ATTACHMENT 1

I. Basic Information

A. Project Title:

Training Workshop on Begomovirus: Detection, Identification and Etiology and Development of Loop-Mediated Isothermal Amplification (LAMP) Kit as a Diagnostic Tool

B. 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

C. 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

D. Implementing Agencies

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
Agricultural Biotechnology Division, MARDI, Ministry of Agriculture and Food Industry, Malaysia

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

Funding Agency

Japan - ASEAN Integration Fund (JAIF) through ASEAN Secretariat

E. Project Duration: Two (2) weeks

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

II. 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* and *Topocuvirus* (Brown et al. 2012). 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 cause to major life-sustaining crops in the world. With this proposed training workshop on the diagnostic of begomovirus, recipients or participants who are working as the forefronts of crop protection agencies and plant quarantine departments will gain pertinent knowledge on basic information about the virus, its importance, detection tools, manner of virus transmission and disease spread.

B. Course Description

This **"Training Workshop on Begomovirus: Detection, Identification and Etiology and Development of Loop-Mediated Isothermal Amplification (LAMP) kit as a Diagnostic Tool"** 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"**. 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 proposed 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 molecular and sero-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 Polymerase Chain Reaction (PCR) assay and Loop-Mediated Isothermal Amplification (LAMP), virus transmission via insect vector, whitefly (*Bemisia tabaci* Genn.) and the epidemiological study of virus spread and vector flight pattern using STELLA model, and management options in avoiding and suppressing disease development. Interactive lecture discussions and practical or hands-on laboratory activities will be imposed to achieve the training workshop's objectives. Field tour or visits will be 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., the Division of Agriculture Biotechnology, 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.

C. Objectives

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 molecular, sero-molecular and LAMP PCR assays; and learn the manner of virus transmission via insect vector(s).

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 gain insight on the epidemiology of Begomovirus and flight pattern of whitefly as insect vector using STELLA model.
8. To learn how to protect crops from Begomoviruses through cultural control, resistant varieties, virus-free planting materials and genetically modified (GM) crops.
9. 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 sero-molecular and molecular assays.
3. To detect Begomoviruses from plant samples and insect vector, whitefly using sero-molecular and Polymerase Chain Reaction (PCR) assays.
4. To demonstrate the application of Loop-Mediated Isothermal Amplification (LAMP) – PCR technique in detecting Begomovirus.
5. To differentiate morphologically common insect-vector of Begomovirus.
6. To demonstrate how Begomoviruses are transmitted into host plants using insect vector, whitefly.

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
- 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 Philippines and neighbouring regions
- Lecture 3. Status and diversity of Begomovirus in East and Southeast Asia
- Practical 1. Preparation of buffer, reagents and other materials for serological and molecular detection assays

SESSION 3. Detection and characterization of Begomoviruses

- Lecture 4. Symptom recognition and disease assessment
- Lecture 5. Detection of Begomovirus(es): Serological Approach
- Lecture 6. Detection of Begomovirus(es): Molecular Approach
- Lecture 7. Detection of Begomovirs(es): LAMP – PCR assay
- Practical 2. Extraction of virus nucleic acid
- Practical 3. Detection of Begomovirus(es) using Enzyme-linked immunosorbent assay (ELISA)
- Practical 4. Detection of Begomovirus(es) using Polymerase chain reaction (PCR) assay.
- Practical 5. Gel electrophoresis and analysis
- Demo 1. Application of LAMP-PCR in the detection and identification of Begomovirus

SESSION 4. Transmission of Begomovirus

- Lecture 8. General concept in the transmission of plant viruses
- Lecture 9. The role of insect-vector whitefly, *Bemisia tabaci* Genn. in the development of diseases and successful spread of Begomoviruses

- Lecture 10. Identification and characterization of whitefly (*Bemisia tabaci* Genn.) and its biotypes
- Lecture 11. Flight pattern of Begomovirus insect vector, *Bemisia tabaci* Genn. and its relationship to the disease spread using STELLA model
- Practical 6. Transmission of begomovirus using insect-vector whitefly, *Bemisia tabaci* Genn.
- Practical 7. Viewing of results
- Demo 2. Introduction to STELLA Model

SESSION 5. Strategies in protecting crops from Begomovirus infection

- Lecture 12. Protecting crops from virus diseases: Integrated Pests Management (IPM)
- Lecture 13. Protecting crops from virus disease: Biological Control Agents against Insect Vectors

E. Training Content and Tentative Schedule

Week 1

Date/Venue/ Time	Topic/ Activity	Resource Person(s)/Facilitator
Pre-Training		
DAY 1. Sunday, September 18, 2022		
	Arrival and billeting at Hotel (TBA)	
Training Proper		
DAY 2. Monday, September 19, 2022		
SESSION 1: OPENING PROGRAM AND INTRODUCTION **Venue: NCPC Auditorium**		
08:00 – 10:00	Registration	Secretariat
	Group Photo	
	Welcome Address	*DOA*
	Message	*MARDI*
10:01 – 10:15	Training Introduction and Overview	Dr. Marita S. Pinili *University Researcher IV, Regional Training Coordinator*
10:16 – 10:30	Introduction of Participants, Trainers and Training Team	*ASEANET*
10:31 – 10:45	Coffee/Tea Break	
10:46 – 11:00	Pre-evaluation Test	Dr. Marita S. Pinili & Ms. Lailatul Jumaiyah Saleh Huddin

