangor, Malaysia

September 19 – 30, 2022

Collaboration between the following institutions:

EXECUTIVE SUMMARY

This **"Training Workshop on Diagnostics of Begomovirus and Development of LAMP PCR"** was implemented by the Plant Biosecurity Division, Department of Agriculture (DOA) Malaysia as the host organization in collaboration with the National Crop Protection Center, Collage of Agriculture and Food Science, University of the Philippines Los Baños (NCPC-UPLB) and Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) through the ASEAN Plant Health Cooperation Network (APHCN) of ASEANET Phase II project on *"Taxonomic capacity building to support market access for agricultural trade in the ASEAN region".* This project is funded by the Japanese Government through the JAPAN – ASEAN Integration Fund (JAIF).

This two-week training workshop which was implemented on September 19 – 30, 2022 in Serdang, Selangor, Malaysia trained 23 plant pathologists and entomologists from 10 Southeast Asian countries including Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. Majority of the representatives are affiliated to the Plant Quarantine Centre and Plant Protection Division under their respective Departments of Agriculture, and some are from the academe.

The training workshop's main objective is to gain in-depth knowledge and awareness about the devastating effect of Begomoviruses on economically important crops, and to draw immediate and long-term solutions to manage such risks through capacity building development among plant quarantine and plant protection experts in the ASEAN region. Capacity building in terms of knowledge and skills on disease diagnostics using conventional serological and molecular tools and the new detection technology, the Loop-mediated isothermal amplification (LAMP). Aside from the serology i.e., Enzyme-linked immunoassay (ELISA) and molecular tool, Polymerase chain reaction (PCR) assay, the development of LAMP provides quick and sensitive tool with direct field application for plant virus detection including the emerging Begomoviruses.

The training course consisted of 12 lectures and 12 practical activities interactively designed to effectively capacitate the plant quarantine and plant protection experts in terms of virus disease diagnostics and detection of Begomovirus using LAMP. In support to the lecture and laboratory activities, a field visit and sample collection was conducted in Kg Ulu Chuchoh and Kg Ulu Teris in Serdang, wherein eggplant and chili pepper were infected by Begomovirus and infested with whitefly, *Bemisia tabaci*, the known insect vector of Begomovirus. To cover the whole training course, eight sessions were devised. These include Introduction, Begomovirus: Its impact on economically important crops, Detection and characterization of Begomovirus using serological and molecular approaches, Transmission, Detection and characterization using LAMP-PCR, Strategies in protecting crops from Begomoviruss infection, Data collection and Post evaluation.

To assess the participants technical background and overall performance on the training course, a pre – and post evaluation tests were administered. Also, the efficiency of the training

program and organizing team was determined through evaluation form given during the workshop.

Aside from the technical course activities, cultural showcase about the host country, Malaysia was organized. A welcome dinner in the panoramic view of PICC in Putrajaya was arranged and this was followed by a whole day field tour to Genting Highlands, Central Market and the iconic Petronas Tower in Kuala Lumpur.

Session 1. Opening Program and Introduction

The 23 participants representing Brunei Darussalam, Cambodia, Indonesia, Laos PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam were welcomed by the Director General of the Department of Agriculture Malaysia (DOA), Dato Zahimi Bin Hassan and Director General of Malaysian Agricultural Research and Development Institute (MARDI), Dato Dr. Mohd Zabawi bin Abdul Ghani. The messages shared by the two Director Generals revolved around the importance of virus diseases caused by Begomovirus including its insect vector on major agricultural crops and the global effects on food security. The timely training workshop was also commended as virus diseases require immediate management action plan through effective and efficient diagnostic systems including the new technology, Loop – mediated isothermal amplification (LAMP). A welcome address was also given by the head of the local organizing team, Ms. Lailatul Jumaiyah Saleh Huddin of DOA. Also, in attendance the Director of the Biotechnology and Nanotechnology Research Centre, MARDI, Dr. Faridah Salam and Mrs. Azean Ahmad, Head of Import Control and Enforcement Act Section, Plant Biosecurity Division. Training workshop background information and mechanics were also briefly discussed and this was followed by a pre-evaluation test for the participants proficiency assessment on the course topics.

Session 2. Begomovirus: Its impact on economically important crops

Three lectures were delivered by Dr. Marita S. Pinili, resource person from NCPC-UPLB and Regional Training Coordinator and Ms. Norhayati Madiha of the Plant Biosecurity Division, DOA and lead Technical Team. Inclusive in the lectures were the world of Geminiviridae in which Begomoviruses belong, its classification and morphology, Status and threat of diseases caused by Begomovirus group on economically important crops in Malaysia and the Status and diversity of Begomovirus in East and Southeast Asia. Supplementary to this session were the country reports presented by each participating country. During the report, organizational and functional structures of each country and the status of diseases of crops due Begomoviruses were presented.

Session 3. Detection and characterization of Begomoviruses using serological and molecular approaches

Lectures on Begomovirus symptom recognition and principles and methods of serological and molecular virus detection assays were discussed during this session. Dr. Sri Hendrastuti Hidayat, Professor and Senior Plant Virologist from IPB University, Bogor, Indonesia delivered the two lectures on virus detection approaches. Participants were able to acquire knowledge and skills on basic virus serological detection assay using the Enzyme-linked immunosorbent assay (ELISA) as well as the molecular tool, Polymerase Chain Reaction assay (PCR).

Laboratory activities done at the Biotechnology and Nanotechnology Research Centre, MARDI and the Plant Biosecurity Laboratory were performed including sample and reagents preparations, virus DNA extraction, amplification of target genes and gel electrophoresis and viewing and interpretation of results. The tomato plant samples used were from the Plant Biosecurity Lab whereas chili pepper dried samples infected with *Pepper yellow leaf curl virus* (PYLCV) collected from Brebes, Central Java, Kulon, Progo, Yogyakarta, and IPB, Bogor provided by Prof. Hidayat.

Session 4. Transmission of Begomovirus

In aid of the symptom recognition of Begomovirus diseases, a field visit in Kg Ulu Chuchoh and Kg Ulu Teris in Sepang was conducted. Farmers field planted to eggplant and chili pepper were visited and infected samples and whiteflies were documented and collected. Collected whiteflies, *B. tabaci* were then kept and used for the succeeding activity on virus detection from insect vector. Also, a greenhouse transmission activity was conducted at MARDI, wherein participants were tasked to perform simple virus transmission experiment using *B. tabaci* from the previously maintained infected tomato plants to a healthy tomato test plant kept in an insect-proof cage. The processes of virus transmission were discussed and demonstrated during this session. Two lectures were also given by Dr. Nursazilawati Saad from University Putra Malaysia and Mr. Mohd Sanusi Mohd Kasim from Plant Biosecurity Division, DOA. Lectures included the basic principle of how plant viruses are being acquired by the insect vector and how it is being transmitted to the healthy host plant. Morphological identification and biology of whiteflies were discussed by Mr. Sanusi with emphasis on how to morphologically differentiate whitefly species naturally occurring in the field. Also, detection of Begomovirus from *B. tabaci* was conducted using PCR assay following the protocol used by the Plant Biosecurity Division.

Session 5. Detection and characterization of Begomovirus using LAMP – PCR assay

Dr. Masashi Ugaki from The University of Tokyo introduced the LAMP technology. The LAMP which is specific for the *Sri Lankan cassava mosaic virus* (SLCMV) was demonstrated as a more sensitive virus detection tool and with direct field application. Dr. Ugaki discussed the principles, applications and limitations of LAMP as well as the primer design in comparison with the conventional PCR assay. Crucial steps such as pipetting errors, volume of reagents required and polymerase needed were deeply conversed. During this session, participants were also able to perform wet LAMP and dried LAMP kit to detect SLCMV. Results were interpreted based on the visual turbidity of the product using UV light and validated in electrophoresis.

Session 6. Strategies in protecting crops from Begomovirus infection

Lectures and discussions on the Integrated Pests Management (IPM) with emphasis on the use of biological controls were highlighted in this session. Since Begomovirus is transmitted by whitefly, *B. tabaci*, the management of the disease is through insect vectors. Biological controls including parasitoids and predators and list of entomopathogenic fungi (EPF) were presented. Commercial formulations and products in the market of those biological control agents were discussed, their field or greenhouse applications as well the demerits.

Session 7. Data collection

All activities during the two-week training workshop were well-documented and interpreted in preparation for a group report.

Session 8. Post evaluation and closing ceremony

Prior to the closing ceremony, a post evaluation test was given to gauge the level of learnings that each participant gained from the training workshop. After the one-hour post evaluation test, each group was given 30 minutes to present their results and recommendations about the activities. During the group report, methodologies including the step-by-step execution of protocols were discussed and commented. Results were analyzed and interpreted based on their methodologies. Several recommendations and conclusions were also tackled. After the group report and discussion, the training workshop was concluded by giving certificates of appreciation and completion to the resource persons, organizing team and participants. The awarding of certificates was spearheaded by the Director of the Plant Biosecurity Division, DOA Malaysia, Mrs. Rosmawati Selamat. Closing remarks from the Regional Training Coordinato, Dr. Pinili and response from the participants represented by Ms. Russ-Uzi Mayenne Ebora and Mr. Muhd Azhari bin Mohammad Zain from the Philippines and Singapore, respectively culminated the ceremony.

Technical and organization evaluations

Consolidated percentage scores on pre- and post-evaluations administered to participants showed impressing results. Post evaluation test results obtained high percentage (100%) on majority of the questions given. In terms of the organizational evaluations, all participants expressed that they have met the training workshop expectations and the learnings that they have gained are relevant to their jobs and will be used to disseminate to their colleagues for their references. Furthermore, through this training workshop they will make recommendations to their organization to further improve their diagnostic laboratories. Evaluations on the resource speakers and facilitators received the highest Excellent rating of 77 – 80%. In terms of logistics, concerns about the accommodation have received 33.3% and 20.8% Good and Unsatisfactory ratings, respectively. But all others like workshop venue, travel arrangements, field trip and food and refreshments garnered high percentage scores of Good to Excellent.

CHAPTER 1

1. Basic Information

1.1. Project Title:

Training Workshop on Begomovirus and Development of LAMP-PCR

1.2. Project Coordination:

Dr. Soetikno S. Sastroutomo – Acting Chairperson, APHCN – ASEANET
Dr. Marita S. Pinili – Regional Training Coordinator & Collaborator, NCPC – UPLB
Ms. Lailatul Jumaiyah Saleh Huddin – Local Coordinator in Malaysia, DOA

1.3. Proponent and Address

Plant Biosecurity Division, Department of Agriculture, Ministry of Agriculture and Food Industry, Jalan Gallagher, 50480 Kuala Lumpur, Malaysia
Tel: +603-2697 7139
Fax: +603-2697 7205

1.4. Implementing Agencies

1.4.1. Lead Agencies

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

Plant Biosecurity Division, Department of Agriculture Malaysia AND
Biotechnology and Nanotechnology Research Centre, MARDI, Ministry of Agriculture and Food Industry, Serdang, Malaysia

National Crop Protection Center (NCPC), College of Agriculture and Food Science
University of the Philippines Los Baños, College, Laguna 4031, Philippines

1.4.2. Funding Agency

This training workshop is supported by the Government of Japan through the Japan - ASEAN Integration Fund (JAIF).

1.5. Project Duration: Two (2) weeks

a. Date Project Started: 18 September, 2022
b. Expected Date of Completion: 1 October 2022

CHAPTER 2

2.1. Technical Description

A. Background

Emerging and re-emerging diseases contribute and further aggravate the current status of economically important crops from attaining high yield and quality of produce. The emerging and re-emerging diseases caused by viruses are perhaps the most devastating ones and require immediate attention and remedies due to the manner of disease transmission, spread and distribution across wide geographical locations. Begomoviruses are remarkably the most successful group of emerging viruses (Briddon et al. 2010; Rojas & Gilbertson 2008) which become important constraints to the production of solanaceous crops such as tomato (*Solanum lycopersicum*) and pepper (*Capsicum* spp.) and cucurbits (*Cucurbitaceae*). Begomovirus belongs to the large and diverse group of plant pathogenic viruses of the Family *Geminiviridae.* Geminiviruses possess a small circular single-stranded DNA (ssDNA) genome encapsidated within characteristic twinned, quasi-isometric virions (Briddon et al. 2010). Aside from Begomovirus the family *Geminiviridae* comprises of *Mastrevirus, Curtovirus, Becurtovirus, Turncurtovirus, Capulavirus, Eragrovirus, Grablovirus, Topocuvirus, Citlodavirus, Maldovirus, Mucrilevirus, Opunvirus,* and *Topilevirus* (Brown et al. 2012; ICTV 2020; Rougmanac et al. 2022). Members of the Begomovirus group infect dicotyledonous plants, and are associated with the polyphagous and virus-vector whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in a persistent, circulative manner. Begomoviruses have either mono- or bipartite genome reported originating from the Old World and New World, respectively. However, in Southeast and East Asia, emergence and diversity of Begomoviruses have been identified from crops like tomato and pepper and shows that Southeast Asia appears to be a major center of diversity (Kenyon et al. 2014). Begomovirus particularly the *Tomato yellow leaf curl virus* (TYLCV) have spread across the region. For instance, the *Tomato yellow leaf curl Thailand virus* (TYLCTHV) have spread from Thailand – Myanmar region into southern China and seems displacing the local species of TYLCV-infecting tomato in Taiwan and the *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) have spread to Java, Indonesia from its origin Thailand-Vietnam region.

