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Attachment Program:
DIAGNOSTICS OF PLANT PARASITIC NEMATODES

(JAIF Funded Project on Taxonomic Capacity Building to Support Market Access for Agricultural Trade in the ASEAN Region)

Organizer:
Ryukoku University, Japan

7th February to 4th April 2023

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BACKGROUND INFORMATION

The ASEAN Plant Health Cooperation Network (APHCN) – ASEANET Project "Taxonomic capacity building to support market access for agricultural trade in the ASEAN region", funded by the Japan ASEAN Integration Fund (JAIF) successfully held its capacity building activity, entitled "Training Workshop on Diagnostics of Plant Parasitic Nematodes", from 23rd February to 7th March 2020 at the Applied Research Institute for Agriculture Quarantine (ARIAQ). This topic was chosen because, as we know, nematodes are very small organism, need microscope to see this and many are considered as pests of crops. Most of the parasitic nematodes can be present in imported agricultural products. The damage that they cause is very destructive and can have an impact on economic aspect. On the other hand, there are many other species of nematodes that attack agricultural crops. Hence, quick and accurate identification is vital in quarantine processes and converted farm areas. Based on the recommendation of the resource persons on the training, two selected participants are from Indonesia and Philippines, which endorsed for the 2nd Phase of the Training. The theme of this attachment training program is "Diagnostics of Plant Parasitic Nematodes".

The attachment program is two months training organized by the ASEAN Plant Health Cooperation Network (APHCN) of ASEANET. Two participants were trained to improve their diagnostic capability and enhance diagnostic resources in the ASEAN. This is also in line with the APHCN-ASEANET project "Taxonomic capacity building to support market access for agricultural trade in the ASEAN region". During two months program, participants are attached in several laboratories in Japan, i.e., Ryukoku University; Yokohama Plant Protection Station; Institute for Plant Protection, NARO; Kyushu Okinawa Agricultural Research Center, NARO; Kumamoto University; Nara Institute of Science and Technology; Kyoto University; Forestry and Fishery Product Research Institute. Through this attachment, the participants would be able to gain more in-depth knowledge on taxonomical identification of nematodes of quarantine importance, to broaden their diagnostic capability, to increase competency to handle all pest diagnosis, and to widen their experience on nematode collection and preservation. The acquired information would be useful to build up the confidence and skills thus contributing to capacity development of each Institution or in the representative country of participant.

1. OBJECTIVES OF THE ATTACHMENT

The objectives of the attachment program are:

a. To transfer skills and knowledge of nematode experts specifically Japanese experts on nematodes to participants so as to increase capacity, experience, and knowledge of the participants in the diagnostics of pests and taxonomic identification and also will be able to learn techniques in identification;
b. To strengthen the diagnostic capacity by providing participants with practical understanding of the concept of nematodes, their identification and current management practices; and
c. To tap these trained participants who would undergo more intensive technical training and subsequently serve as potential ASEAN resource persons on the identification of nematodes using their gained expertise to the benefit of all ASEAN member states and the ASEAN Diagnostic Network.

2. PROGRAM OF THE ATTACHMENT

The Attachment program is funded by the Japan ASEAN Integration Fund (JAIF) in cooperation with The ASEAN Plant Health Cooperation Network (APHCN) - ASEANET in Project "Taxonomic capacity building to support market access for agricultural trade in the ASEAN region" which organized by Ryukoku University. This program is the continuation of the previous program that successfully held from 23rd February to 7th March 2020 at the Applied Research Institute for Agriculture Quarantine (ARIAQ), entitled "Training Workshop on Diagnostics of Plant Parasitic Nematodes".

Duration

The attachment program was conducted from 7th February to 4th April 2023 in Japan.

Participants

The attachment program consisted of 2 (two) participants from: Indonesia and Philippines. Table 1 shows the participants of the attachment program.