SESSION 2. BEGOMOVIRUS: ITS IMPACT ON ECONOMICALLY IMPORTANT CROPS		
11:01 – 12:00	**Lecture 1.** Geminiviridae: Begomovirus group – Classification and Morphology	Dr. Marita S. Pinili *University Researcher IV, NCPC*
12:01 – 13:00	Lunch Break	
13:01 – 14:00	**Lecture 2.** Diseases of economically important crops caused by Begomovirus group: Status and threat in Malaysia and neighbouring regions	Ms. Lailatul Jumaiyah Saleh Huddin *DOA*
14:01 – 15:00	**Lecture 3.** Status and diversity of Begomovirus in East and Southeast Asia	Dr. Marita S. Pinili *University Researcher IV, NCPC*
15:01 – 15:15	Tea/Coffee Break	
15:16 – 17:00	In-country Report	All Participants
18:00 – 20:30	Dinner Reception Venue: TBA	Participants, Resource Persons, Training Team
DAY 3. Tuesday, September 20, 2022		
SESSION 3. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES		
08:00 – 09:00	**Lecture 4.** Symptom recognition and disease assessment	Dr. Marita S. Pinili *University Researcher IV, NCPC*
09:01 – 09:30	Tea/Coffee Break	
09:31 – 10:30	**Lecture 5.** Detection of Begomovirus(es): Serological Approach	Dr. Sri Hendrastuti Hidayat *Professor, IPB Bogor*
10:31 – 12:00	**Lecture 6.** Detection of Begomovirus(es): Molecular Approach	Dr. Sri Hendrastuti Hidayat *Professor, IPB Bogor*
12:01 – 13:00	Lunch Break	
13:01 – 15:00	**Practical 1**. Preparation of buffer, reagents and other materials for serological and molecular detection assays	Training Team (NCPC, UPLB and DOA)
15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:00	**Practical 1**. Preparation of buffer, reagents ….*continuation*	Training Team (NCPC, UPLB and DOA)

DAY 4. Wednesday, September 21, 2022		
SESSION 3. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES **SESSION 4. TRANSMISSION OF BEGOMOVIRUS**		
08:00 – 09:30	**Lecture 7.** Introduction to LAMP-PCR: Principles, Applications and Limitations	Dr. Masashi Ugaki *Professor, University of Tokyo*
09:31 – 10:00	Tea/Coffee Break	
10:01 – 11:00	**Lecture 8.** Detection of Begomovirs(es) using LAMP – PCR assay	Dr. Masashi Ugaki *Professor, University of Tokyo*
11:01 – 12:00	**Lecture 9.** 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. Marita S. Pinili/Entomologist from DOA
12:01 – 13:00	Lunch Break	
13:01 – 15:00	**Practical 2.** Extraction of virus nucleic acid	Dr. Sri Hendrastuti Hidayat *Professor, IPB Bogor* Training Team (NCPC & DOA)
15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:00	**Practical 3**. Detection of Begomovirus(es) using Enzyme-linked immunosorbent assay (ELISA)	Dr. Sri Hendrastuti Hidayat *Professor, IPB Bogor* Training Team (NCPC & DOA)
DAY 5. Thursday, September 22, 2022		
SESSION 3. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES **SESSION 4. TRANSMISSION OF BEGOMOVIRUS**		
08:00 – 09:30	**Lecture 10**. Identification and characterization of whitefly (*Bemisia tabaci* Genn.) and its biotypes	*Dr. Marita S. Pinili/Entomologist from DOA
09:31 – 10:00	Tea/Coffee Break	
10:01 – 12:00	***Lecture 11.** Flight pattern of Begomovirus insect vector, *Bemisia tabaci* Genn. and its relationship to the disease spread using STELLA model (*To be decided*)	*Disease Epidemiology Expert from DOA

12:01 – 13:00	Lunch Break	
13:01 – 15:00	**Practical 3/Demo1**. Application of LAMP-PCR in the detection and identification of Begomovirus	Dr. Masashi Ugaki *Professor, University of Tokyo*

15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:00	**Demo 1**. Application of LAMP-PCR in the detection and identification of Begomovirus *…continuation*	Dr. Masashi Ugaki *Professor, University of Tokyo*
DAY 6. Friday, September 23, 2022		
Field Visit/Sample Collection		
08:00	Leave MARDI Guest House	*Logistic Team*
09:00	Arrival at Farm	
09:01 – 09:30	Tea/Coffee Break	
09:31 – 12:00	Sample collection	
12:01 – 14:00	Lunch Break	
14:01 – 16:00	Sample Collection	
17:00	Arrive MARDI Guest House	
DAY 7. Saturday, September 24, 2022		
Leisure Trip, Kuala Lumpur		
DAY 8. Sunday September 25, 2022		
REST DAY		