The successful ability of Begomoviruses to infect local weed species together with the intensification and expansion of production of solanaceous crops across Asia have resulted to the abundance of whiteflies thus aids the increase and spread of more aggressive or crop-adapted species and strains of Begomovirus. The variants of Begomovirus in Asia may have arisen through mutation, recombination, pseudo-recombination, and acquisition of satellite DNA molecules (Kenyon et al. 2014). Thus, this ability of the virus to modify its genetic make-up will be of great challenge in designing the appropriate, effective and sound management

strategies to address the disease, in addition to the increase in population of biotypes of whitefly as the virus' efficient insect-vector.

This apparent scenario of wide and diverse distribution of Begomoviruses among Asian countries would require expertise in the detection, identification and molecular characterization of the virus, identification of potential alternate hosts including weed species as well as biotype identification of whitefly. Moreover, the flight pattern and spatial and temporal population spread or dynamics of the insect vector through computer models are highly necessary in understanding the spread of the disease and more importantly in the disease forecasting.

This proposed project will be an eye-opener not only to Asian countries but worldwide in obtaining a better understanding about the economic significance of Begomoviruses and the devastating disease(s) it caused to major life-sustaining crops in the world. With this training workshop on the diagnostic of Begomovirus, recipients or participants who are working as the forefronts of crop protection and plant quarantine agencies will gain pertinent knowledge on the basic information about the virus, its importance, detection tools, manner of virus transmission, potential alternate hosts and disease spread.

2.2. Course Description

This **"Training Workshop on Diagnostic of Begomovirus and Development of LAMP-PCR"** is coordinated by the Plant Biosecurity Division, Department of Agriculture Malaysia through the ASEAN Plant Health Cooperation Network (APHCN) of ASEANET Project Phase 2 on **"Taxonomic capacity building to support market access for agricultural trade in the ASEAN region"** and in collaboration with the National Crop Protection Center, University of the Philippines Los Baños. 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 workshop aims to; (1) provide basic and practical understanding on Begomoviruses, (2) importance of the virus, (3) identity and major characteristics of the virus group, (4) diagnosis of diseases of economically important crops caused by Begomoviruses, (5) mode(s) or manner of virus transmission, (6) identification of insect – vector, whitefly *Bemisia tabaci* Gen., (7) detection and identification of the virus both from infected samples and insect – vector(s) using serological and molecular assays, and (8) application of Loop-Mediated Isothermal Amplification (LAMP) – PCR detection kit, and (9) selected strategies in managing diseases due to Begomoviruses.

The topics to cover include the following: knowledge on the basic classification and morphology of Begomovirus group, importance of Begomoviruses on major agricultural crops in the tropics and sub-tropics, virus transmission, diagnosis based on symptomatology, detection using Enzyme-linked immunosorbent assay (ELISA), Polymerase Chain Reaction (PCR) assay and Loop-Mediated Isothermal Amplification (LAMP), virus transmission via insect vector, whitefly (*Bemisia tabaci*

Genn.) and the management options in avoiding and suppressing disease development.

Interactive lecture discussions and practical or hands-on laboratory activities were imposed to achieve the training workshop's objectives. Field tour or visit was done on major crop-growing areas in Malaysia where high occurrence and incidence of Begomovirus – associated diseases are observed. Actual disease assessment and sample collection are also part of the training workshop for symptom familiarization and insect-vector identification.

The knowledge stated above will aid the participants in establishing standard protocol in identifying diseases caused by Begomovirus, characterizing the virus using available detection assays, and choosing appropriate disease management strategy(ies).

The venue of the training-workshop *i.e.,* Biotechnology and Nanotechnology Research Centre, MARDI, Ministry of Agriculture and Food Industry, Malaysia has been chosen since the institute can provide the required facilities to conduct both lecture and hands-on activities needed by the training-workshop, and its nearness to various field locations where abundant virus diseases of crops are being observed.

2.3. Objectives

2.3.1. General Objectives

Lecture: At the end of the training, it envisioned that the participants will acquire fundamental knowledge on the global importance of Begomoviruses under tropical and sub-tropical agriculture; and how to mitigate or manage diseases caused by Begomoviruses; and relevant issues on the exchange of planting materials that may pose threat to the geographical distribution and spread of the virus.

Laboratory: At the end of the training, the participants will acquire diagnostics skills in recognizing symptoms expressed by Begomoviruses; learn the techniques from fundamental to advance methodologies in detection and characterization of the virus using serological, molecular, and LAMP PCR assays; and learn the manner of virus transmission via insect vector(s).

2.3.2. Specific Objectives

Lecture:

1. To acquire knowledge on the taxonomy and classification of Begomovirus group.
2. To become aware on the economic importance of diseases caused by Begomoviruses in tropical and sub-tropical crops.

3. To gain knowledge on the manner of Begomovirus transmission and its associated insect vector, whitefly *Bemisia tabaci.*
4. To familiarize with the symptoms on Begomovirus – infected crops.
5. To gain knowledge on simple to advance detection tools in detecting Begomovirus.
6. To acquire basic information on the molecular characteristics of Begomovirus based on the gene sequence profile.
7. To learn how to protect crops from Begomoviruses through cultural control, resistant varieties, virus-free planting materials and genetically modified (GM) crops.
8. To acquire knowledge on current issues on potential emerging/re-emerging diseases caused by Begomovirus and their importance in the exchange of planting materials.

Laboratory:

1. To learn the typical symptoms expressed in Begomovirus-infected plants.
2. To learn how to prepare buffer and other reagents used for serological and molecular assays.
3. To detect Begomoviruses from plant samples and insect vector, whitefly (*Bemisia tabaci*) using serological (Enzyme-linked immunosorbent assay, ELISA) and Polymerase Chain Reaction (PCR) assay.
4. To demonstrate the application of Loop-Mediated Isothermal Amplification (LAMP) – PCR technique in detecting Begomovirus.
5. To differentiate morphologically the common insect-vector of Begomovirus.
6. To demonstrate how Begomoviruses are transmitted into host plants using insect vector, *Bemisia tabaci.*

2.4. 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
- Pre-evaluation Test
- Country Report

SESSION 2. Begomovirus: Its impact on economically important crops

- Lecture 1. Geminiviridae: Begomovirus group – Classification and Morphology
- Lecture 2. Diseases of economically important crops caused by Begomovirus group: Status and threat in the Malaysia and neighboring regions
- Lecture 3. Status and diversity of Begomovirus in East and Southeast Asia

SESSION 3. Detection and characterization of Begomoviruses using Serological and Molecular Approaches

- Lecture 4. Symptom recognition and disease assessment
- Lecture 5. Detection of Begomovirus(es): Serological Approach
- Lecture 6. Detection of Begomovirus(es): Molecular Approach
- Practical 1. Stuffing for DNA Extraction
- Practical 2. Buffer Preparation and Serological Analysis
- Practical 3. Extraction of virus nucleic acid
- Practical 4. DNA Quality Check.
- Practical 5. Detection of Begomovirus using Polymerase Chain Reaction Assay
- Practical 6. Gel Electrophoresis

SESSION 4. Transmission of Begomovirus

- Lecture 7. General concept in the transmission of plant viruses: The role of insect-vector whitefly, *Bemisia tabaci* Genn. In the development of diseases and successful spread of Begomoviruses
- Lecture 8. Identification and characterization of whitefly (*Bemisia tabaci* Genn.) and its biotypes
- Practical 8. Transmission of Begomovirus using insect-vector whitefly, *Bemisia tabaci* Genn.
- Practical 9. Detection of Begomovirus from insect-vector
- Practical 10. Viewing of results

SESSION 5. Detection and Characterization of Begomovirus using LAMP-PCR Assay

- Lecture 9. Introduction to LAMP-PCR: Principles, Applications and Limitations
- Lecture 10. Detection of Begomovirs(es) using LAMP – PCR assay
- Lecture 11. Primer Design for LAMP -PCR
- Practical 7. Application of LAMP-PCR for the detection and identification of Begomovirus

SESSION 6. Strategies in protecting crops from Begomovirus infection

- Lecture 11. Protecting crops from virus diseases: Integrated Pests Management (IPM)
- Lecture 12. Protecting crops from virus disease: Biological Control Agents against Insect Vectors

SESSION 7. Data Collection

- Practical 11. Consolidation of data

SESSION 8. Post – Evaluation and Closing Ceremony

- Practical 12. Group Report

2.5. Training Content and Schedule

Week 1

Date/Venue/ Time	Topic/ Activity	Resource Person(s)/Facilitator
Pre-Training		
DAY 1. Sunday, September 18, 2022		
	Arrival and billeting at MARDI Guest House	*Logistic Team*
Training Proper		
DAY 2. Monday, September 19, 2022		
SESSION 1: OPENING PROGRAM AND INTRODUCTION **Venue: Auditorium Kompleks Latihan MARDI, Serdang**		
08:30 – 09:30	Registration Group Photo	Secretariat
	Welcome Address	*Director General, Department of Agriculture, Malaysia*
	Message	*Director General, Malaysia Agriculture Research and Development Institute (MARDI)*
09:31 – 10:00	Training Introduction and Overview	Dr. Marita S. Pinili *University Researcher IV, Regional Training Coordinator ASEANET*
10:01 – 10:30	Introduction of Participants, Trainers and Training Team	
10:31 – 10:45	Coffee/Tea Break	
10:46 – 11:45	Pre-evaluation Test	Dr. Marita S. Pinili & Ms. Lailatul Jumaiyah Saleh Huddin
SESSION 2. BEGOMOVIRUS: ITS IMPACT ON ECONOMICALLY IMPORTANT CROPS **Venue: Auditorium Kompleks Latihan MARDI, Serdang**		
11:46 – 12:30	**Lecture 1.** Geminiviridae: Begomovirus group – Classification and Morphology	Dr. Marita S. Pinili *University Researcher IV, NCPC*
12:31 – 14:00	Lunch Break	
14:01 – 14:45	**Lecture 2.** Diseases of economically important crops caused by Begomovirus group: Status and threat in Malaysia and neighboring regions	Ms. Norhayati Madiha *MARDI*
14:46 – 15:30	**Lecture 3.** Status and diversity of Begomovirus in East and Southeast Asia	Dr. Marita S. Pinili *University Researcher IV, NCPC*
15:31 – 15:45	Tea/Coffee Break	
15:46 – 17:30	In-country Report	All Participants
DAY 3. Tuesday, September 20, 2022		
SESSION 3. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES		
08:30 – 09:15	**Lecture 4.** Symptom recognition and disease assessment	Dr. Marita S. Pinili

