

# TRAINING WORKSHOP ON DIAGNOSTICS OF PLANT PARASITIC NEMATODES

ARIAQ, Bekasi, Indonesia, 23rd February to 7th March 2020

## Objective/Outcome

Plant-parasitic nematodes (PPNs) cause serious problems in agricultural crops throughout the world from tropical to cold part of agricultural region. Especially, in tropical and subtropical region, such as South-East Asia where agriculture occupies an important position in the economy, PPNs proliferate so quickly at high growth rate that crops sometimes suffer considerably high yield loss. Because the symptoms caused by PPNs resemble to that of plant disease or physiological disorders, diagnosis is very important to deal with the damage appropriately.

There are lots of species of PPNs, such as root-knot nematodes (*Meloidogyne* spp.), root-lesion nematodes (*Pratylenchus* spp.) and cyst nematodes (*Heterodera* and *Grobodera* spp.). However, detection and identification of PPNs are difficult even for researchers because they are so tiny, have few morphological characters that microscopes and special skills in nematology are needed. Even in such situations, there are very few researchers of PPN in South-East Asia. For this reason, fostering of nematologists is urgently required in this area.

In this workshop, participants are going to study the biology and ecology of PPNs and the importance and difficulties in control through lectures. In the laboratory and field work, participants are going to practice to collect and extract PPNs from the field and learn morphological and DNA analysis techniques for identifying PPNs.

## Results/Output

This workshop aims to provide participants with the knowledge of basic and practical understanding of general nematology and PPNs, how to identify important PPN species and several management methods for them, such as chemical, physical, biological, cultural methods, and so on. To achieve these aims, the training course covers the following topics: collection method and theory, basic taxonomy and ecology of major species, families and genera of PPNs, collecting and preparation of specimens, identification based on morphological characters and DNA analysis, impacts of PPNs on major agricultural crops, and available options of management. Thereby, upon completion of training, participants will have enough knowledge on nematology to design effective management plans for controlling PPNs.

## Main Activities

### Lecture:

1. To become aware of the importance of PPNs on agriculture in the world, especially in tropical and sub-tropical area.
2. To acquire knowledge on the biology, ecology, and population distribution of PPNs.
3. To learn how to identify the symptoms (above and below grounds) of PPN-infected crops as one cause of poor growth due to continuous cropping.
4. To acquire knowledge on the taxonomy and classification of PPNs in relation to other nematode taxon; morphological characteristics and DNA barcoding of major genera and species.

4. To acquire knowledge on recent advances in identifying PPNs; focused on DNA extraction method, and analyses such as PCR, RFLP, real-time PCR and DNA sequencing method.
5. To learn how to protect crops from PPNs by chemical, physical, biological, cultural (including use of crop rotation, resistant varieties, nematode-suppressive or non-host plants) methods, and so on.
6. To become aware of the importance of international migration of PPNs as a serious problem on plant quarantine.

*Laboratory:*

1. To learn the symptoms (shoot wilting, root gall, root lesion, etc.) caused by different genera of PPNs.
2. To learn the basic techniques in soil and plant sampling, and proper handling of samples.
3. To learn how to extract PPN from soil and plant parts (Maceration - Baermann funnel method and sieving method)
4. To learn the morphological characters of major genera of PPNs for tentative identification and how to assess their population by nematode count them under a microscope.
5. To learn how to extract DNA from an individual nematode, perform PCR and RFLP assay for species identification.

### Training Methodology

The training will be conducted by a combination of interactive lectures, laboratory practices and field visits. Field observation and sampling will be carried out in crop fields.