**Tentative*

Week 2

Date/Venue/ Time	**Topic/ Activity**	**Resource Person(s)/Facilitator**
DAY 9. Monday, September 27, 2022		
SESSION 3. DETECTION AND CHARACTERIZATION OF BEGOMOVIRUSES **SESSION 4. TRANSMISSION OF BEGOMOVIRUS**		
08:00 – 09:30	**Practical 4**. Detection of Begomovirus(es) using Polymerase chain reaction (PCR) assay.	Dr. Sri Hendrastuti Hidayat *Professor, IPB Bogor* and Training Team (NCPC & DOA)
09:31 – 10:00	Tea/Coffee Break	
10:01 – 12:00	**Practical 4**. Detection of Begomovirus(es) using Polymerase chain reaction (PCR) assay…*continuation*	Dr. Sri Hendrastuti Hidayat *Professor, IPB Bogor* and Training Team (NCPC & DOA)
12:01 – 13:00	Lunch Break	

13:01 – 15:00	**Practical 4**. Detection of Begomovirus(es) using Polymerase chain reaction (PCR) assay…*continuation*	Dr. Sri Hendrastuti Hidayat *Professor, IPB Bogor* and Training Team (NCPC & DOA)
15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:00	**Practical 5.** Gel electrophoresis and analysis	Dr. Sri Hendrastuti Hidayat *Professor, IPB Bogor* and Training Team (NCPC & DOA)
DAY 10. Tuesday, September 27, 2022		
SESSION 4. TRANSMISSION OF BEGOMOVIRUS		
08:00 – 09:30	**Practical 6**. Transmission of begomovirus using insect-vector whitefly, *Bemisia tabaci* Genn.	Training Team (NCPC & DOA)
09:31 – 10:00	Tea/Coffee Break	
10:01 – 12:00	**Practical 6**. Transmission of begomovirus using insect-vector whitefly, *Bemisia tabaci* Genn….*continuation*	Training Team (NCPC & DOA)
12:01 – 13:00	Lunch Break	
13:01 – 15:00	**Practical 6**. Transmission of begomovirus using insect-vector whitefly, *Bemisia tabaci* Genn….*continuation*	Training Team (NCPC & DOA)
15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:00	**Practical 6**. Transmission of begomovirus using insect-vector whitefly, *Bemisia tabaci* Genn….*continuation*	Training Team (NCPC & DOA)
DAY 11. Wednesday, September 28, 2022		
SESSION 5. STRATEGIES IN PROTECTING CROPS FROM BEGOMOVIRUS INFECTION		
08:00 – 09:00	**Lecture 12.** Protecting crops from virus diseases: Integrated Pests Management (IPM)	Dr. Marita S. Pinili *University Researcher IV, NCPC*
09:01 – 09:30	Tea/Coffee Break	
09:31 – 10:30	**Lecture 13.** Protecting crops from virus diseases: Biological control agents insect vectors	Dr. Marita S. Pinili *University Researcher IV, NCPC*
10:31 – 12:00	***Demo 2.** Introduction to STELLA Model SPIDTECH for insect-vector identification	Disease Epidemiology Expert
12:01 – 13:00	Lunch Break	

13:01 – 15:00	**Practical 7**. Detection of Begomovirus from insect-vector	Training Team (NCPC & DOA)
15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:00	**Practical 7.** Detection of Begomovirus from insect-vector…*continuation*	Training Team (NCPC & DOA)
DAY 12. Thursday, September 29, 2022		
SESSION 6. DATA COLLECTION		
08:00 – 09:00	**Practical 7.** Detection of Begomovirus from insect-vector…*continuation*	Training Team (NCPC & DOA)
09:01 – 09:30	Tea/Coffee Break	
09:31 – 12:00	**Practical 8.** Viewing of Results	Training Team (NCPC & DOA)
12:01 – 13:00	Lunch Break	
13:01 – 15:00	**Practical 9.** Consolidation of data	All Participants
15:01 – 15:30	Tea/Coffee Break	
15:31 – 17:00	**Practical 9.** Consolidation of data….*continuation*	All Participants
DAY 13. Friday, September 30, 2022		
SESSION 7. POST-EVALUATION AND CLOSING CEREMONY		
08:00 – 09:00	Post-test evaluation	Dr. Marita S. Pinili
09:01 – 09:30	Tea/Coffee Break	
09:31 – 12:00	**Practical 10**. Group Report	Groups 1 & 2
12:01 – 13:00	Lunch Break	
13:01 – 15:00	**Practical 10**. Group Report…*continuation*	Groups 3, 4 & 5
15:01 -15:30	Tea/Coffee Break	
15:31 – 16:30	Presentation of Certificates	Dr. Marita S. Pinili & Ms. Lailatul Jumaiyah Saleh Huddin
16: 31 – 16:45	Response from Participants	Two representatives
16: 46 – 17:00	Closing Message	Dr. Soetikno Sastroutomo *Secretary, APHCN –ASEANET*
DAY 14. Saturday, October 1, 2022		
DEPARTURE		