		University Researcher IV, NCPC
09:16 – 09:30	Tea/Coffee Break	
09:31 – 11:00	**Lecture 5.** Detection of Begomovirus(es): Serological Approach	Dr. Sri Hendrastuti Hidayat *Professor, IPB University, Bogor*
11:01 – 12:30	**Lecture 6.** Detection of Begomovirus(es): Molecular Approach	Dr. Sri Hendrastuti Hidayat *Professor, IPB University, Bogor*
12:31 – 14:00	Lunch Break	
14:01 – 15:00	**Practical 1**. Stuffing for DNA Extraction *Venue: CMDV Laboratory, MARDI*	Training Team (NCPC, UPLB and DOA)
15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:30	**Practical 2**. Buffer Preparation and Serological Analysis *Venue: Plant Biosecurity Lab, Serdang*	Training Team (NCPC, UPLB and DOA)
18:00 – 20:30	Dinner Reception *Venue: PICC, Putrajaya*	Participants, Resource Persons, Training Team
DAY 4. Wednesday, September 21, 2022		
SESSION 3. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES		
08:30 – 10:30	**Practical 3.** Extraction of virus nucleic acid. *Venue: CMDV Laboratory, MARDI, Serdang*	Dr. Sri Hendrastuti Hidayat *Professor, IPB University, Bogor* and Training Team (NCPC & DOA)
10:31 – 10:45	Tea/Coffee Break	
10:46 – 12:30	**Practical 3**. Extraction of virus nucleic acid…*continuation* *Venue: CMDV Laboratory, MARDI, Serdang*	Dr. Sri Hendrastuti Hidayat *Professor, IPB University, Bogor* and Training Team (NCPC & DOA)
12:31 – 14:00	Lunch Break	
14:01 – 15:30	**Practical 3**. Extraction of virus nucleic acid…*continuation* *Venue: CMDV Laboratory, MARDI, Serdang*	*Dr. Sri Hendrastuti Hidayat Professor, IPB University, Bogor* Training Team (NCPC & DOA)
15:31 – 15:45	Tea/Coffee Break	
15:46 – 17:30	**Practical 4**. DNA Quality Check *Venue: CMDV Laboratory, MARDI, Serdang*	Dr. Sri Hendrastuti Hidayat *Professor, IPB University, Bogor* Training Team (NCPC & DOA)
DAY 5. Thursday, September 22, 2022		
SESSION 3. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES **SESSION 4. TRANSMISSION OF BEGOMOVIRUSES**		
08:30 – 10:30	**Practical 5**. Detection of Begomovirus(es) using Polymerase chain reaction (PCR) assay	Dr. Sri Hendrastuti Hidayat *Professor, IPB University, Bogor*

	Venue: CMDV Laboratory, MARDI, Serdang	and Training Team (NCPC & DOA)
10:31 – 10:45	Tea/Coffee Break	
10:46 – 12:30	**Practical 6.** Gel electrophoresis and analysis *Venue: CMDV Laboratory, MARDI, Serdang*	Dr. Sri Hendrastuti Hidayat *Professor, IPB University, Bogor* and Training Team (NCPC & DOA)
12:31 – 14:00	Lunch Break	
14:01 – 16:00	**Lecture 7.** General concept in the transmission of plant viruses: The role of insect-vector whitefly, *Bemisia tabaci* Genn. in the development of diseases and successful spread of Begomoviruses	Dr. Norsazilawati Saad *Senior Lecturer, University Putra Malaysia*
16:01 – 16:15	Tea/Coffee Break	
16:16 – 17:30	**Lecture 8**. Identification and characterization of whitefly (*Bemisia tabaci* Genn.) and its biotypes	Mohd Sanusi Mohd Kasim *Agriculture Officer, Department of Agriculture, Malaysia*
DAY 6. Friday, September 23, 2022		
Field Visit/Sample Collection		
08:00	Leave MARDI Guest House	*Logistic Team*
09:00	Arrival at Hulu Langat, Selangor.	
09:01 – 09:30	Tea/Coffee Break	
09:31 – 12:00	Sample collection	
12:01 – 14:00	Lunch Break & Prayer Break	
14:01 – 16:00	Sample Collection	
17:00	Arrive MARDI Guest House	
DAY 7. Saturday, September 24, 2022		
Sample Inspection and Pre-processing		
08:30 – 12:00	Inspection and pre-processing of samples	
12:01 – 13:00	Lunch Break	
13:01 – 17:00	Tour (Venue TBA)	
DAY 8. Sunday September 25, 2022		
REST DAY		

Week 2

Date/Venue/ Time	Topic/ Activity	Resource Person(s)/Facilitator
DAY 9. Monday, September 26, 2022		
SESSION 5. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES USING LAMP-PCR ASSAY		

08:30 – 10:00	**Lecture 9.** Introduction to LAMP-PCR: Principles, Applications and Limitations	Dr. Masashi Ugaki *Professor Emeritus* *The University of Tokyo*
10:01 – 10:15	Tea/Coffee Break	
10:16 – 12:30	**Lecture 10.** Detection of Begomovirs(es) using LAMP – PCR assay	Dr. Masashi Ugaki *Professor Emeritus* *The University of Tokyo*
12:31 – 14:00	Lunch Break	
14:01 – 15:30	**Practical 7.** Application of LAMP-PCR for the detection and identification of Begomovirus	Dr. Masashi Ugaki *Professor Emeritus* *The University of Tokyo*
15:31 – 15:45	Tea/Coffee Break	
15:46 – 17:30	**Practical 7.** Application of LAMP-PCR for the detection and identification of Begomovirus *…continuation*	Dr. Masashi Ugaki *Professor Emeritus* *The University of Tokyo*
DAY 10. Tuesday, September 27, 2022		
SESSION 5. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES USING LAMP-PCR ASSAY		
08:30 – 10:00	**Lecture 11.** Primer Design for LAMP -PCR	Dr. Masashi Ugaki *Professor Emeritus* *The University of Tokyo*
10:01 – 10:15	Tea/Coffee Break	
10:16 – 12:30	**Practical 7.** Application of LAMP-PCR in the detection and identification of Begomovirus *…continuation*	Dr. Masashi Ugaki *Professor Emeritus* *The University of Tokyo*
12:31 – 14:00	Lunch Break	
14:01 – 15:45	**Practical 7.** Application of LAMP-PCR in the detection and identification of Begomovirus *…continuation*	Dr. Masashi Ugaki *Professor Emeritus* *The University of Tokyo*
15:46 – 16:00	Tea/Coffee Break	
16:01 – 17:30	**Practical 8**. Transmission of Begomovirus using insect-vector whitefly, *Bemisia tabaci* Genn. *Venue: CMDV Glasshouse, MARDI, Serdang*	Training Team (NCPC & DOA)
DAY 11. Wednesday, September 28, 2022		
SESSION 6. STRATEGIES IN PROTECTING CROPS FROM BEGOMOVIRUS INFECTION		
08:30 – 09:15	**Lecture 12.** Protecting crops from virus diseases: Integrated Pests Management (IPM)	Dr. Marita S. Pinili *University Researcher IV, NCPC*
09:16 – 09:30	Tea/Coffee Break	
09:31 – 10:15	**Lecture 13.** Protecting crops from virus diseases: Biological control agents of insect vectors	Dr. Marita S. Pinili *University Researcher IV, NCPC*
10:16 – 12:30	**Practical 8**. Transmission of Begomovirus using insect-vector	Training Team (NCPC & DOA)

	whitefly, *Bemisia tabaci* Genn…*continuation* *Venue: CMDV Glasshouse, MARDI, Serdang*	
12:31 – 14:00	Lunch Break	
14:01 – 15:30	**Practical 9**. Detection of Begomovirus from insect-vector	Training Team (NCPC & DOA)
15:31 – 15:45	Tea/Coffee Break	
15:46 – 17:30	**Practical 9.** Detection of Begomovirus from insect-vector…*continuation*	Training Team (NCPC & DOA)
DAY 12. Thursday, September 29, 2022		
SESSION 7. DATA COLLECTION		
08:30 – 09:30	**Practical 9.** Detection of Begomovirus from insect-vector…*continuation*	Training Team (NCPC & DOA)
09:31 – 09:45	Tea/Coffee Break	
09:46 – 12:30	**Practical 10.** Viewing of Results	Training Team (NCPC & DOA)
12:31 – 14:00	Lunch Break	
14:01 – 15:00	**Practical 11** Consolidation of data	All Participants
15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:30	**Practical 11.** Consolidation of data….*continuation*	All Participants
DAY 13. Friday, September 30, 2022		
SESSION 8. POST-EVALUATION AND CLOSING CEREMONY		
08:30 – 9:45	Post-test evaluation	Dr. Marita S. Pinili & Ms. Lailatul Jumaiyah Saleh Huddin
09:46 – 10:00	Tea/Coffee Break	
10:01 – 12:30	**Practical 12**. Group Report	Groups 1&2
12:31 – 14:30	Lunch Break & Prayer Break	
14:31 – 15:45	**Practical 12**. Group Report…*continuation*	Groups 3,4 & 5
15:46 - 16:00	Tea/Coffee Break	
16:01 – 17:00	Presentation of Certificates	Dr. Soetikno S. Sastroutomo Dr. Marita S. Pinili Dr. Masashi Ugaki Ms. Lailatul Jumaiyah Saleh Huddin
17:01 – 17:30	Response from Participants	Two representatives
17:31 – 18:00	Closing Message	Dr. Marita S. Pinili *Regional Training Coordinator*
DAY 14. Saturday, October 1, 2022		
DEPARTURE		

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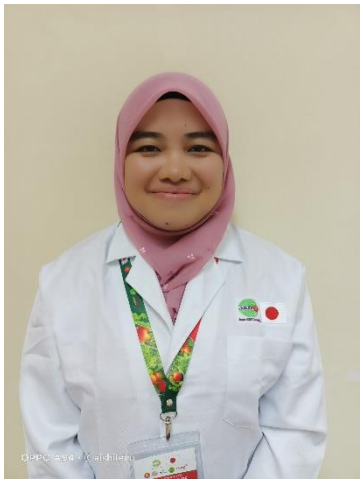

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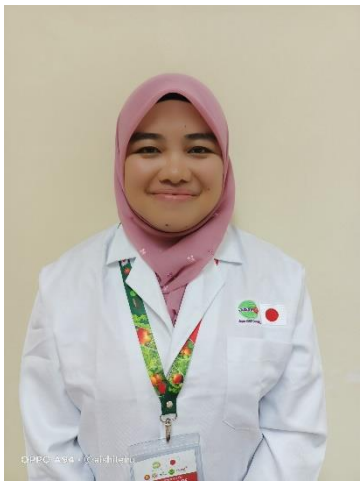

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2.9. Groupings

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	Dr. Win Than	Myanmar
	Ms. Russ-Uzi Mayenne Ebora	Philippines
Group 2	Ms. Nurul Hanisah binti Morni	Brunei Darussalam
	Mr. Phanhnouleth Nonthilath	Laos PDR
	Dr. Phone Kyaw Myint	Myanmar
	Mr. Muhd Azhari Bin Mohammad Zain	Singapore
	Ms. Andika Septiana Suryanighsih	*Indonesia*
Group 3	Mr. Oeurn Samoul	Cambodia
	Ms. Khonesavanh Chittarath	Laos PDR
	Dr. Yuvarin Boontoop	Thailand
	Ms. Troung Thi Ly	Vietnam
	Ms. Norhayati binti Madiha	*Malaysia*
Group 4	Mr. Mang Socheat	Cambodia
	Mr. Saiffulbahri bin Abdul Mutalib	Malaysia
	Dr. Phoowanarth Maneechoat	Thailand
	Ms. Mary Joy C. Mendoza	*Philippines*
	Ms. Lai Lee San	*Malaysia*
Group 5	Dr. Jati Adiputra	Indonesia
	Dr. Razean Haireen Binti Mohd Razali	Malaysia
	Mr. John M. Ermina	Philippines
	Mr. Nguyen Hoang Trung Anh	Vietnam

CHAPTER 3

Methodology

This training workshop utilized the two-week interactive activities including lectures, laboratory and greenhouse hands-on, and field sampling. The training course was divided into eight (8) sessions inclusive of pre- and post- test evaluation, data collection and opening and closing ceremonies. Five (5) major sessions were devoted to 12 lectures and 11 practical activities. Session 1. Opening Program and Introduction, Session 2. Begomovirus: Its impact on economically important crops, Session 3. Detection and characterization of Begomovirus using Serological and Molecular Approaches, Session 4. Transmission of Begomovirus, Session 5. Detection and Characterization of the Begomovirus using LAMP-PCR Assay, Session 6. Strategies in protecting crops from Begomovirus infection, Session 7. Data Collection, and Session 8. Post-Evaluation and Closing Ceremony were the major activities. Each session consisted of at least two lectures delivered in 45 min to an hour followed by laboratory hands-on which took at a minimum of 3 hours per activity. During week 1, lectures were delivered by Dr. Marita S. Pinili, Researcher and Senior Plant Virologist from the National Crop Protection Center (NCPC), University of the Philippines Los Baños, (UPLB) Philippines and Dr. Sri Hendrastuti Hidayat, Professor and Senior Plant Virologist from IPB University in Bogor, Indonesia, and a special lecture from Ms. Norhayati Madiha, Plant Virologist from the Plant Biosecurity Division, Department of Agriculture Malaysia (DOA). During the second week, lectures were given by Dr. Masashi Ugaki, Professor Emeritus from The University of Tokyo, Japan and two special lectures from Dr. Norsazilawati Saad, Senior Lecturer from the University of Putra Malaysia (UPM) and Mr. Mohd Sanusi Mohd Kasim, Agriculture Officer also from the Plant Biosecurity Division, DOA Malaysia. All practical were handled and supervised by the resource persons and assisted by the Technical Training Team members headed by Ms. Norhayati Madiha and Mr. Freddiewebb B. Signabon, University Researcher from NCPC-UPLB, and technical staff from the Biotechnology and Nanotechnology Research Centre, MARDI and Plant Biosecurity Division, DOA.