## **WORKSHOP PROGRAM**

<b>DAY 1 – 24 February 2020 (Monday) OPENING AND INTRODUCTION</b>		
	<b>Activity</b>	<b>Resource Person(s)/Facilitator</b>
08.30 – 08.45	<b>Registration</b>	Dr. Ummu S. Rustiani (and her team), <i>ARIAQ, Bekasi</i>
	<b>OPENING AND INTRODUCTION</b>	
08.45 – 09.00	Workshop Introduction Purpose and scope of workshop	Dr. Soetikno Sastroutomo <i>Technical Secretary APHCN-ASEANET, Malaysia</i>
09.00 – 10.00	Opening Remarks and Official Opening	Dr. Ali Jamil, <i>Director General, IAQA, Jakarta</i> represented by Mr. Maman Suparman, Head of Seed Quarantine Division, Centre for Plant Quarantine & Biosafety
	<b>Participants and Training Team Introductions –</b> Participants will be invited to introduce themselves, identify the agencies they work for and state the nature of their work.	Dr. Marita S. Pinili <i>Regional Training Coordinator National Crop Protection Center, University of the Philippines Los Baños</i>
	Group photograph	

<b>DAY 1 – 24 February 2020 (Monday) OPENING AND INTRODUCTION</b>		
<b>10.00 – 10.30</b>	<b>Morning tea</b>	
10.30 – 11.00	Administration of pre-evaluation tests	Dr. Nurjanah
11.00 – 11.45	Introduction to the JAIF Funded Capacity Building Project.	Dr. Soetikno Sastroutomo
11.45 – 12.15	Lecture 1: General introduction to nematology	Dr. Hideaki Iwahori <i>Ryukoku University, Japan</i>
<b>12.15 – 13.15</b>	<b>Lunch</b>	
13.15 – 13.45	Lecture 2: Diseases caused by plant-parasitic nematodes: Signs and Symptoms	Dr. Marita S. Pinili
13.45 – 14.15	Lecture 3: Economically important plant-parasitic nematodes	Dr. Hideaki Iwahori
14.15 – 15.00	Lecture 4: Sampling: Principles and Methods	Dr. Marita S. Pinili
<b>15.00 – 15.30</b>	<b>Afternoon tea</b>	
15.30 – 16.00	Lecture 5: Nematode physiology and anatomy, and their implications for quarantine and survival	Dr. Hideaki Iwahori
16.00 – 17.00	Lecture 6: Introduction to nematode extraction: Passive and active techniques	Dr. Nurjanah
<b>19:00 - 21:00</b>	<b>Official Workshop/Welcome Dinner</b>	

<b>DAY 2 – 25 February 2020 (Tuesday) FIELD TRIP &amp; NEMATODE EXTRACTION</b>		
08.30 – 09.30	Field trip to collect nematode specimens - Set up extractions facilities	Dr. Nurjanah
09.30 – 10.00	Preparation for field trip to several agricultural farms	All
10.00	Depart for nematode collection	
<b>12.00 – 13.00</b>	<b>Lunch</b> (in the field)	
13.00 – 17.00	On-site field sampling and collection of root and soil samples	All
17.00	Return to accommodation.	
<b>DAY 3 – 26 February 2020 (Wednesday) SYMPTOM ASSESSMENT AND NEMATODE EXTRACTION</b>		
08.30 – 10.30	Practical 1: Below ground and above ground symptom assessment	Dr. Marita S. Pinili
<b>10.30 – 11.00</b>	<b>Morning tea</b>	
11.00 – 12.00	Discussion	Dr. Nurjanah
<b>12.30 – 13.30</b>	<b>Lunch</b>	
13.30 – 15.30	Practical 2: Nematode extraction from soil	Dr. Nurjanah
<b>15.30 – 16.00</b>	<b>Afternoon tea</b>	
16.00 – 17.00	Practical 2: Nematode extraction from soil (continuation)	Dr. Nurjanah
<b>DAY 4 – 27 February 2020 (Thursday) EXTRACTION TECHNIQUES</b>		

