Report of Attachment Program
Advanced Diagnostics of Plant Viruses

at
Laboratory of Tropical Plant Protection
Tokyo University of Agriculture (Tokyo NODAI), Japan
October 26 – December 25, 2015

By

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Organized by:

Tokyo University of Agriculture
(Tokyo NODAI), Japan

In Collaboration with:

bionet aseanet

ASEAN Network on Taxonomy

2016

Attachment Program:
Advanced Diagnostic on Plant Viruses

(Taxonomic Capacity Building to Support Market Access for Agricultural Trade in the ASEAN Region)
Tokyo University of Agriculture, Japan

Duration:
26th October 2015 – 25th December 2015

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1. Background Information

This training ("Training Workshop on the Diagnostics of Plant Viruses") was coordinated by the Institute of Plant Breeding - Crop Science Cluster, College of Agriculture, and the University of the Philippines Los Banos through ASEANET project and Plant Health ASEAN Cooperation Network (APHCN) namely "Taxonomic capacity building to support market access for agricultural trade in the ASEAN region". The project is sponsored by the Japan - ASEAN Integration Fund (JAIF) which will be implemented within two years, includes a number of activities related to trainings and attachment programs.

The project aims to provide a basic understanding of the concepts and practical plant virus, a diagnosis of infected plants, the latest technology and management of diseases involving viral diseases. The topics are as follows: basic knowledge of virus classification, morphology genus of plant viruses, virus transmission, diagnosis is based on symptoms, detection using serological tests (Enzyme-linked immunosorbent assay, ELISA) and molecular techniques (polymerase chain reaction, PCR), virus interests to plant crops in tropical and sub-tropical and virus management methods to prevent the spread of disease.

2. Objectives

The objectives of the attachment program are:

1. To provide diagnostic skills for identification of plant virus disease based on symptoms.
2. To study detection and identification of plant viruses using advanced diagnostic techniques; serological and molecular techniques.
3. To study the transmission of plant viruses from the sources to target hosts.
4. To apply the suitable techniques for plant virus disease management in ASEAN countries.

3. Daily Programs

Date	Programs	Remarks
26th Oct 2015 (Monday)	• Arrived in Tokyo. • Moved into Tokyo NODAI Guest House and visited to the lab. • Short discussion with Prof. Natsuaki on the coming two months attachment schedules.	
27th Oct 2015 (Tuesday)	• All participants were meet up inProf. Hogoken's Laboratory. • Tour around NODAI campus. • Buffer preparation for ELISA test - Phosphate buffer - General Extraction Buffer. - 5X PBST.	The tour was guided by Prof. Natsuaki's students.We have visited the library, Student Coop, administration office and Museum.
28th Oct 2015 (Wednesday)	• Sample collection and symptoms observation on Bamboo virus around NODAI campus. • Detection on Potyvirus from Bamboo samples.	Detection of Potyvirus using ELISA test.
29th Oct 2015 (Thursday)	• Sample collection at the greenhouse (passion fruit, *Passiflora edulis*) • Sap inoculation (back inoculation) of unknown passion fruit virus on healthy passion fruit seedlings. • Sow the 3 types of seeds (passion fruit and beans).	
30th Oct 2015 (Friday)	• Visited to NODAI Museum and Biorium with Prof. Natsuaki. • Attending Tea Ceremony during Festival.	
31st Oct 2015 (Saturday)	• Attending NODAI Festival and welcome party with Professor and students from Laboratory of Tropical Plant Protection.	