Lecture program, schedules and laboratory manual were provided to each participant before the training proper. Other supplies such as laboratory gown and writing notes/materials were also given during the registration.

A written pre-evaluation test was administered before the start of the lecture session to determine and gauge the participants' background on the subject matter and expectations on the training workshop. Afterwards, a country report was also initiated wherein each participating country presented briefly about the nature of their work as well as the status of Begomovirus-related diseases of crops on their respective region.

Lecture hand-outs were provided either before or after each lecture and were compiled at the end of the training workshop. A total of eight lectures were delivered accompanied by a discussion after each lecture. A pre-laboratory discussion was done aside from the pre-reading of the laboratory manual provided. This is for further clarification or slight modification in the scheduled activities. In performing the laboratory activities, participants were divided into 5 groups with 4 to 5 members including the observers from Malaysia, Philippines and

Indonesia. A total of 12 practical activities were conducted including the data collection and group discussion. A group report was also imposed for an intensive discussion and exchange of ideas regarding Begomovirus diseases, detection protocol and disease management.

A field tour which includes Begomovirus disease sample and insect vector collections was integrated in the training workshop. This activity entailed disease familiarity based on symptoms, disease and insect vector assessment in the area, as well as supplementary knowledge on how farmers manage such virus diseases. A one-day field visit was conducted in Kg Ulu Chuchoh and Kg Ulu Teris in Sepang planted to eggplant and chili pepper, respectively.

Technical and organizational evaluations were given to each participant in a form of questionnaires provided by the resource persons and organizing team. Technical pre-evaluation and organizational evaluation questionnaires were given at the start of the training workshop, whereas post-evaluation test was administered during the last day of the training workshop before the closing ceremony. Questions were designed to determine the level of confidence in performing the related activities taught, objectives met, future plans, and recommendations. Also, the three main resource speakers were rated according to their level of expertise on the subject matter, whereas the overall logistics were graded according to the set criteria.

CHAPTER 4

Accomplishments and Major Findings

4.1. Opening Program and Introduction

A total of 23 participants representing Brunei Darussalam, Cambodia, Indonesia, Laos PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam actively joined and welcomed by the Director General of the Department of Agriculture Malaysia (DOA), Dato Zahimi Bin Hassan and Director General of Malaysian Agricultural Research and Development Institute (MARDI), Dato Dr. Mohd Zabawi bin Abdul Ghani (Fig. 1 left & right, respectively) during the Opening Program hosted by Ms. Norhayati Madiha.

Fig. 1. Director General of the Department of Agriculture Malaysia (DOA), Dato Zahimi Bin Hassan (left) and Director General of Malaysian Agricultural Research and Development Institute (MARDI), Dato Dr. Mohd Zabawi bin Abdul Ghani (right) giving their messages during the workshop Opening Program.

The two Director Generals expressed their gratitude to the ASEAN Plant Health Cooperation Network (APHCN) of ASEANET and to the Japanese Government through the Japan-ASEAN Integration Fund (JAIF) as well as to the collaborations of various institutions namely; National Crop Protection Center, University of the Philippines Los Baños (NCPC-UPLB), Plant Biosecurity Division of DOA, and Biotechnology and Nanotechnology Research Centre of MARDI for bringing the training workshop in Malaysia as the host country after a two-year of controlled movement due to COVID19 pandemic. They also mentioned the importance of the training workshop relative to food crop loss due to pests and diseases and the evolving plant viruses and insect vectors. Thus, empowering plant disease diagnostics technology in crop protection and plant quarantine is necessary to ensure the prevention and management of such diseases. Dato Zahimi bin Hassan also emphasized the importance of molecular

techniques in virus detection in particular the promising application of LAMP-PCR as rapid diagnostic tool.

Aside from the first two resource persons, Dr. Sri Hendrastuti Hidayat and Dr. Marita S. Pinili who is also acting as the Regional Training Coordinator present during the opening ceremony, the event was also attended by the Director of Biotechnology and Nanotechnology Research Centre, MARDI, Dr. Faridah Salam and Deputy Director of Plant Biosecurity Division of DOA, Mrs. Azean Ahmad, Head of Import Control and Enforcement Act Section, Plant Biosecurity Division.

The local organizing team headed by Ms. Lailatul Jumaiyah Saleh Huddin, Senior Principal Assistant Director of Plant Biosecurity Division, DOA was also present during the ceremony and gave her message on behalf of the whole technical and administrative support staff (Fig. 2).

After the opening messages from the distinguished guests, a group photo was conducted and this was followed by the training workshop mechanics which was given by Dr. Marita S. Pinili, Regional Training Coordinator (Fig. 3).

Before the formal lecture, a one-hour pre-evaluation test was administered to the participants. Sets of questions about the topics included in the training workshop were given.

Fig. 2. Ms. Lailatul Jumaiyah Saleh Huddin, head of the local organizing team of the training workshop expressed her warm welcome to the guests and participants on behalf of the whole technical and administrative support staff.

Figure 3. Group photo during the Opening Program headed by the Director General of the Department of Agriculture Malaysia, Dato Zahimi Bin Hassan (center right) and Director General of the Malaysian Agricultural Research and Development Institute, Dato Dr. Mohd Zabawi bin Abdul Ghani (center left).

4.2. Session 2. Begomovirus: Its Impact on Economically Important Crops

After the pre-evaluation test, session 2 started by the series of lectures. Session 2 includes discussion on the impact of Begomovirus on economically important crops. Dr. Marita S. Pinili delivered the first lecture about *Geminiviridae* in which the Begomovirus group belongs including their classification and virus morphology (Fig. 4). This was followed by a lecture on the status of diseases of economically important crops caused by Begomovirus in Malaysia and neighboring regions. The topic was discussed by Ms. Norhayati Madiha in the afternoon session (Fig. 5). Also, during day 1, a third lecture on the status and diversity of Begomovirus in East and Southeast Asia was tackled and it was presented by Dr. Pinili. In between lectures, an active exchange of questions and discussions happened due to the interesting and timely conduct of this training workshop. Most participants shared their knowledge and experiences regarding various diseases involving Begomovirus.

4.2.1. Country Report

The day 1 session ended with an In-country report in which each participating country prepared a 15-minute lecture presentation about the background and mandate of their respective institution or organization as well as the update on the current status of Begomovirus – related diseases in their country (Fig. 6).

Country reported started with Brunei Darussalam represented by Ms. Adi Lisea binti Mohd Addly. She mentioned the organizational structure of their institution and presented updates on the current status of viruses in Brunei. She also shared the use of serological technique such as ELISA and immunostrips in detecting plant viruses, and the renovation of the laboratory facilities in the Plant Pathology Unit. The report was followed by Mr. Oeurn Samoul

from Cambodia. Mr. Samoul comprehensively presented the surveillance protocols they are currently doing in their country to address the increasing problem of cassava Begomovirus. Similarly, Laos PDR shared the same Begomovirus disease of cassava i.e., Cassava mosaic virus with Cambodia. Ms. Knonesavanh Chittarath discussed the rampant occurrence of Cassava mosaic virus and their collaboration with the Australian government to help them manage the disease using the Droid LAMP device. She was also very proud to share that after her training on the diagnostics of plant viruses in the Philippines way back 2015, their laboratory has been improved and started to gain external technical support from other organizations.

Dr. Jati Adiputra of Indonesia presented the plant quarantine detection of Begomovirus in their country. He mentioned some diagnostic tools that they are currently using to check incoming seeds and other plant materials for possible virus infection. On the other hand, Mr. Saifflubahri bin Abdul Mutalib and Dr. Win Than from Malaysia and Myanmar, respectively gave brief information about their organization in terms of structure and functions and updates on virus diseases of selected crops in their countries.

Representatives from the Philippines, Ms. Russ-Uzi Mayenne Ebora and Mr. John Ermina presented individually their respective offices. Ms. Russ discussed the organizational structure and functions of the Crop Pest Management Division under the Bureau of Plant Industry situated in Manila, whereas Mr. John expound the crucial functions of the National Plant Quarantine Service Division in Cagayan de Oro. Both offices are under the Department of Agriculture but performing separate but with interlinked mandates (Fig. 7).

Country report from Singapore was presented by Mr. Muhd Azhari bin Mohammad Zain of the Animal and Plant Health Centre, National Parks Board. Unlike the rest of the participating countries, Singapore has a limited production of crops due to small land area. Vertical farming is being utilized in Singapore including hydroponics. But despite of that, plant disease diagnostics are being done to intercept possible importation of pests and diseases.

Dr. Yuvarin Boontoop of the Plant Protection Research in the Department of Agriculture Thailand discussed the devastating occurrence and incidence of *Pepper yellow leaf curl virus* (PYLCV). PYLCV is a Begomovirus currently creating havoc on chili pepper in Thailand particularly in Kanchanaburi region. She also presented the next generation sequencing that they are using for virus detection and characterization.

Lastly, Ms. Troung Thi Ly from Plant Quarantine Diagnostic Centre (PQDC) of the Ministry of Agriculture and Rural Development in Vietnam. Ms. Ly mentioned that in Vietnam there are so much to do when it comes to documenting Begomoviruses and its insect vector. Thus, the training workshop is very timely and would help them update their records on plant viruses.

Fig. 4. During day 1, Dr. Marita S. Pinili delivered the lectures on Geminiviridae: Begomovirus group Classification and Morphology and Lecture 3. Status and diversity of Begomovirus in East and Southeast Asia.

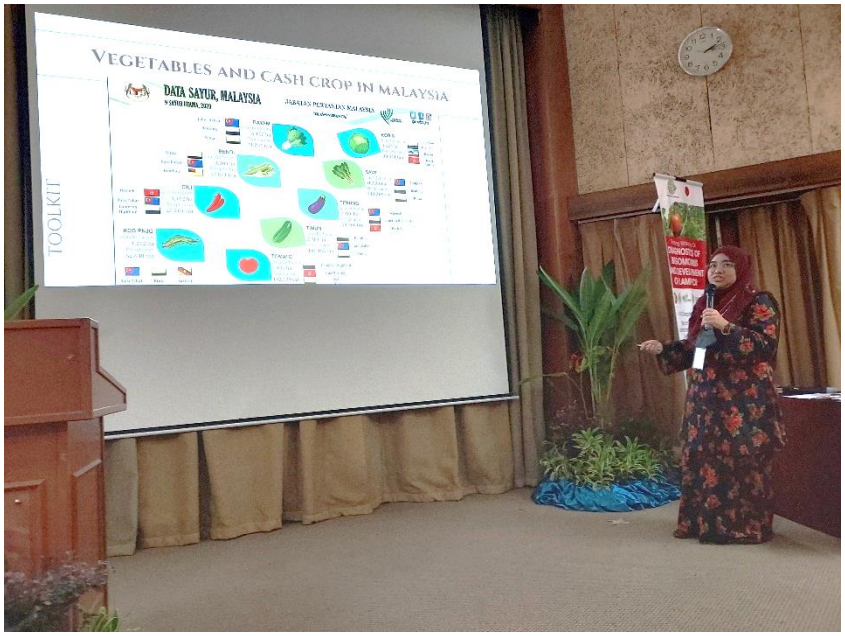

Fig. 5. Ms. Norhayati Madiha presenting the Lecture 2 on Diseases of economically important crops caused by Begomovirus group: Status and threat in Malaysia and neighboring regions.

Fig. 6. Country reports presented by Ms. Adi Lisea binti Mohd Addly of Bruei Darussalam (upper left), Mr. Oeurn Samoul of Cambodia (upper right), Ms. Khonesavanh Chittarath of Laos PDR (middle left), Dr. Jati Adiputra of Indonesia (middle right), Mr. Saiffulbahri bin Abdul Mutalib of Malaysia and Dr. Win Than of Myanmar (lower left and right, respectively).