Table 1. The participants of the attachment

No.	Name of participant	Occupation	Country
1.	Happy Cahya Nugrahana	Plant Quarantine Officer (Indonesian Agricultural Quarantine Agency Ministry of Agriculture)	Indonesia
2.	Aldwin C Mendoza	Philippines Quarantine Officer	Philippines

Instructure/Facilitator

The instructors or facilitator of the attachment were from: (1) Ryukoku University; (2) Yokohama Plant Protection Station; (3) Institute for Plant Protection, NARO; (4) Kyushu Okinawa Agricultural Research Center, NARO; (5) Kumamoto University; (6) Nara Institute of Science and Technology; (7) Kyoto University; (8) Forestry and Fishery Product Research Institute.

Program Activities

The program of the attachment consisted of 3 (three) activities namely: lecture, laboratory practices, and field trip, and these divided into 8 (eight) locations including: Ryukoku University; Yokohama Plant Protection Station; Institute for Plant Protection, NARO; Kyushu Okinawa Agricultural Research Center, NARO; Kumamoto University; Nara Institute of Science and Technology; Kyoto University; Forestry and Fishery Product Research Institute. Table 2. shows the activities in each location.

Table 2. The program of the attachment in each location

Date	Location	Activities	Responsible Person/Scientist (s)
February 7, 2023 [Tuesday]	Kansai Airport	Arrival in Osaka	Prof. Iwahori
February 8, 2023 [Wednesday]	Ryukoku University	Briefing and orientation (Campus tour)	Prof. Iwahori
February 9, 2023 [Thursday]	Ryukoku University	Collect nematodes at Univ experimental farm and forest	Prof. Iwahori
February 10, 2023 [Friday]	Ryukoku University	Learn laboratory equipment/Extract nematode	Prof. Iwahori
February 11, 2023 [Saturday]	Kusatsu	Free day	--
February 12, 2023 [Sunday]	Kusatsu	Free day	--
February 13, 2023 [Monday]	Ryukoku University	Lecture of plant-parasitic nematodes in the world and Japan	Prof. Iwahori
February 14, 2023 [Tuesday]	Ryukoku University	Make specimen and microscopy of nematodes	Prof. Iwahori

February 15, 2023 [Wednesday]	Ryukoku University	Make specimen and microscopy of nematodes	Prof. Iwahori
February 16, 2023 [Thursday]	Ryukoku University	Make specimen and microscopy of nematodes	Prof. Iwahori
February 17, 2023 [Friday]	Kusatsu	Tour to suburban agricultural facilities / Extract nematodes	Prof. Iwahori and Farmer
February 18, 2023 [Saturday]	Kusatsu	Free day	--
February 19, 2023 [Sunday]	Kusatsu	Free day	--
February 20, 2023 [Monday]	Ryukoku University	Learn how to do infection experiment / Inoculation of nematodes to tomato	Prof. Iwahori
February 21, 2023 [Tuesday]	Ryukoku University	Identify nematodes by PCR-RFLP methods	Prof. Iwahori
February 22, 2023 [Wednesday]	Ryukoku University	Identify nematodes by PCR-RFLP methods	Prof. Iwahori
February 23, 2023 [Thursday]	Kusatsu	Free day [National day in Japan]	--
February 24, 2023 [Friday]	Ryukoku University	Identify nematodes by PCR-RFLP methods	Prof. Iwahori
February 25, 2023 [Saturday]	Kusatsu	Free day	--
February 26, 2023 [Sunday]	Kusatsu	Free day	--
February 27, 2023 [Monday]	Ryukoku University	Examine plants infected by root-knot nematode	Prof. Iwahori
February 28, 2023 [Tuesday]	Ryukoku University	Examine plants infected by root-knot nematode	Prof. Iwahori
March 1, 2023 [Wednesday]	Ryukoku University	Examine plants infected by root-knot nematode	Prof. Iwahori
March 2, 2023 [Thursday]	Ryukoku University	Examine plants infected by root-knot nematode	Prof. Iwahori
March 3, 2023 [Friday]	Ryukoku University	Examine plants infected by root-knot nematode	Prof. Iwahori
March 4, 2023 [Saturday]	Kusatsu	Free day	--
March 5, 2023 [Sunday]	Kusatsu	Free day	--
March 6, 2023 [Monday]	Tsukuba	Visit to Central Region Agricultural Research Center	Prof. Iwahori
March 7, 2023 [Tuesday]	Yokohama	Visit to Plant Quarantine Office	Prof. Iwahori
March 8, 2023 [Wednesday]	Ryukoku University	Make specimen and microscopy of nematodes	Prof. Iwahori
March 9, 2023 [Thursday]	Kusatsu	Tour to Shionogi Pharmaceutical Company	Prof. Iwahori dan Company officer
March 10, 2023 [Friday]	Ryukoku University	Meeting - Overview of the first half of training	Prof. Iwahori