08.30 – 09.00	Lecture 7: Extraction technique for endoparasitic nematodes	Dr. Marita S. Pinili
09.00 – 10.00	Practical 3: Extraction of plant-parasitic nematodes from roots, seeds, and bulbs	Dr. Marita S. Pinili
<b>10.00 – 10.30</b>	<b>Morning tea</b>	
10.30 – 12:30	Practical 3: Extraction of plant-parasitic nematodes from roots, seeds, and bulbs (continuation)	Dr. Nurjanah
<b>12.30 – 13.30</b>	<b>Lunch</b>	
13.30 – 14.00	Lecture 9 : Extraction using Sucrose-centrifuge method	Dr. Hideaki Iwahori
14.00 – 15.30	Practical 5: Sucrose-centrifuge method	Dr. Hideaki Iwahori
<b>15.30 – 16.00</b>	<b>Afternoon tea</b>	
16.00 – 17.00	Lecture 8 and Practical Demo: Extraction using Mist chamber technique	Dr. Nurjanah
<b>DAY 5 – 28 February 2020 (Friday) NEMATODE FIXATION AND MOUNTING</b>		
08.30 – 09.30	Lecture 10: Miscellaneous techniques: Handling, killing, fixing, staining, and mounting	Dr. Marita S. Pinili
09.30 – 10.00	Practical 6: Killing-fixation of nematodes	Dr. Marita S. Pinili
<b>10.00 – 10.30</b>	<b>Morning tea</b>	
10.30 – 11.00	Practical 6: Killing – fixation of nematodes (continuation)	Dr. Marita S. Pinili
11.00 – 12.00	Practical 7: Mounting of nematodes	Dr. Nurjanah
<b>12.00 – 13.00</b>	<b>Lunch</b>	
13.00 – 15.00	Practical 7: Mounting of nematodes (continuation)	Dr. Nurjanah
<b>15.00 – 15.30</b>	<b>Afternoon tea</b>	
15.30 – 17.00	Practical 7: Mounting of nematodes (continuation)	Dr. Nurjanah
13.00 – 15.00	Practical 7: Mounting of nematodes (continuation)	Dr. Nurjanah
<b>DAY 6 – 29 February 2020 (Saturday) NEMATODE FIXATION AND MOUNTING</b>		
08.30 – 10.00	Practical 7: Mounting of nematodes (continuation)	Dr. Nurjanah/Dr. Marita S. Pinili
<b>10.00 – 10.30</b>	<b>Morning tea</b>	
10.30 – 12.00	Practical 8: Tentative identification of nematodes based on morphological features	Dr. Nurjanah/Dr. Marita S. Pinili
<b>12.00 – 13.00</b>	<b>Lunch</b>	

**01 March 2020 (Sunday) - Holiday**

**DAY 7 – 02 March 2020 (Monday) NEMATODE IDENTIFICATION AND CLASSIFICATION**

08.30 – 09.00	Lecture 11: Digestive System of Nematodes: Key to Morphological Identification	Dr. Marita S. Pinili
09.00 – 10.00	Practical 9: Morphological Identification of nematode specimens collected during survey	All
<b>10.00 – 10.30</b>	<b>Morning tea</b>	
10.30 – 11.30	Lecture 12: Useful Taxonomic Characters used in Nematode Identification: Keys and other identification resources	Dr. Hideaki Iwahori
11.30 – 12.30	Practical 9: Morphological Identification of nematodes collected from S-E countries	All
<b>12.30 – 13.30</b>	<b>Lunch</b>	
13.30 – 17.00	Practical 9: Morphological Identification of nematodes collected from S-E countries	All

<b>DAY 8 – 03 March 2020 (Tuesday) NEMATODE BIOLOGY</b>		
08.30 – 09.00	Lecture 13: Plant-parasitic nematode life cycle	Dr. Marita S. Pinili
09.00 – 10.00	Practical 9: Morphological Identification of fixed nematodes	All
<b>10.00 – 10.30</b>	<b>Morning tea</b>	
10.30 – 11.30	Lecture 14: Plant-parasitic nematode bionomics	Dr. Hideaki Iwahori
11.30 – 12.30	Practical 9: Morphological Identification of nematodes	All
<b>12.30 – 13.30</b>	<b>Lunch</b>	
13.30 – 14.00	Lecture 15: Extraction and <i>in vitro</i> culture techniques: Case of <i>Radopholus similis</i>	Dr. Marita S. Pinili
14.00 – 15.00	Practical 10: Observing, counting and population assessment of nematodes	Dr. Marita S. Pinili
<b>15.00 – 15.30</b>	<b>Afternoon tea</b>	
15.30 – 16.30	Lecture 16: Molecular Techniques: Progress and Limitations	Dr. Hideaki Iwahori