Fig. 7. Country reports were presented by Ms. Russ-Uzi Mayenne Ebora and Mr. John Ermina of the Philippines (upper right and left, respectively), Mr. Muhd Azhari bin Mohammad Zain of Singapore (middle left), Dr. Yuvarin Boontoop of Thailand (middle right), and Ms. Troung Thi Ly of Vietnam (bottom).

4.3. Session 3. Detection and Characterization of Begomovirus

Dr. Pinili and Prof. Hidayat led the lecture discussions on Begomovirus detection and characterization. Lecture 4 on symptom recognition and disease assessment was given by Dr. Pinili wherein she tackled common symptoms of a Begomovirus-infected plants. The importance of familiarity with typical symptoms of Begomovirus such as upward curling and yellowing of leaves, mottling, mosaic and vein -clearing and stunted growth are apparent. Accompanying these symptoms are the presence of whitefly, *Bemisia tabaci*, the known vector of the Begomoviruses. The lecture was subsequently followed by lectures of Prof. Hidayat on serological and molecular approaches for detecting Begomoviruses (Fig. 8).

Fig. 9. A laboratory practical was then performed at the Biotechnology and Nanotechnology Centre, MARDI for the preparation of plant sample and stuffing for DNA extraction. Each group was assigned to prepare healthy and Begomovirus – suspected tomato leaf sample for grinding using a stainless ball then subjected to a -80°C incubation. Additional samples i.e., chili pepper from IPB, Bogor, Indonesia were also processed for comparison and detection of *Pepper yellow leaf curl virus* (PYLCV). While waiting for the overnight incubation, the group proceed to the Plant Biosecurity Laboratory in Serdang for the serological assay. Same source of samples used in DNA extraction were processed for the Enzyme-linked immunoassay (ELISA) except the dried chili pepper samples from Indonesia. Prior to ELISA, a briefer was given to the participants to avoid confusion in sample and buffer preparations (Fig. 10).

Fig. 8. Prof. Hidayat giving lectures on the principles and methods of virus detection using serological and molecular approaches during the Day 2 of the training workshop.

Each group were given sets of ELISA reagents and plate for antibody coating, washing and sample loading (Fig. 11). After the sample loading, the ELISA plate was incubated for overnight at 4°C. While waiting for the incubation, Ms. Norhayati Madiha demonstrated to the group how to use the ELISA reader and how to determine the absorbance reading for the interpretation of results (Fig. 12).

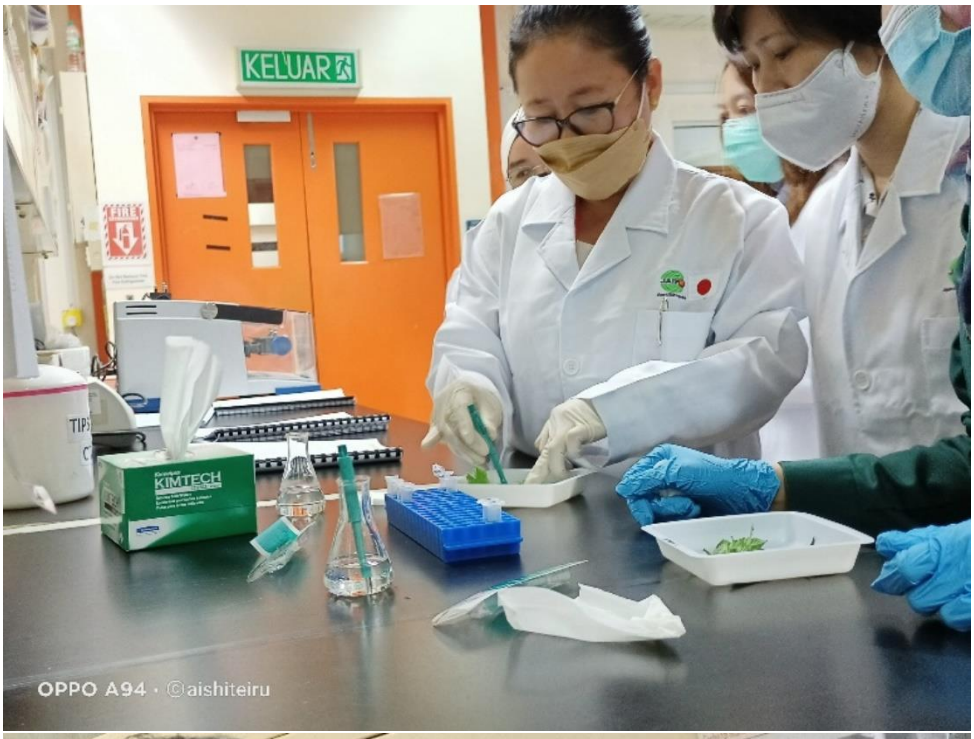

Fig. 9. Performing sample preparation for DNA extraction. Healthy and symptomatic tomato leaf samples were provided for the PCR assay to detect *Tomato yellow leaf curl virus* (TYLCV).

Fig. 10. Resource person, Dr. Pinili giving briefer prior to the laboratory hands-on activity.

Fig. 11. Participants performing the Enzyme-linked immunsorbent assay (ELISA) at the Plant Biosecurity Laboratory, Serdang. Computation of buffers relative to the number of samples is being done prior to loading of samples (above). Loading of coating buffer IgG into ELISA Plate (below left), and loading of antigen from the plant sample extract for and overnight incubation (below right).

Fig. 12. Ms. Norhayati Madiha demonstrating how to use the ELISA reader and how to interpret the data based on the absorbance reading.

4.3.1. Reception Dinner

The first part of the session 3 was then followed by a reception dinner held at PICC, Putrajaya. The reception dinner organized by the local training workshop team headed by Ms. Lailatul Jumaiyah Saleh Huddin was enormous and was able to showcase to the participants the astonishing 360° panoramic view of Putrajaya. Prior to the dinner, short messages were given by Ms. Lailatul and Dr. Pinili. All participants showed their appreciation to the efforts of the local organizing team by giving musical performances led by Dr. Win Than, all delegates from the Philippines, Malaysia and a dance number initiated by Dr. Hidayat. A surprise was also given by the team to the birthday celebrators, Ms. Mary Joy Mendoza, Mr. John Ermina, Mr. Nguyen Hoang Trung Anh and Mr. Phanhnouleth Nonthilath. The dinner was concluded with an ample of delicious food served during the entire event (Fig. 13, 14 & 15).

Fig. 13. A dinner reception at PICC, Putrajaya arranged by the local organizing team of the Plant Biosecurity Division of the Department of Agriculture, Malaysia.

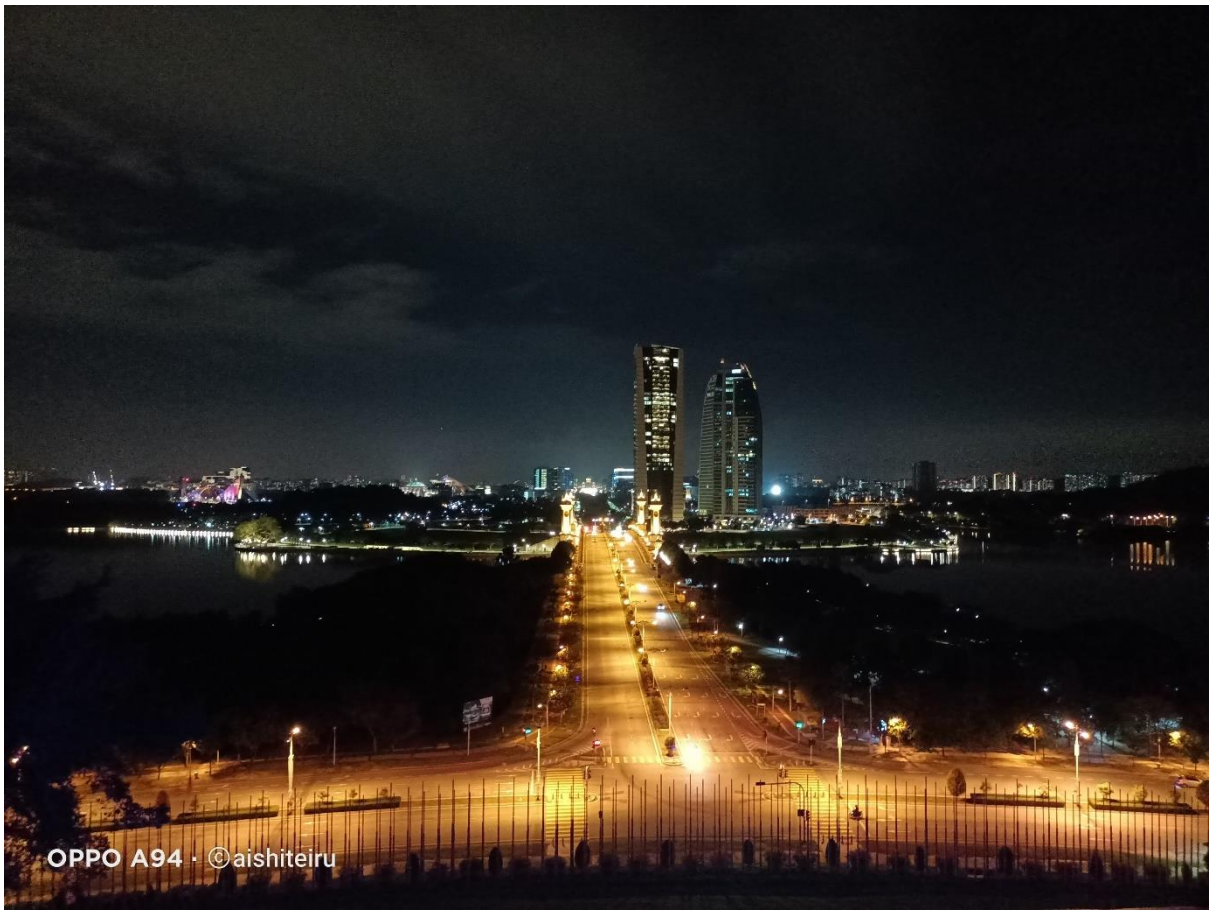

Fig. 14. Panoramic view of Putrajaya taken from PICC.

Fig. 15. Enjoying the food during the dinner reception at PICC, Putrajaya. A surprised given to birth celebrators (above right) and musical performances from the Philippine delegates (below left) and Malaysian participant and administrative staff (below right).

The session 3 was continued the next day (Day 4) and resumed the serological and molecular detection assays. To maximize time, one representative from each group joined the Plant Biosecurity Division in Serdang to continue the ELISA. Incubated plates were washed with wash buffer and an enzyme-labelled antibody conjugate was added and incubated for 5 hours. Then, joined the rest of the group at the Biotechnology and Nanotechnology laboratory afterwards (Fig. 16.). Results of the ELISA were then checked by the assigned group representative after 5 hours. Color reaction was then inspected after 30 minutes and one hour incubation with substrate para-nitrophenylphosphate (pNPP). Two readings were also conducted using the ELISA reader to verify the validity of the color change observed (Fig. 17.).

For the molecular detection assay, a pre-lab discussion was given by Mr. Freddiewebb Signabon prior to DNA extraction activity. DNA extraction of suspected begomovirus from tomato (*Tomato yellow leaf curl virus*, TYLCV) and chili pepper, TYLCV were done following the protocol set by the Centre for Marker Discovery and Validation (CMDV). Sets of primers were also prepared to detect TYLCV using the TYLCV F/R which targets the coat protein gene (˜543 bp), and PYLCV primers for DNA-A and DNA-B and a universal primer for begomovirus. Respective thermal cycle conditions were set and used to determine the presence of the target Begomoviruses.

Day 5 continued the molecular assay. Each group loaded their PCR products on the prepared 1.5% agarose gel. PCR products were gel electrophoresed at 110V for 30 minutes and then viewed in a transilluminator gel documentation system. Results were analyzed based on the appearance of the target or expected bands (Fig. 18).