March 11, 2023 [Saturday]	Kusatsu	Free day	--
March 12, 2023 [Sunday]	Kusatsu	Free day	--
March 13, 2023 [Monday]	Ryukoku University	Identify nematodes by PCR-RFLP methods or DNA sequencing	Prof. Iwahori
March 14, 2023 [Tuesday]	Ryukoku University	Identify nematodes by PCR-RFLP methods or DNA sequencing	Prof. Iwahori
March 15, 2023 [Wednesday]	Ryukoku University	Identify nematodes by PCR-RFLP methods or DNA sequencing	Prof. Iwahori
March 16, 2023 [Thursday]	Ryukoku University	Identify nematodes by PCR-RFLP methods or DNA sequencing	Prof. Iwahori
March 17, 2023 [Friday]	Ryukoku University	Identify nematodes by PCR-RFLP methods or DNA sequencing	Prof. Iwahori
March 18, 2023 [Saturday]	Kusatsu	Free day	--
March 19, 2023 [Sunday]	Kusatsu	Free day	--
March 20, 2023 [Monday]	Ryukoku University	Identify nematodes by PCR-RFLP methods or DNA sequencing	Prof. Iwahori
March 21, 2023 [Tuesday]	Kusatsu	Nationa Holiday	--
March 22, 2023 [Wednesday]	Kyushu	Visit to Kyushu Okinawa Agricultural Research Center	Prof. Iwahori
March 23, 2023 [Thursday]	Kumamoto	Visit to agricultural farm in Kumamoto	Prof. Iwahori
March 24, 2023 [Friday]	Kumamoto University	Visit Kumamoto University	Prof. Iwahori
March 25, 2023 [Saturday]	Kusatsu	Free day	--
March 26, 2023 [Sunday]	Kusatsu	Free day	--
March 27, 2023 [Monday]	Nara Institute of Science and Technology	Visit Nara Institute of Science and Technology	Dr. Mina Ohtsu
March 28, 2023 [Tuesday]	Kyoto University	Visit Kyoto University	Dr. Yuuko Takeuchi
March 29, 2023 [Wednesday]	Ryukoku University	Pine Wilt Nematode	Prof. Iwahori
March 30, 2023 [Thursday]	Forestry and Fishery Product Research Institute.	Visit Forestry and Fishery Product Research Institute.	Prof. Natsumi Kanzaki
March 31, 2023 [Friday]	Ryukoku University	Make a presentation	--

April 1, 2023 [Saturday]	Kusatsu	Free day	--
April 2, 2023 [Sunday]	Kusatsu	Free day	--
April 3, 2023 [Monday]	Ryukoku University	Final Presentation Farewell party	Prof. Iwahori Dr. Marita S. Pinili Dr. SoetiknoS. Sastroutomo Mr. Happy Cahya Nugrahana Mr. Aldwin C. Mendoza
April 4, 2023 [Tuesday]	Departure of participants		

3. DAILY ACTIVITIES

3.1 Ryukoku University

Ryukoku University is a private university that was established as a Buddhist institution of high education for monks and has a rich history date back as far as 1639. The university suffered from a city fire in 1788 and was restored in 1792. In 1905 the institution was renamed Bukkyo Daigaku (Buddhist College). It became Ryukoku University when it was granted a university status in 1922.