2nd Nov 2015 (Monday)	• Preparation of negative stain reagent (PTA buffer). • Sample preparation for electron microscope observation to detect plant virus particles.	
3rd Nov 2015 (Tuesday)	-	National holiday
4th Nov 2015 (Wednesday)	• Detection of Potyvirus from passion fruit using Indirect ELISA technique.	
5th Nov 2015 (Thursday)	• Detection of Potyvirus from taro using Indirect ELISA technique.	
7th–9thNov 2015 (Sat– Mon)	• ISSAAS Conference and trip to Mount Fuji.	
10th Nov 2015 (Tuesday)	-	Off day
11th Nov 2015 (Wednesday)	• Briefing and orientation • DNA extraction from BBTV-infected banana. • PCR and gel electrophoresis.	
12th Nov 2015 (Thursday)	• Pre-lab discussion • Short topic presentation on plant parasitic nematodes. • Gel electrophoresis. • Extraction and detection of BBTV from fresh and old banana sample and abaca samples.	
13th Nov 2015 (Friday)	Post-lab discussion	
16th Nov 2015 (Monday)	• Gel electrophoresis of BBTV PCR products. • Impregnation and extraction of virus nucleic acid from FTA plant card.	
17th Nov 2015 (Tuesday)	• PCR assay of DNA from FTA plant card. • Gel electrophoresis. • Gel cut, purification of DNA products.	
18th Nov 2015 (Wednesday)	• Extraction of BBTV directly from insect vector, *Pentalonia nigronervosa*. • PCR assay.	

	• Ligation of purified DNA using pGEM vector.	
19th Nov 2015 (Thursday)	• Extraction of BBTV DNA from aphids impregnated on FTA card. • PCR assay. • Gel electrophoresis. • Transformation of ligated plasmid.	
20th Nov 2015 (Friday)	• Checking of colonies/transformants. • Post-lab discussion.	
23rd Nov 2015 (Monday)	• Picking of transformed colonies. • Culturing of *E. coli* colonies into LB medium.	
24th Nov 2015 (Tuesday)	• Mini-prep. • Insert check. • Visit to electron microscope laboratory.	
25th Nov 2015 (Wednesday)	• Precipitation. • DNA sequencing.	
26th Nov 2015	• Special lecture. • Sequence analysis.	Lecture on bioanalysis and phylogenetic tree by Dr. Noriko Furuya from DDBJ.
27th Nov 2015 (Friday)	Post-lab discussion.	
30th Nov 2015 (Monday)	• Discussion with Sensei about the Yokohama Trip and December schedules. • Halal Seminar	
1st Dec 2015 (Tuesday)	• Analyze the sequencing results using Bioseq software, BLAST.	
2ndDec 2015 (Wednesday)	• Preparation of LB Medium	
3rd Dec 2015 (Thursday)	• Attachment at Utsunomiya University on dsRNA Extraction of Cucumber Mosaic Virus (CMV).	Under supervision by Prof.Tomohide Natsuaki.
4th Dec 2015 (Friday)	Post-lab discussion	
7th Dec 2015 (Monday)	• Potyvirus RNA Extraction from passion fruit	
8th Dec 2015 (Tuesday)	• cDNA synthesis	

9th Dec 2015 (Wednesday)	• Gel electrophoresis of Potyvirus cDNA	
10th Dec 2015 (Thursday)	• cDNA synthesis from Potyvirus (repeat). • Preparation of chemical (chloride for TB) and TB medium.	cDNA synthesis was repeated for second time because we could not get the good quality of cDNA product.
11th Dec 2015 (Friday)	• Visit to Yokohama Plant Protection Section and Research Centre.	
14th Dec 2015 (Monday)	• Gel electrophoresis • Gel Extraction (Purification of cDNA)	
15th Dec 2015 (Tuesday)	• Ligation • SDS-Page Protocol • Western Blot Protocol • Preparation of reports and final presentation.	SDS-Page and Western Blot laboratory protocol was conducted by Chong Sensei.
15th Dec 2015 (Wednesday)	• Transformation of *E. coli*	National holiday
16th Dec 2015 (Thursday)	• Checking the *E. coli* colonies. • Culturing of *E. coli* on TB Medium (Repeat 2nd time).	The colonies obtained on TB medium is not enough to be used for Mini Prep and sequencing. Therefore, 3rd time ligation was done to get better result.
17th Dec 2015 (Friday)	• Purification of dsRNA • Attending PhD Thesis Defends from Ayaka Uke.	
21st Dec 2015 (Monday)	• Final presentation for 3 ASEAN participants on attachment program. • Ligation (3rd time)	
22nd Dec 2015 (Tuesday)	• Transformation (3rd time) • Finishing all reports and submit to Sensei.	
23rd Dec 2015 (Wednesday)	• Checking the *E. coli* colonies. • Culturing *E. coli* in TB medium (3rd time).	
24th Dec 2015 (Thursday)	• Mini Prep. • Move out from NODAI Guest House and went to Narita Hotel.	
25th Dec 2015 (Friday)	• Going back to Malaysia.	