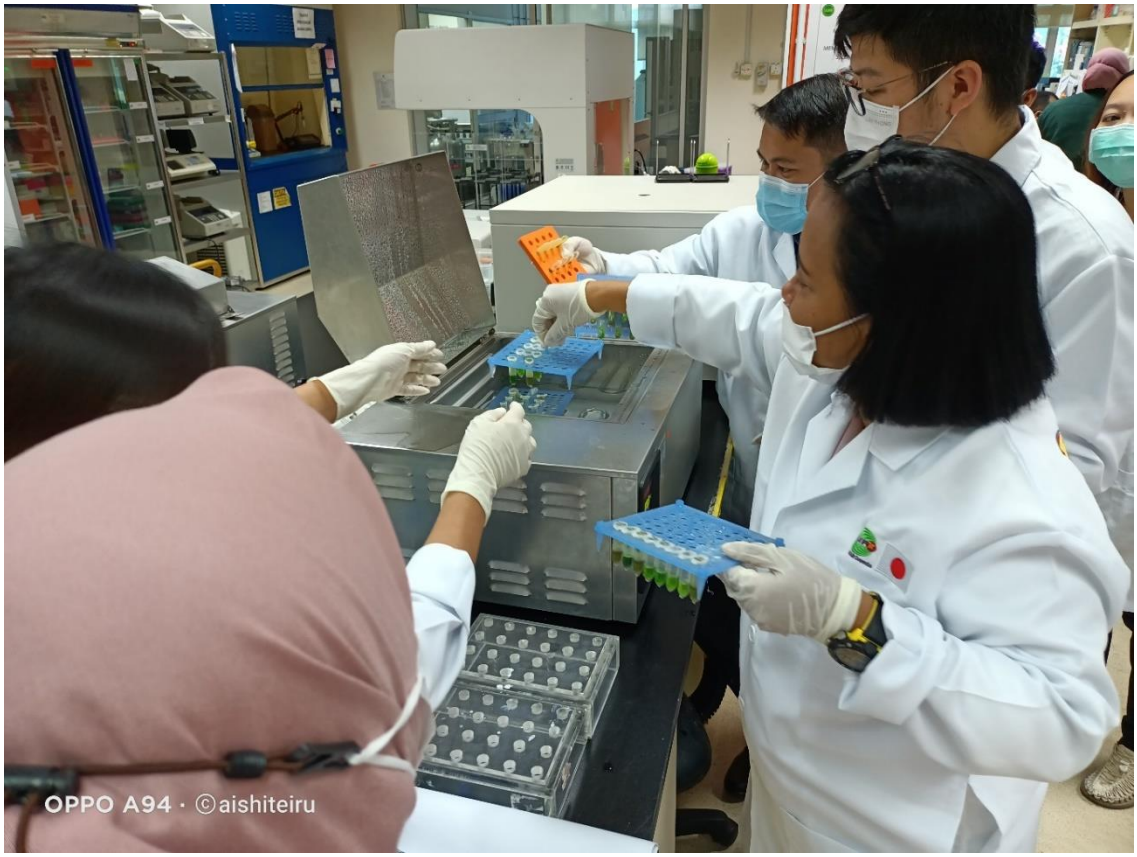

Fig. 16. Day 4 continuation of the PCR assay at the Biotechnology and Nanotechnology Laboratory, MARDI.

Fig. 17. Application of substrate para-nitrophenylphosphate (pNPP) on ELISA plate to observe color reaction after a 30-minute incubation.

Fig. 18. Day 5 continuation of PCR assay. Preparation of PCR cocktail for the thermal cycler (above) and loading of PCR products into get electrophoresis (below).

4.4. Session 4. Transmission of Begomovirus

Main activity on Begomovirus transmission includes greenhouse and field practical on catching whitefly, *Bemisia tabaci* using an improvised insect collector. Each group were provided with an insect collector and instructed to capture 10 viruliferous whiteflies for the transmission experiment. Whiteflies were kept in a screened cages with TYLCV-infected tomato as their source of the virus. Healthy tomato plants of about one-month old were maintained individually in a separate cage for inoculation. Viruliferous whiteflies were then allowed to feed onto healthy tomato plants for inoculation and symptom development (Fig. 19 & 20).

Fig. 19. Collecting whiteflies in the greenhouse using an improvised insect collector apparatus.

In line with this topic, two lectures were also given by Dr. Norsazilawati Saad and Mr. Mohd Sanusi Mohd Kasim (Fig. 21.). Dr. Saad discussed the general concept in plant virus transmission, particularly the role of whitefly, *B. tabaci*, the sole insect-vector of begomovirus relative to the disease development and successful spread of the virus. She emphasized the processes involved during virus acquisition, latent period, incubation and transmission of the virus to the plant cell from the insect stylet. Types of virus transmission i.e., persistent and non-persistent were also mentioned.

On the other hand, Mr. Sanusi, the senior Entomologist from DOA Malaysia lectured about the identification and characterization of whitefly, *B. tabaci*. He also tackled what are the biotypes

of *B. tabaci* currently identified so far and gave some basic information on how to distinguish morphologically between whitefly, *B. tabaci* and *Trialeurodes vaporariorum* or the greenhouse whitefly. His discussion gave more emphasis on the biology and ecology of *B. tabaci*.

Fig. 20. Greenhouse set-up for the virus transmission experiment using whitefly, *B. tabaci*. Begomovirus infected eggplant as virus source of whiteflies (above left) and the healthy tomato plant maintained in insect-proof cage for virus inoculation (above right). Inoculation of Begomovirus by transferring viruliferous whiteflies directly into the test plants (below).

Fig. 21. Prof. Norzasilawati (above) giving lecture on virus transmission whereas Mr. Mohd Sanusi Mohd Kasim discussing about the morphological identification and characterization of whitefly (below)

4.4.1. Field Sampling

Week 1 of the training workshop was concluded by a field visit to Kg Ulu Chuchoh and Kg Ulu Teris in Selangor planted to eggplant and chili pepper, respectively (Fig. 22). In Kg Chuchoh, a farmer's field which started to practice minimal application of pesticides was visited and checked for the presence of Begomovirus disease and whiteflies on eggplant. Each group was given an hour to document the symptoms and collect whiteflies using the improvised insect collector. A lot of whiteflies were collected and kept in a vial for identification and to be used for the Begomovirus detection. Right after the sample collection, a token was given to the farm owner as a sign of gratitude. Then, the whole team proceed to the next site i.e., Kg Ulu Teris to check the chili peppers. In the site, the group was welcomed by the owner who is also the leader of the area and diligently following the Good Agricultural Practices (GAP). Chili peppers showed severe yellowing and curling of leaves with mosaic and mottling symptoms. Some chilies were apparently deformed and undersized but still able to produce considerable harvests. Unfortunately, no whiteflies were collected due to hot weather condition and close to noontime sampling, wherein most whiteflies flew and hide to avoid direct sunlight. Likewise, a token of appreciation was given to the farm owner for accommodating the participants and for allowing to do field visit (Fig. 23 & 24)

After a tiring field visits, the group visited a family-owned business shop, Agro Chips (Jamirah Food Industries) that is selling processed root crops like cassava chips. The participants were able to taste and buy chips and nuts as souvenirs After the one-stop shop, a lunch was served at Banghuris Homestay in Sepang, known for the Nasi Ambeng D' Rebung. The owner welcomed all the participants and the organizing team with some freebies and delicious food (Fig. 25).

After a heavy lunch, a tour at HL Dragon Fruit Eco Farm. The farm owner welcomed the participants by introducing the farm history, a lecture about dragon fruit production and product processing, and a farm tour. Right after the tour, a refreshing dragon fruit shake, chips and bread stuffed with chicken curry were served (Fig. 26).

Fig. 22. Field visits to Kg Ulu Chuchoh (above) and Kg. Ulu Teris (below) in Serdang. Prior to field sampling farm owners give briefings above the crop planted and their field practices including the adoption of less pesticides use and good agricultural practices (GAP).

Fig. 23. Eggplant and chili pepper plants cultivated in Kg Uluh Chuchoh and Kg Ulu Teris, respectively.

Fig. 24. Symptomatic eggplant observed in Kg. Ulu Chuchoh (above) and severely infected chili pepper showing apparent leaf curling, vein-clearing ang yellowing planted in Kg Ulu Teris (below).

Fig. 25. A quick tour in Agro Chips (Jamirah Food Industries), a family-owned business selling cassava chips and other nuts and chips (above). Having lunch at Banghuris Homestay in Sepang, known for the Nasi Ambeng D' Rebung (below).

Fig. 26. At HL Dragon Fruit Eco Farm and Restaurant in Sepang.

4.4.2. Field Tour at Genting Highlands and Kuala Lumpur

A field tour was organized for the participants to learn more about Malaysia and enjoy the hidden sceneries and famous tourist spots.

Genting Highlands is a hill station situated in the peak of Mt. Ulu Kali in the state of Pahang and is known for its exciting adventure and theme park. Cable car riding was the forefront of the tour in which participants enjoyed more than 30-minute ride up to the mountaintop of about 1,800 meters above sea level. Various shops including designer's clothes, food courts and others were the highlights of the Genting tour. After a two-hour stay in the highlands, the group were toured around Kuala Lumpur. Souvenir shops in the Central Market was visited followed by city tour at the famous Petronas Tower. Participants enjoyed the taking photos in the iconic tower (Fig. 27 & 28).

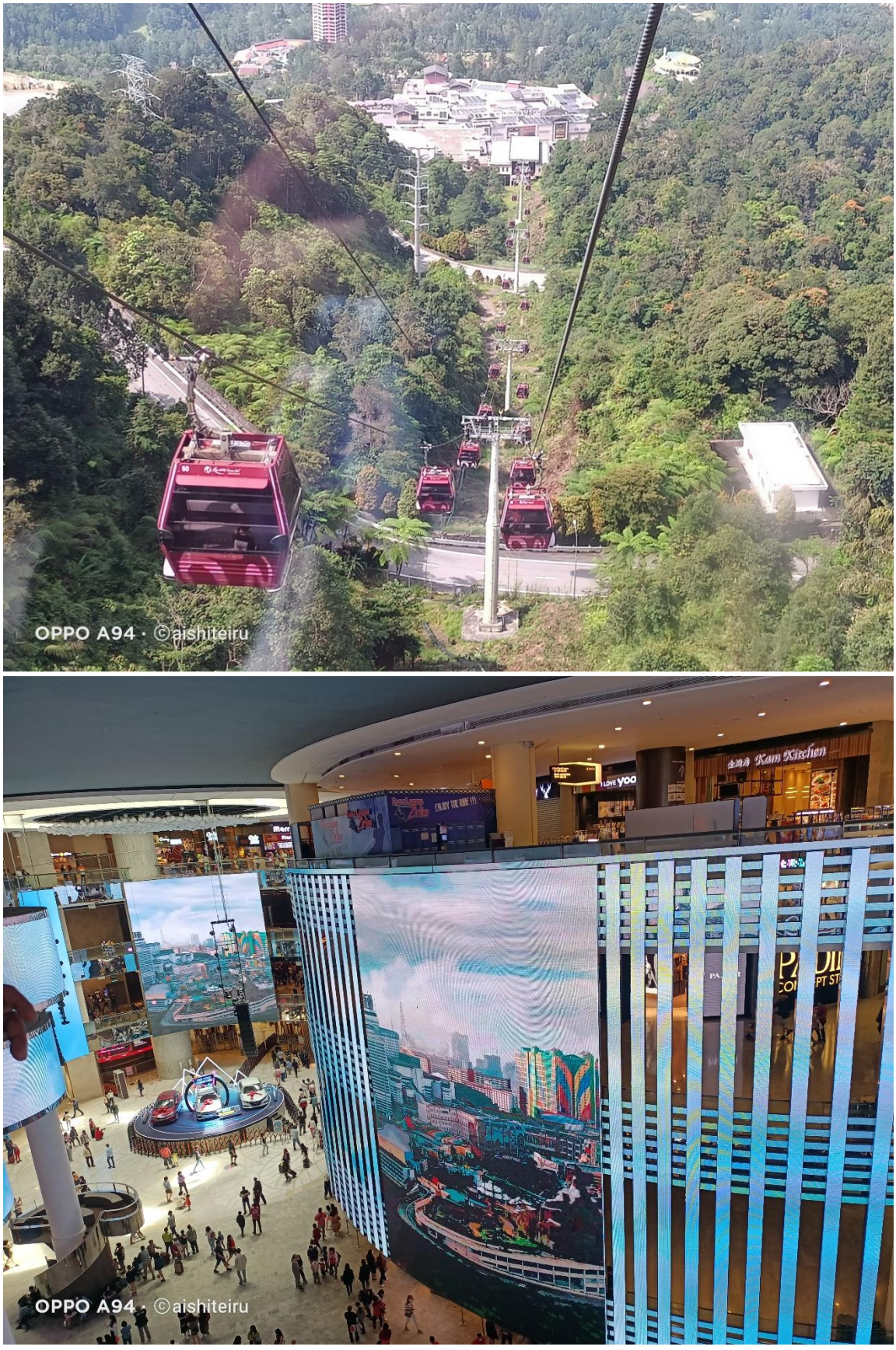

Fig. 27. Serene view and astonishing shopping mall of Genting Highlands in Mt. Ulu Kali in Pahang state.