The university is divided into nine (9) faculties, which specialize in humanities, economics, international studies, advanced science and technology, business administration, sociology, law, agriculture, and policy science. Each faculty offers a number of courses at both undergraduate and graduate level. The university also offers a Japanese Culture and Language Programme for international students.

The university was inspired by the Buddhist tradition. Its spirit is the desire to wake all living beings from ignorance to enlightenment. The university achieves this by educating its students to become people who "seek truth, live in truth, and reveal truth". The university also teaches its students to value five key principles: equality, independence, contemplation, gratitude and peace in order to become wise and successful individuals.

The activities were conducted during at Ryukoku University of Agriculture are shown in Table 3:

Table 3. The activities at Ryukoku University of Agriculture

No.	Activity	Result
1.	Briefing and orientation **Instructor:** **Prof. Iwahori**	Introduction of: • Ryukoku University of Agriculture, • Learn daily necessities: shops, restaurants, etc.
2.	Orientation of Ryukoku University of Agriculture - Seta Campus **Instructor:** **Prof. Iwahori**	Orientation of: • Laboratory, participants got more information of Nematology Laboratory at Ryukoku University of Agriculture, also have a good reference related to the laboratory facilities in Ryukoku University of Agriculture, such as: room, tools, microscopes, book/journals collections related to nematodes, etc. • Building • Library • Education system • Other facilities • etc.
3.	Collect nematodes at University experimental farm and forest **Instructor:** **Prof. Iwahori**	• To find out what nematodes are being studied at Ryukoku University and on which host plants. The research was conducted in a greenhouse and growth chamber. • How to collect nematodes correctly and without contamination. • How to handle samples i.e., roots and soil which were placed in plastic bag and then autoclaved before samples disposal.
4.	Extract nematode egg masses **Instructor:** **Prof. Iwahori**	• How to extract egg masses nematode from roots. • How to stain nematodes using brilliant blue technique so that the egg masses will turn into blue in color. Making it easier to distinguish for extraction without using a microscope. • This extraction is used to reproduce second juveniles from a single parent. It used for the PCR assay and obtaining pure nematode cultures. This process is also used for nematode pure culture inoculation process.

5.	Extract cyst nematode **Instructor:** **Prof. Iwahori**	How to extract cyst nematode from soil using Fenwick Can method. This method is suitable for dried soil samples. Extraction is based on the floating properties of dried cysts (containing air) and the difference in size between other fractions of the sample. Most of the cysts in the soil sample were collected this way. The soil at the base of the Fenwick can is elutriated by means of water flowing rapidly through a long glass or metal tube which is inserted deep into the can. The water flow stirs the sediment and releases any trapped cysts. The cysts move upward, into the collar and end up on the sieve.
6.	Extracting nematode from the roots **Instructor:** **Prof. Iwahori**	This extaction is used for PCR assay purposes. The roots just cleaned from any adhering soil by washing it in running water. Clean roots are immediately cut on the part with gall of 1 cm and done with bead smash 12 machine.
7.	Extracting nematode from soils **Instructor:** **Prof. Iwahori**	To extract second juvenile of nematode using Baermann Funnel technique. The principle of Baermann technique is that the nematode wiggle out of the biological material, cannot swim against gravity and will fall through the water to the area of clamped off tubing. The clamp is released to collect the nematode suspension for nematode identification.
8.	Extracting nematode from pine stem **Instructor:** **Prof. Iwahori**	This extaction is used for LAMP assay. The stem of pine tree that is infected by nematode wase just drilled using a hand drille, collected and ready to use for identification using LAMP assay.
9.	Pick up the nematode **Instructor:** **Prof. Iwahori**	Hand picking of nematode is used to collect the same nematode group or species (parasitic nematode), not mixed with free-living nematodes so that the identification process with PCR machine will be more accurate. In this training, only one nematode was used for each PCR process.
10.	Purification the DNA **Instructor:** **Prof. Iwahori**	We learn how to purify the DNA. DNA analysis by multiplex and real-time PCR, the importance of high-quality, purified DNA cannot be underestimated. Finding a suitable DNA isolation system to satisfy the downstream application needs is vital for the successful completion of experiments. There are five basic steps of DNA extraction that are consistent across all the possible DNA purification chemistries: 1) disruption of the cellular structure to create a lysate, 2) separation of the soluble DNA from cell debris and other insoluble material, 3) binding the DNA of interest to a purification matrix, 4) washing proteins and