Saturday and Sunday – Free day

4. Activities during Attachment Program

(a) Laboratory Studies

(i) Buffer Preparation for ELISA Test

We were assisted by the Hogoken's students for the preparation of phosphate buffer, general extraction buffer, and 5X PBST for ELISA test. We also learned the preparation culture medium for the *E. coli* (TB and LB medium). These two types of media will be used for *E. coli* culturing and cloning procedure. All ingredients and preparation protocols for buffers are shown in Appendix 1 – 2.

(ii) Symptoms Observation and Sample Collection (Refer to Appendix 3 – 4)

Several types of plants were collected from NODAI and outside the university campus for detection of the virus infection. First sample which was collected around the NODAI is bamboo plant (*Pleioblastus chino)*. The bamboo leaves were suspected to be infected by Potyvirus. The symptoms were showed streaking along the leaves and yellow mosaic pattern compare with healthy bamboo leaves. These symptoms are similar to the sugarcane viruses.By using ELISA method, it will help to differentiate the viruses.

Second sample was taro leaves. The samples were taken by one of the student from other prefecture and from the growth chamber at Hogoken's laboratory. The leaves were showed mosaic pattern on the leaves. The samples were subjected for Potyvirus detection using ELISA test

Third sample was passion fruit. The leaves were taken from glasshouse at NODAI. The leaves also used for sap inoculation on healthy passion fruit leaves. The symptoms were not obvious.The leaves were used for ELISA test for detection of Potyvirus.

Banana leaves which were infected with Banana bunchy top virus (BBTV) were taken inform the growth chamber at Hogoken's laboratory. The plants were highly infested with the aphid (*Pentalonia negronervosa*). Only

leaveswere used for the detection and identification of BBTV using molecular technique and FTA cards. For the aphids, sample was taken from the same infested plants and subjected for FTA cards impregnation and molecular identification.

(iii) Detection of Plant Viruses using Indirect ELISA

Samples taken (bamboo, taro and passion fruit) were subjected for the detection of Potyvirus using Indirect ELISA. The ELISA procedures were shown in Appendix 5 – 7.

(iv) Identification of Plant Viruses using Molecular Techniques (Extraction of DNA, RNA and dsRNA, Polymerase Chain Reaction (PCR), Reverse Transcriptase (RT) PCR, Gel electrophoresis, Purification of PCR Product, Cloning and Sequencing)

Samples from banana infected with BBTV, aphids and Cucurbit leaves (Cucumber mosaic virus) were used for identification of virus using all the techniques mentioned above. The samples were extracted from fresh infected leaves, fresh aphids and samples impregnation on FTA cards. Full procedures were shown in Appendix 8 – 12.

(v) SDS-PAGE and Western Blot

SDS-PAGE and western blot is one of the method can be used for detection of virus protein instead of using ELISA and molecular techniques by separating the protein mixture according to their molecular weight. This training was conducted by Mr. Chong from NODAI. Details protocol was shown in Appendix 13.

(b) Field Study/ Visit

(i) Visit to NODAI's Museum and Biorium

The visit was done with Prof Keiko T. Natsuaki. During the visit, we get to know about the history of Tokyo University of Agriculture and Japanese tradition.

(ii) **Visit to Utsunomiya University**

The visit was done in early of December for 2 days. This attachment was supervised by Prof. Tomohide Natsuaki and his students. During the 2 days attachment, we learned about dsRNA extraction for detection of dsRNA of Cucumber mosaic virus (CMV) from Cucurbit plant. Protocol details were shoen in Appendix 14.