Fig. 28. The iconic Petronas Tower in Kuala lumpur, Malaysia.

4.5. Session 5. Detection and Characterization of Begomovirus using LAMP-PCR Assay

The second week of the training workshop started with another interesting activity on Begomovirus detection, the Loop-mediated isothermal amplification (LAMP) which was introduced by Dr. Masashi Ugaki. Dr. Ugaki lectured two important topics on LAMP; 1) Introduction to LAMP-PCR: Principles, Applications and Limitations, and 2) Detection of Begomovirus using LAMP-PCR assay. During his first lecture, participants were able to know more about LAMP, how the technology was first developed and its potential application to other pathogens aside from plant viruses. Dr. Ugaki emphasized the principles of LAMP and how this new technology could be used under field condition with compromising the detection sensitivity. He showed the actual field use of LAMP using the recorded video done by his research group (Fig. 29). The lecture also discussed simple tools that can be applied in the absence of electricity in the field. After the two introductory lectures, a laboratory practical was conducted. Dr. Ugaki first demonstrated the Dry LAMP kit and the Wet LAMP protocol and how to prepare and store the required reagents. Due to the expensive reagents of LAMP kit, only limited number of samples were processed. Each group was assigned to perform dry and wet LAMP protocols with positive and negative samples. Following the provided protocol, participants were able to check the sensitivity of LAMP via visual inspection in UV lamp and through gel electrophoresis (Fig. 30).

On the next day, another lecture was given by Dr. Ugaki on primer design for LAMP. He mentioned the importance of at least four sets of primers including the loop primers for the specific detection of plant viruses. He also compared the LAMP primers with that of the conventional PCR primers. He emphasized how those primers work and amplify with high sensitivity the target specific viruses.

Due to the false positive and negative results observed during the first run of LAMP assay, a revised protocol was followed. Homemade wet LAMP and Dry LAMP were performed by each group and were able to obtained the expected result. During this activity participants learned the importance of pipetting errors that are crucial in any DNA detection assays. Pipetting of samples and reagents has brought to the consciousness of each participant that it may play a very important role in the sensitivity of LAMP and even in conventional PCR assay (Fig. 31).

Fig. 29. Prof. Masashi Ugaki discussing about the principles and application of the Loop-mediated isothermal amplification or LAMP.

Fig. 30. Prior to LAMP hands-on activity, Prof. Ugaki explained every detail about the reagents composition and required volume for the dried LAMP kit (above). Careful pipetting of reagents is crucial in performing LAMP (below).

Fig. 31. More of the LAMP hands-on activities (top to middle left) and discussion from Prof. Ugaki (middle right). Sample LAMP results viewed under UV lamp and gel electrophoresis (below left and right, respectively).

4.6. Session 6. Strategies in Protecting Crops from Begomovirus

This session focused on the integrated pest management (IPM) approaches to lessen the incidence of Begomovirus-related diseases on crops. Lectures on IPM with emphasis on biological control agents against insect vectors were discussed by Dr. Pinili. She discussed the commercially available biological control agents including parasitoids and predators of whitefly as well as some entomopathogenic fungi (EPF) of *B. tabaci*. How such biological control agents kill and parasitize the insect vector was also mentioned. A supplemental lecture was also given by Dr. Pinili on the use of FTA plant card for storage and its downstream application on PCR assay.

Also, part of this session was the direct detection of Begomovirus from *B. tabaci* collected during the field visit and from the greenhouse transmission experiment. Using the DNA extraction protocol provided by the Plant Biosecurity Division, *B. tabaci* from the greenhouse were found infected with Begomovirus (Fig. 32).

Fig. 32. Some of the activity highlights during DNA extraction from whitefly, *Bemisia tabaci* and detection of Begomovirus using universal primer

4.7. Session 7. Data Collection

Viewing of results and interpretation of data were done during this session. Each group was tasked to prepare a report of all the practical laboratory and field activities during the two-week training workshop course. Results were consolidated, interpreted and a conclusion and recommendations were drawn in a PowerPoint presentation.

4.8. Session 8. Post-Evaluation and Closing Ceremony

A post-evaluation test was given prior to group reporting. Sets of questions were solicited from each resource person to determine how each participant learned and understand the topics discussed during lecture and laboratory sessions (Fig. 33). After the post-evaluation test, each group was given a 30-minute presentation and 2 minutes for the question and answer. Group reports consisted of introduction, methodology on how they execute each activity, presentation of results based on their analyses, and conclusion and recommendation (Fig. 34 & 35).

After the fruitful discussions during the group report, the venue at CMDV was arranged for the closing ceremony. The closing ceremony was attended by the Director of the Plant Biosecurity Division Mrs. Rosmawati Selamat and the rest of the technical and administrative support staff of DOA. Mrs. Selamat expressed here gratitude to the organizing team particularly to APHCN-ASEANET and JAIF (Fig. 36). Then, followed by a closing remark from Dr. Pinili as the Regional Training Coordinator. An utmost appreciation and sincere thanks to all the people and organizations behind the success of the training workshop were highlighted. Dr. Pinili also emphasized that despite of some shortcomings of the concluded training workshop, the most important thing is the learning principles behind those techniques being taught despite of some differences. The whole training team expressed high expectations to each participant to further cultivate their skills and disseminate the knowledge they have learned during the training course.

Two participants were invited to give their response on behalf of all participating countries. Ms. Russ-Uzi Mayenne Ebor from the Philippines and Mr. Muhd Azhari Bin Mohammad Zain from Singapore expressed their overwhelming thanks to the organizing team and collaborating institutes. They also showed how this training workshop would help their home country and institutions in terms of plant virus diagnosis. They are also looking forward to another training workshop in the future (Fig. 37).

Issuance of certificate of appreciation was done starting from the Chairperson of APHCN-ASEANET, Dr. Soetikno Sastroutomo, Training Coordinator, Dr. Pinili and to the resource person Dr. Ugaki. Certificates of completion were then handed over to each participant and another certificate of appreciation to the technical team members (Fig. 38 to 41).

The ceremony was concluded by a group photo with all the participants and resource persons and organizing team (Fig. 42).

Fig. 33. Post-evaluation test given during the last day of the training workshop.

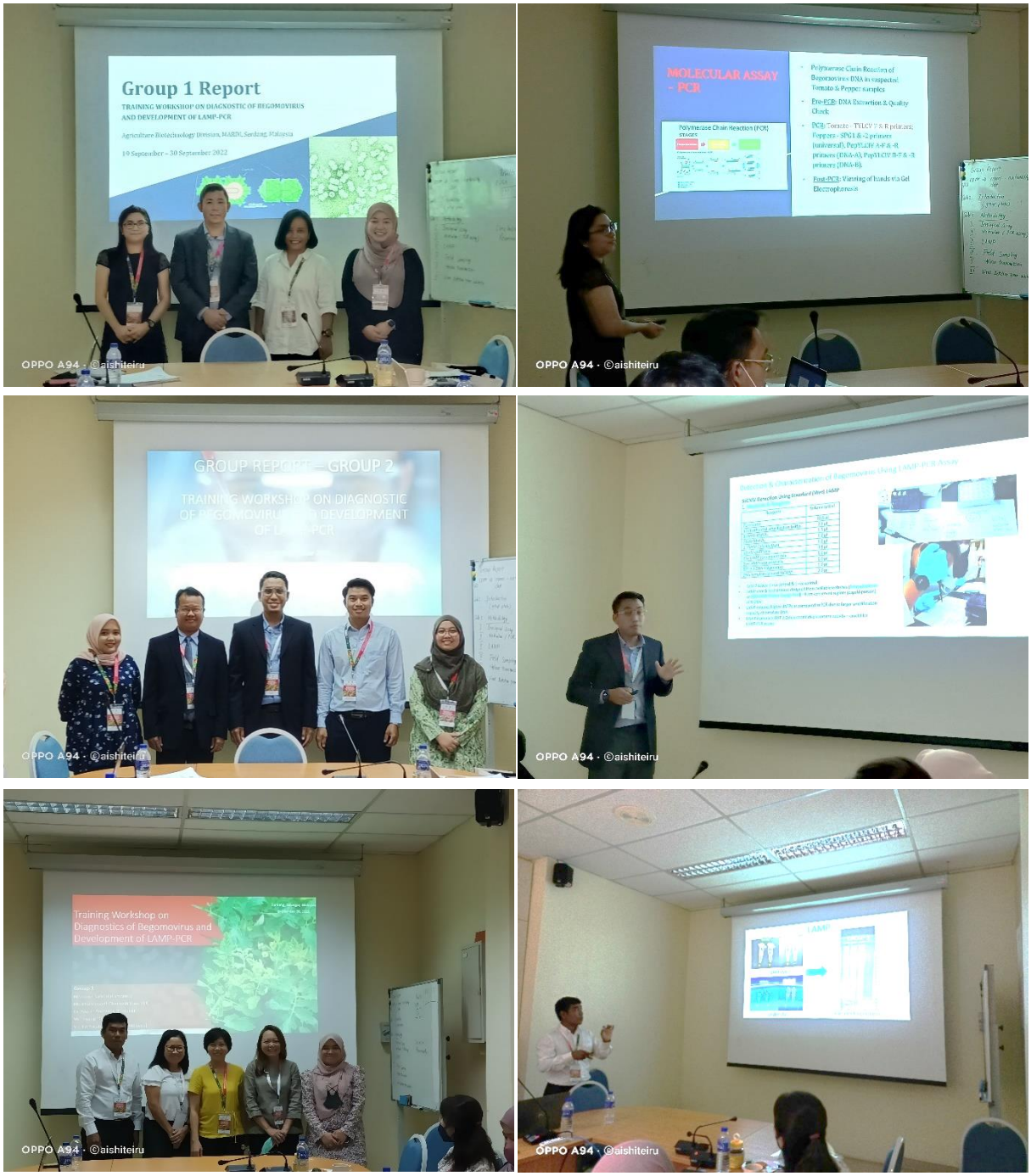

Fig. 34. Activity accomplishment reports from Group 1 (top), Group 2 (middle) and Group 3 (bottom).

Fig. 35. Accomplishment report from Group 4 (top) and Group 5 (middle) and more discussions from Prof. Ugaki regarding the LAMP technology (bottom).

Fig. 36. During the ceremony the Director of Plant Biosecurity Division, Department of Agriculture Malaysia, Mrs. Rosmawati Selamat delivers her congratulatory message to the participants and organizing team, and a closing remarks from the Regional Training Coordinator, Dr. Marita S. Pinili.

Fig. 37. Response on behalf of the 23 participants is represented by Mr. Muhd Azhari Bin Mohammad Zain (Singapore) and Ms. Russ-Uzi Mayenne Ebora (Philippines), top left and right, respectively. Awarding of certificate of appreciation to Dr. Marita S. Pinili (middle left), Prof. Masashi Ugaki (middle right), Dr. Sastroumo Soetikno (bottom left), and a certificate of completion to Ms. Adi Lisea binti Mohd Addly (Brunei Darussalam) (bottom right).

Fig. 38. Awarding of Certificates of Completion to Ms. Nurul Hanisah binti Morni (Brunei Darussalam), Mr. Oeurn Samoul (Cambodia), Mr. Mang Socheat (Cambodia), Dr. Jati Adiputra (Indonesia), Ms. Marisa Purba (Indonesia) and Ms. Andika Septiana Suryanighsih (Indonesia), from top to bottom left to right.

Fig. 39. Awarding of Certificates of Completion to Ms. Khonesavanh Chittarath (Laos PDR), Mr. Phanhnouleth Nonthilath (Laos PDR), Mr. Saiffulbahri bin Abdul Mutalib (Malaysia), Dr. Razean Haireen Binti Mohd Razali (Malaysia), Ms. Lai Lee San (Malaysia), Dr. Phone Kyaw Myint (Myanmar), from top to bottom left to right.

Fig. 40. Awarding of Certificates of Completion to Dr. Win Than (Myanmar), Mr. John Ermina (Philippines), Ms. Russ-Uzi Mayenne Ebora (Philippines), Ms. Mary Joy C, Mendoza (Philippines), Mr. Muhd Azhari Bin Mohammad Zain (Singapore), and Dr. Phoowanarth Maneechoat (Thailand), from top to bottom left to right.