		other contaminants away from the matrix and 5) elution of the DNA.
11.	Identification of nematode **Instructor:** **Prof. Iwahori**	We learn how to identify nematodes by molecular method i.e., PCR , RFLP and LAMP **PCR conventional** The polymerase chain reaction (PCR) provides a highly sensitive method for DNA amplification and identification. Previously, we utilized PCR to amplify mitochondrial DNA (mtDNA) from individual Meloidogyne second juvenile. We used Harris and Powers protocols for molecular analysis and for identification of Meloidogyne species. In this case, we used the universal primer, and subjected the PCR products for sequencing analysis. **Real time PCR** In real-time PCR, the accumulation of amplification product is measured as the reaction progresses, in real time, with product quantification after each cycle. The main advantage of real-time PCR over PCR is that real-time PCR allows to determine the initial number of copies of template DNA (the amplification target sequence) with accuracy and high sensitivity over a wide dynamic range. Real-time PCR results can either be qualitative (the presence or absence of a sequence) or quantitative (copy number). Quantitative real-time PCR is thus also known as qPCR analysis. **RFLP** In molecular biology, restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences, known as polymorphisms, in order to distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence. We used the Harris and Powers protocols for molecular analysis and. identification of *Meloidogyne* species. **LAMP** The recent invention of loop-mediated isothermal amplification (LAMP) provides a new alternative for molecular diagnosis. LAMP uses a set of four to six primers and a DNA polymerase with strand displacement activity (BstDNA polymerase) to amplify DNA with high specificity under isothermal conditions in the field or in under-equipped laboratories. This method is very simple, only

		extracting DNA from nematodes in a wood sample. This method is easy and highly sensitive, and requires no specialized equipment or techniques.
12.	How to plant **Instructor:** **Prof. Iwahori**	• Participants were taught how to plant lotus, and inoculating nematodes *Hirschmanniella* sp. into lotus plants. • Participants were also taught how to plant sweet potatoes using stem cuttings. Stem cuttings are cut 5 cm above the soil surface, leaving a few knuckles to ensure the growth of the next shoot which can be harvested again after 4 weeks.
13.	Learning Activities **Instructor:** **Prof. Iwahori**	Participants were given lectures on Potato Cyst Nematode and Pine Wilt Nematode thus increasing knowledge about these nematode species.
14.	Final presentation **Instructor:** **Prof. Hideaki Iwahori** **Dr. Soetikno Sastroutomo** **Dr. Marita Pinili**	Presentation of final report by participants from: • Indonesia • Philippines The content of presentation, consisted of: background, objectives, activities, and conclusion of the attachment program.

3.2 Yokohama Plant Protection Station

Plant Protection System in Japan performs import inspections for plants to prevent the entry of harmful pests from overseas, and export inspections based on plant quarantine requirements specified by the destination countries in order to prevent similar problems abroad. Plant Protection Organization in Japan consists of five (5) Head Offices, these are: (1) Yokohama Plant Protection; (2) Kobe Plant Protection; (3) Nagoya Plant Protection; (4) Moji Plant Protection; and (5) Naha Plant Protection. This Attachment Program conducted in one (1) location: Yokohama Plant Protection Station.