(iii) **Visit to Yokohama Plant Protection Station**

The visit was started at the main office of Yokohama Plant Protection Station situated in Yokohama. During the visit, the plant quarantine officer giving a tutorialon the Japan's Plant Quarantine Policies, regulations and implementation of Plant Quarantine Act for all the importation and exportation items, electronic system for application of import permit and others.

In the afternoon, we moved to the Research Centre of Yokohama Plant Quarantine Station. In this Research Centre, we have been shown on their roles to conduct researchers on pests, diseases, pest risk analysis and plant quarantine treatments. The research conducted by this centre helps to provide beneficial information for the application of plant quarantine regulation in Japan.Refer to Appendix 15 for full report.

(c) Program of Conferences

(i) **International Society for Southeast Asian Agricultural Sciences (ISSAAS) – International Congress**

The congress was held on 7 – 9 November at NODAI. From the congress, we gained more knowledge from all the lectures presented by ASEAN and Japanese participants from quarantine agencies and universities. I have a good chance to attend their presentation regarding to the research on pests and diseases, biocontrol of plant pathogens, nutrient application on oil palm, diversity of plant viruses and current methodology in identification of pathogens such as detection of plant viruses using immunochromatographic strip developed by researcher from Thailand. Besides that I met colleagues from Malaysia and other countries. We were able to share knowledge and opinions together during the congress.

On the final day of the congress, we participated in the field trip to Kawaguchi Lake and Mount Fuji.

(ii) **Halal Seminar**

This seminar was held on 30 November in NODAI. I was attended one of the lecture presented by Dr. Dzulkifly Mat Hashim from Universiti Putra Malaysia on Halal Food Authentication in Malaysia. The purpose of this seminar is to introduce the halal industry, halal certificates, technology for halal test and others. This is important for Malaysia to introduce the halal concept to the Japanese and increase their understanding on the application of halal for the Muslim and food industry and become one of the attraction for tourists to visit Japan.

5. Summary and Recommendations

In general, the attachment program has reached the main objectives to provide capacity building for the ASEAN countries to learn and gain knowledge specifically on diagnostic of plant viruses. The training was conducted and supervised by expert resource persons on plant viruses and as a chosen participant, I have good experiences in order to learn the basic and advance knowledge in plant viruses diagnostic. I was able to create a networking and sharing some ideas with Japan's agencies, professors and students and build cooperation between ASEAN countries and Japan in plant quarantine enforcement. With the relationship built up, it may help to improve our diagnostic skills in plant quarantine test procedures starting from symptoms observation until identification of the plant viruses for agriculture products trade, understanding the importance of plant viruses and applying the correct management techniques in order to control the spread of plant virus infections within the country.

In conclusion, this program provided me a good exposure to new technology and improved my technical skills in detection and identification of plant viruses. I will applied and share the skills and techniques learned during the attachment program to my colleagues in my department. In addition, my recommendation for next training is to conduct training program on plant parasitic nematodes. The limitation of expertise in ASEAN countries especially for identification of plant parasitic nematodes is a constraint for us in managing the nematodes infection. Such training will helps us to increase our understanding and technical skills for early detection of disease infestation, species identification and suitable control measures to prevent the infection by plant parasitic nematodes.

6. Acknowledgement

I would like to express my deepest appreciation to the sponsor of this project, Japan-ASEAN Integration Fund (JAIF) for giving me the opportunity to ASEAN countries to involve in this capacity building program.

I place on record my sincere gratitude to Dr.Lum Keng Yeang and Dr. Soetikno Selamat for giving me chance to be selected for the attachment program in Japan. Grateful acknowledgement to Prof. Keiko T. Natsuaki sensei for her time and effort, guidance, sharing her experiences, taking care our needs and accommodate us with comfortable accommodations and giving us advices.

I would like to thank Dr. Marita S. Panili, Prof. Tomohide Natsuaki, Mr. Chong sensei and all the Hogoken's students for sharing the valuable knowledges, experiences, encouragement and guidance during our short attachment with them. Thank you very much for everything.

LIST OF APPENDIXES