<b>DAY 9 – 04 March 2020 (Wednesday) EXTRACTION TECHNIQUES AND NEMATODE ASSAY</b>		
08.30 – 10.30	Practical 11: Extraction of Nematode DNA	Dr. Hideaki Iwahori
<b>10.30 – 11.00</b>	<b>Morning tea</b>	
11.00 – 12.30	Practical 11: Extraction of Nematode DNA (continuation)	Dr. Hideaki Iwahori
<b>12.30 – 13.30</b>	<b>Lunch</b>	

13.30 – 15.00	Practical 12: Molecular Identification of Nematodes: PCR assay	Dr. Hideaki Iwahori
<b>15.00 - 15.30</b>	<b>Afternoon tea</b>	
15.30 – 17.00	Practical 12: Molecular Identification of Nematodes: RFLP assay	Dr. Hideaki Iwahori

**DAY 10 - 05 March 2020 (Thursday) MOLECULAR IDENTIFICATION OF NEMATODES**

08.30 – 11.00	Practical 13: Gel Electrophoresis and Analysis	Dr. Hideaki Iwahori
11.00 - 12.00	Practical 14: Viewing and Interpretation of RFLP Results	Dr. Hideaki Iwahori
<b>12.00 – 13.30</b>	<b>Lunch</b>	
13.30 – 14.30	Lecture 17: Nematodes recorded worldwide and their global distribution	Dr. Hideaki Iwahori
14.30 - 15.30	Lecture 18: Management Strategies on Plant-Parasitic Nematodes I – Physical and Cultural Approaches	Dr. Nurjanah
<b>15.30 – 16.00</b>	<b>Afternoon tea</b>	
16.00 – 17.00	Lecture 19: Management Strategies on Plant-Parasitic Nematodes II – Biological and Plant Resistance Approaches	Dr. Marita S. Pinili

**DAY 11 – 06 March 2020 (Friday) NEMATODE DAMAGE ASSESSMENT**

<b>TIME</b>	<b>EVENT</b>	
08.30 – 09.30	Discussion 2: Storage and cataloguing of images and specimen	Dr. Nurjanah
09.30 – 10.00	Group Report (Group 1)	Representative
10.00 - 10.30	Group Report (Group 2)	Representative
<b>10.30 – 11.00</b>	<b>Morning tea</b>	
11.00 – 11.30	Group Report (Group 3)	Representative
<b>11.30 – 14.00</b>	<b>Lunch Break and Friday Prayer</b>	
14.00 - 14.30	Group Report (Group 4)	Representative
14.30 - 15.00	Group Report (Group 5)	Representative
<b>15.00 – 15.30</b>	<b>Afternoon tea</b>	
<b>POST-EVALUATION AND CLOSING SESSION</b>		
15.30 – 16.00	Post-workshop evaluation test	Dr. Nurjanah
16.00 – 16.30	Group Discussion and Workshop conclusion (Testimony from participants)	Dr. Marita S. Pinili

16.30 – 17.30	Presentation of Certificates and Closing remarks	Dr. Wawan Sutian, Director of ARIAQ, Bekasi Dr. Soetikno Sastroutomo
<b>17.30</b>	<b>Workshop Close</b>	

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## RESOURCE PERSONS

- *Prof. Hideaki Iwahori, Department of Bioresource Science, Faculty of Agriculture, Ryukoku University, 1-5 Yokotani, Seta Oe-cho, Otsu, Shiga 520-2194, Japan. E-mail: [iwahori@agr.ryukoku.ac.jp](mailto:iwahori@agr.ryukoku.ac.jp)*
- *Dr. Marita S. Pinili, Senior Researcher, National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna 4031 Philippines. E-mail: [maripinili@gmail.com](mailto:maripinili@gmail.com)*
- *Dr. Nurjanah & Dr. Ummu Salamah, ARIAC, Bekasi, Indonesia (for venue and logistic preparation)*