Generally, the activities carried out at the Yokohama Plant Protection Station consists of: (1) Introduction of Plant Protection System in Japan and Japan Plant Quarantine; (2) visiting plant parasitic nematodes specimen collections; and (3) visiting greenhouse experiment with full of plants with nematode infections. The activities conducted during at Yokohama Plant Protection Station visit are shown Table 4.

Table 4. The activities at the Yokohama Plant Protection Station

No.	Activity	Result
1.	Briefing and orientation **Instructor:** **Yokohama Plant Protection Station Officer**	Briefing and orientation were divided into: 1. Presentation of: • Plant protection system in Japan, • International/regional arrangements and Japan's contributions 2. Introduction of Yokohama Plant Protection Station (structure of organization, facilities, human resources, laboratories, management, etc.)
2.	Visiting plant parasitic nematodes specimen collections **Instructor:** **Yokohama Plant Protection Station Officer**	Participants received information on plant parasitic nematodes specimen collection at Yokohama Plant Protection Station. These collections were acquired during the interception at the port.
3.	Visiting nematology laboratory **Instructor:** **Yokohama Plant Protection Station Officer**	Participants received information about the modern machine and tools to identify the nematodes
4.	Visiting the green house **Instructor:** **Yokohama Plant Protection Station Officer**	Participants visited the greenhouse which contains a collection of plants that have been inoculated with various species of nematodes. This green house is also a means to reproduce nematodes and a means of recognizing symptoms in plants.
5.	Presentation	Presentation on agricultural quarantine in Indonesia

3.3 Institute for Plant Protection, NARO

The activities also carried out at Tsukuba which consists of visit to the Institute for Plant Protection, NARO. The activities are presented in Table 5.

Table 5. The activities at the Institute for Plant Protection, NARO

No.	Activity	Result
1.	Briefing and orientation **Instructor:** **Mr. Okada**	• Introduction of Institute for Plant Protection, NARO. • Introduction of Division of core technology for Pest Control Research.
2.	Visiting plant parasitic nematodes specimen collections **Instructor:** **Mr. Okada**	Participants received information about plant parasitic nematodes specimen collection at the Institute for Plant Protection, NARO.
3.	Visiting the laboratory for nematode **Instructor:** **Mr. Okada**	Participants received information about the modern machine and tool to identify the nematodes. There is a laboratory that specifically handles alien pests with a very high level of biosecurity.
4.	Visiting the green house **Instructor:** **Mr. Okada**	Participants visited the greenhouse which contains a collection of plants that have been inoculated with various species of nematodes. This green house is also a means to reproduce nematodes and a means of recognizing symptoms in plants.
5.	Presentation	Presentation on agricultural quarantine in Indonesia.

3.4 Kyushu Okinawa Agricultural Research Center, NARO

The Kyushu Okinawa Agricultural Research Center, NARO is one of the R&D regional agricultural centers of the National Agriculture and Food Research Organization (NARO) that serves as the frontline in bringing the technology developed by NARO. The center aims to contribute to the development of agricultural technologies, promote related industries and create new food cultures for the Kyushu-Okinawa region. Research activities focus on contributing to the development of stable food resources, improvement of food safety and consumer confidence, development of global warming measures and promotion of biomass use, creation of new demands, and other goals set forth in the 4th mid-term plan of NARO. The KARC/NARO facilitates local agricultural research through the establishment of crop development and utilization, livestock and grassland, production environment, rice cultivation and horticulture, and upland farming (*https://www.naro.go.jp/english/laboratory/karc/index.html*).

The activities conducted at Kyushu Okinawa Agricultural Research Center, NARO are shown in Table 6.