Fig. 41. Awarding of Certificates of Completion to Dr. Yuvarin Boontoop (Thailand), Ms. Troung Thi Ly (Vietnam), Mr. Nguyen Hoang Trung Anh (Vietnam), and Certificate of Appreciation to Mr. Freddiewebb B. Signabon (Philippines), from top to bottom left to right.

Fig. 42. Group photo with the 23 participants, training team, resource persons and Director of Plant Biosecurity Division, DOA Malaysia right after the closing ceremony.

4.9. Technical Evaluation

Table 1. showed the pre- and post-evaluation percentage results taken from the sets of questions given during the first and last day of the training workshop. Apparently, majority of the participants were not familiar with the Begomovirus group but they have background serological (ELISA) and molecular (PCR) assays in detecting plant viruses. Based on the scores obtained in the pre-evaluation test, have more knowledge when it comes to managing diseases caused by Begomovirus. High percentage scores (100%) were generated in the post-evaluation which showed mastery and familiarity with topics discussed particularly about Geminiviridae and the associated insect vectors. Understanding the concept and principles of ELISA, PCR and LAMP were also enhanced and clearly comprehended based on the percentage scores (78 – 95%) obtained from the related questions. However, when it comes to types of samples used for PCR detection of plant viruses and types of virus transmission, the percentage scores were the same both in the pre- and post-evaluation tests. Majority of the answers pertained to components of PCR assay and modes of virus transmission.

Participants were also asked to evaluate the workshop based on logistical arrangement. All respondents expressed that they met their expectations wherein the topics given in the program were covered comprehensively and they will adopt the different techniques for Begomovirus detection and identification in their home country. Some also commended the workshop in terms of having them good connection with the Japanese and ASEAN experts on Begomovirus. Some of the learnings that they have gained from the training workshop are the following:

- Apply the skills and knowledge learned to colleagues, junior staff including co-researchers and technicians.
- Adopt the LAMP assay in their country for the quarantine services.
- Develop plant virus and insect vector detection laboratory and propose to upgrade their facilities for more activities on virus detection using PCR and LAMP, and request additional funds.
- Organize similar workshop for regional and/or provincial crop protection offices.
- Disseminate the training materials to institutions/offices as reference materials and give recommendations on their organizations.

Lecture and laboratory sessions were rated separately based on the rating scale provided (5 – Excellent, 4 – Good, 3 – Average, 2 – Fair, and 1 – Poor). Lectures sessions received 63.0% Excellent, 33.6% Good, and 3.4% Average ratings based on the set criteria (Table 2.1.). Similarly, the laboratory sessions obtained an average of 58.7% Excellent, 38.1% Good, and 3.2% Average ratings (Table 2.2.). Majority of the excellent ratings was based on the learning objectives that the participants met, relevance of the activities to their job, and an opportunity to build a network with colleagues.

Regarding the evaluation of the main speakers or facilitators, all resource speakers obtained high percentage scores with 77.0 – 80.0% Excellent rating and 19.2 – 23.2% Good rating. High Excellent ratings reflect to the resource persons mastery or expertise on the assigned topics, ability to engage and maintain the audience interest, organization and preparedness during the lecture and laboratory sessions.

Evaluation on the logistics was also consolidated. This will guide the training team on their future activities and will help set the standards for conducting similar or related training workshops. Majority of the comments were from the accommodation, changing of venue for the lecture and laboratory activities, and insufficient samples and equipment during the practical. These factors somehow extended the waiting period and did not maximize the time schedule in the program. Despite of these the training workshop received an overall rating of 33.3% Good rating in the accommodation, 44.4% Good rating in the training venue, 58.3% Good in travel arrangements, 68.0% Excellent rating in field trip, and 41.7% Good rating when it comes to food and refreshments.

Table 1. Percentage pre – and post – evaluation results.

Questions	Pre-Evaluation (%)	Post Evaluation (%)
1. Aside from Begomovirus, give four (4) other genera of plant viruses that belong to the Family *Geminiviridae*.		
a. All correct answers	13.0	60.9
b. 3 correct answers		26.1
c. 2 correct answers		4.3
d. 1 correct answer	8.7	
e. No correct answer	47.8	8.7
f. No answer	30.4	
2. The name Begomovirus is derived from what virus type species?		
a. Correct answer	17.4	69.6
b. No correct answer	34.8	30.4
c. No correct answer	47.8	
3. What are the three (3) known insect vectors of viruses under the Family *Geminiviridae*?		
a. 3 correct answers	17.4	100.0
b. 2 correct answers	52.2	
c. 1 correct answer	21.7	
d. No correct answer	4.3	
e. No answer	4.3	
4. Give one virus species under the Begomovirus group.		
a. Correct answer	65.2	100.0
b. No correct answer	21.7	
c. No answer	13.1	
5. What is the principle of serology-based detection method?		
a. Correct answer	52.2	95.7
b. No correct answer	26.1	4.3
c. No answer	21.7	
6. What does ELISA stand for?		
a. Correct answer	30.4	78.3
b. No correct answer	60.9	21.7
c. No answer	8.7	
7. What laboratory equipment are needed to run ELISA method?		
a. At least 2 correct answers	30.4	65.2
b. Only 1 correct answer	52.2	8.7
c. No correct answer	13.1	26.1
d. No answer	4.3	

8.	What is the most important component in the PCR method that determine the specificity of the detection?		
	a. Correct answer	82.6	91.3
	b. No correct answer	8.7	8.7
	c. No answer	8.7	
9.	Give examples of several sample types that can be used for the detection of plant pathogens (especially viruses) using PCR method.		
	a. At least 2 correct answers	34.7	39.1
	b. Only 1 correct answer	13.1	4.3
	c. No correct answer	26.1	47.8
	d. No answer	26.1	8.7
10.	What does LAMP stand for?		
	a. Correct answer	39.1	78.3
	b. No correct answer	39.1	21.7
	c. No answer	21.8	
11.	Describe one of the differences between PCR and LAMP.		
	a. Correct answer	47.8	95.7
	b. No correct answer	21.7	4.3
	c. No answer	30.4	
12.	What is the reason/basis for the difference you listed to question 11 above?		
	a. Correct answer	43.5	73.7
	b. No correct answer	8.9	17.4
	c. No answer	47.8	8.9
13.	One day you want to run LAMP reactions in an open field to detect putative virus pathogen, but you cannot operate any electric apparatus controlling LAMP reaction temperature (including heating block, a thermal cycler, or an air incubator etc.), since there is no electric source in the field. How can you control LAMP reaction temperature there?		
	a. Correct answer	39.2	100.0
	b. No correct answer	26.1	
	c. No answer	34.7	
14.	Describe one of the limitations/drawbacks of LAMP.		
	a. Correct answer	47.8	95.7
	b. No correct answer	8.9	
	c. No answer	33.3	4.3
15.	How are you going to manage crops from possible Begomovirus infection?		
	a. All correct answers	52.2	95.7
	b. At least 2 correct answers		
	c. 1 correct answer	30.4	4.3
	d. No correct answer	8.9	
	e. No answer	8.9	
16.	What are the general processes involved in the transmission of plant viruses by their insect vector?		
	a. All correct answers	8.9	59.4
	b. At least 2 correct answers	34.7	21.7
	c. 1 correct answer	26.1	
	d. No correct answer	8.9	8.9
	e. No answer	21.7	
17.	List the different types of plant virus transmission		
	a. All correct answers	8.9	
	b. At least 2 correct answers	13.1	26.1
	c. 1 correct answer	8.9	

	d. No correct answer/ answer mode of transmission instead	34.7	17.4
	e. No answer	30.4	
18.	Briefly explain the process of persistent, circulative transmission of viruses		
	a. Correct answer	47.8	82.6
	b. No correct answer	26.1	
	c. No answer	26.1	17.4
19.	How would you distinguish between sweet potato whitefly (*Bemisia tabaci*) and greenhouse whitefly (*Trialeurodes vaporarium*)?		
	a. Correct answer	n/a	82.6
	b. No correct answer	n/a	13.0
	c. No answer	n/a	4.4

Table 2. Post – evaluation summary

1. Lecture Sessions

Criteria	Percentage obtained relative to the Rating Scale				
	5 - Excellent	4 - Good	3- Average	2 – Fair	1 - Poor
• Activity met the stated learning objectives	73.9	26.1			
• Content was relevant to my job	69.5	30.5			
• Content was presented in a well-paced, structured format	54.5	40.9	4.6		
• Content was pitched at the right level for the audience	45.5	50.0	4.5		
• An appropriate mix of techniques was used to convey the content (e.g., methods, group work, discussion, lecture, etc.,)	68.2	31.8			
• Notes/materials were good quality and will be useful for my job	59.1	27.3	13.6		
• I can immediately apply my learning to my job	60.9	34.8	4.3		
• I had ample opportunity to network with colleagues	72.7	27.3			
Average	63.0	33.6	3.4		

2. Laboratory Sessions

Criteria	Percentage obtained relative to the Rating Scale				
	5 - Excellent	4 - Good	3- Average	2 – Fair	1 - Poor
• Activity met the stated learning objectives	65.2	34.8			
• Content was relevant to my job	69.6	30.4			
• Content was presented in a well-paced, structured format	47.8	47.8	4.4		
• Content was pitched at the right level for the audience	52.2	43.7	4.3		

• An appropriate mix of techniques was used to convey the content (e.g., methods, group work, discussion, lecture, etc.,)	56.5	39.1	4.4
• Notes/materials were good quality and will be useful for my job	52.2	43.5	
• I can immediately apply my learning to my job	60.9	30.4	4.4
• I had ample opportunity to network with colleagues	65.2	34.8	
Average	58.7	38.1	3.2

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

Prof. Masashi Ugaki

Rating Scale	Percentage
5 - Excellent	77.0
4 - Good	23.0
3 - Average	
2 - Fair	
1 - Poor	

Prof. Sri Hendrastuti Hidayat

Rating Scale	Percentage
5 - Excellent	77.1
4 - Good	22.9
3 - Average	
2 - Fair	
1 - Poor	

Dr. Marita S. Pinili

Rating Scale	Percentage
5 - Excellent	80.1
4 - Good	19.2
3 - Average	0.7
2 - Fair	
1 - Poor	

4. This activity might be more useful if:

- the TW extended to 3 – 4 weeks
- the discussion on primer design for PCR and LAMP PCR was given more time
- all the participants get to do all the steps and protocol by themselves
- enough equipment was given to participants during the practical to maximize the time
- individual samples i.e., Begomovirus-infected plants from each participating country were permitted to bring and shared during practical

5. General arrangements – Logistics, field trip, etc.,

- Very good and nice logistics arrangement including location for the field trip
- Laboratory activities should not be conducted at Biotechnology Lab, instead do it at Plant Biosecurity Lab of DOA and should have proper coordination between technical staff of MARDI and resource persons.
- Lectures and practical should be conducted in the same venue.
- Arrangement of activities outside the training schedules were a bit messy i.e., maximized the short period of time for too many sites (Genting Highlands, KL Central Market, KLCC)
- Accommodation at MARDI is quite isolated and should be cleaned all the time.

6. Recommendations to future training re: logistical arrangement

- Accommodations should be near to the lecture and laboratory venue, clean and properly maintained.
- Food and refreshments are of good quality but the quantity sometimes not enough for all participants.
- Hard copies of presentations should be ahead of the training/during the start of the training.

7. Overall Rating

Criteria	Percentage obtained relative to the Rating Scale				
	5 - Excellent	4 - Good	3- Average	2 – Fair	1 - Poor
Accommodation	16.7	33.3	16.7	20.8	12.5
Workshop venue/training venue facilities	33.3	44.4	18.5	3.7	
Travel arrangements	41.7	58.3			
Field trip	68.0	32.0			
Food/refreshments	29.2	41.7	20.8	8.3	

8. Other Comments
    - The training workshop was successfully organized.
    - Set up a networking group for all participants of this JAIF funded training workshop
    - Food in the cafeteria and training venue mostly good and delicious but the quantities were not sufficient for all participants.

CHAPTER 5

Recommendations

To further strengthen the participants capability on diagnostics and development of LAMP for Begomovirus detection, the technical team recommends the following training course program for future implementation;

1. Training workshop on primer design for LAMP PCR.
2. Training workshop on LAMP for Pepper yellow leaf curl virus (PYLCV), Rice tungro virus, and other viruses transmitted by Mealy bugs
3. Advance course on Phytobacteriology, Nematodes and Scale Insects
4. Detection of seed-borne disease