Table 6. The activities at Kyushu Okinawa Agricultural Research Center, NARO

No.	Activity	Result
1.	Briefing and orientation **Instructor:** **Murata Gaku** **Yoshida Mutsuhiro**	Briefing and orientation were divided into: • Introduction of Kyushu Okinawa Agricultural Research Center, NARO. • Introduction of published papers on nematodes.
2.	Visiting plant parasitic nematodes specimen collections **Instructor:** **Murata Gaku** **Yoshida Mutsuhiro**	Participants received information of plant parasitic nematodes specimen collection at Kyushu Okinawa Agricultural Research Center, NARO. .
3.	Visiting laboratory of nematode **Instructor:** **Murata Gaku** **Yoshida Mutsuhiro**	Participants received information about modern machine and tools to identify the nematodes
4.	Visiting the green house **Instructor:** **Murata Gaku** **Yoshida Mutsuhiro**	Participants visited the greenhouse which contains a collection of plants that have been inoculated with various species of nematodes. This green house is also a means to reproduce nematodes and a means of recognizing symptoms in plants.
5.	Presentation	Presentation on agricultural quarantine in Indonesia.

3.5 Kumamoto University

Kumamoto University, abbreviated to Kumadai, is a Japanese national university located in Kumamoto, Kumamoto Prefecture in the Kyushu region of Japan. The activities conducted during the field trip to Kumamoto University are presented in Table 7.

Table 7. The activities at Kumamoto University

No.	Activity	Result
1.	Hydroponic method **Instructor:** **Prof. Shinichiro Sawa**	Participants were able to see the process of propagation of *Meloidogyne incognita* by hydroponic method, which can produce 1 million nematodes in 1 day.
2.	Method of Meloidogyne infection	Participants were also shown the method of Meloidogyne infection in Arabidopsis. As a result,

	Instructor: **Prof. Shinichiro Sawa**	plant stem cell function seemed to be abnormally activated by nematode infestation. Although many of the higher plants have a small amount of stem cells at the boundary between xylem and phloem cells, the activity of these stem cells is strictly controlled. However, when these stem cells start growing out of control, abnormal tissues are formed, just like cancer, and it becomes impossible for the plant to form a normal shape. In the galls, the researchers found that the activity control of the stem cells, which should be held very precisely under normal conditions, exhibited runaway conditions when infected by the parasitic worms. In other words, they thought that the nematodes manipulated the plant stem cells to build their nests.

3.6 Nara Institute of Science and Technology

Nara Institute of Science and Technology (NAIST) is a Japanese national university located in Kansai Science City, a border region between Nara, Osaka, and Kyoto. Founded in 1991, NAIST consisted of graduate schools in three integrated areas: information science, biological sciences, and materials science. In 2018, NAIST underwent an organizational transformation to continue research in these areas while promoting interdisciplinary research and education across traditional fields. With this new single graduate school organization, NAIST strives forward with the objectives of conducting cutting-edge research in frontier areas and training students to become tomorrow's leaders in science and technology.

The activities conducted at Nara Institute of Science and Technology are shown Table 8.

Table 8. The activities at Nara Institute of Science and Technology

No.	Activity	Result
1.	Orientation of Nara Institute of Science and Technology **Instructor:** **Dr. Mina Ohtsu**	Orientation of: • Laboratory, Participants got more information of Laboratory at Nara Institute of Science and Technology • Building • Other facilities, etc.
2.	Visiting laboratory of nematode **Instructor:** **Dr. Mina Ohtsu**	Participants received information about modern machine and tools to identify the nematodes.

3.	Visiting the growth chamber **Instructor:** **Dr. Mina Ohtsu**	Participants were introduced to parasitic plants, plants collection that had been inoculated with Meloidogyne sp., and plant models using agar media.

3.7 Kyoto University

Kyoto University is a public research university located in Kyoto, Japan. Founded in 1897, it is one of the former Imperial Universities and the second oldest university in Japan. Kyoto U is consistently ranked amongst the top two in Japan, the top ten in Asia, and the world's top fifty institutions of higher education. Founded upon the principles of its motto, "freedom of academic culture", Kyoto U is currently composed of three campuses with ten Faculties, eighteen Graduate Schools, thirteen Research Institutes, and twenty-two Research and Educational Centers. The Kyoto University Library, boasting over 7 million volumes, is Japan's second-largest academic library. Furthermore, Kyoto U was one of the first three Designated National Universities and is categorized by the Japanese government as a Top Type university in the Top Global University Project (*https://en.wikipedia.org/wiki/Kyoto_University*).

The activities conducted during at Kyoto Universty are shown in Table 9.

Table 9. The activities at Kyoto University

No.	Activity	Result
1.	Sharing **Instructor:** Dr. Yuuko Takeuchi	Participants were introduced to Pine Wilt Nematodes
2.	Visiting pine collection **Instructure:** Dr. Yuuko Takeuchi	Participants were shown pine collection at Kyoto University, i.e., Japanese and red pine trees.

3.8 Forestry and Fishery Product Research Institute

Forestry and Fishery Product Research Institute has been conducting interdisciplinary research on forests, forestry, the timber industry, and tree breeding. Forestry and Fishery Product Research Institute have paved the way for transdisciplinary research with existing stakeholders. The activities conducted at Forestry and Fishery Product Research Institute are shown in Table 10.

Table 10. The activities at Forestry and Fishery Product Research Institute

No.	Activity	Result
1.	Sharing **Instructor:** **Prof. Natsumi Kanzaki**	Participants were introduced to participants what nematodes were transported by insects, nematodes that are dormant in the trachea of insects, and pine nematodes.
2.	Visiting plant parasitic nematodes specimen collections **Instructor:** **Prof. Natsumi Kanzaki**	Participants received information of nematodes specimen collection.
3.	Visiting laboratory of nematode **Instructor:** **Prof. Natsumi Kanzaki**	Participants received information about modern machine and toll to identify the nematodes.

3.9 Visit farm

During the training the participants were given the opportunity to visit agricultural farm. The activities conducted during the field visit in agricultural farm are presented in Table 11.

Table 11. The activities during the farm visit.

No.	Activity	Result
1.	Visit to strawberry farm **Instructor:** **Farmer**	In this opportunity, participants get an opportunity to visit strawberry farm and have a lot information about how to plant strawberry with the green house, and modern technology
2.	Visit to Shionogi Pharmaceutical Company farm **Instructor:** **Shionogi Pharmaceutical Company Staff**	In this opportunity, participants get an opportunity to visit Shionogi Pharmaceutical Company farm and have a lot information about how to plant strawberry with no pesticide application. Participants were also shown fields that are usually planted with sweet potatoes, but at this time it was winter so there were no crops.
3.	Visit to sweet potato farm **Instructor:** **Farmer**	In this opportunity, participants get an opportunity to visit strawberry farm and have a lot information about how to plant strawberry with the green house, and modern technology

3.10. Visit to Factory

During the training the participants were given the opportunity to visit the factory. The activities conducted during the said visit to the factory are presented in Table 12.

Table 12. The activities during the farm visit.

No.	Activity	Result
1.	Visit to Kirishima factory **Instructor:** **Kirishima factory staff**	In this opportunity, participants get to tours to factory, where we can view the process behind the manufacture of Honkaku Shochu, including Shiro Kirishima and Kuro Kirishima. This tour is made to make us learn more about how shochu is made.

4. SUMMARY OF THE ATTACHMENT

- In this attachment program participants gain new knowledge related to molecular identification of nematodes. Identification was done by PCR , RFLP and LAMP methods.
- Participants were also invited to visit Yokohama Plant Protection Station; Institute for Plant Protection, NARO; Kyushu Okinawa Agricultural Research Center, NARO; Kumamoto University; Nara Institute of Science and Technology; Kyoto University; Forestry and Fishery Product Research Institute so that participant's insights broaden.

5. RECOMMENDATION FOR FUTURE ACTIVITIES

For the next program, please conduct more frequent training in each ASEAN countries, so that more people will be trained who are experts in nematode identification, also please provided adequate supporting equipment for the identification of nematodes

6. ACKNOWLEDGEMENTS

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8. IMAGES/PHOTOS

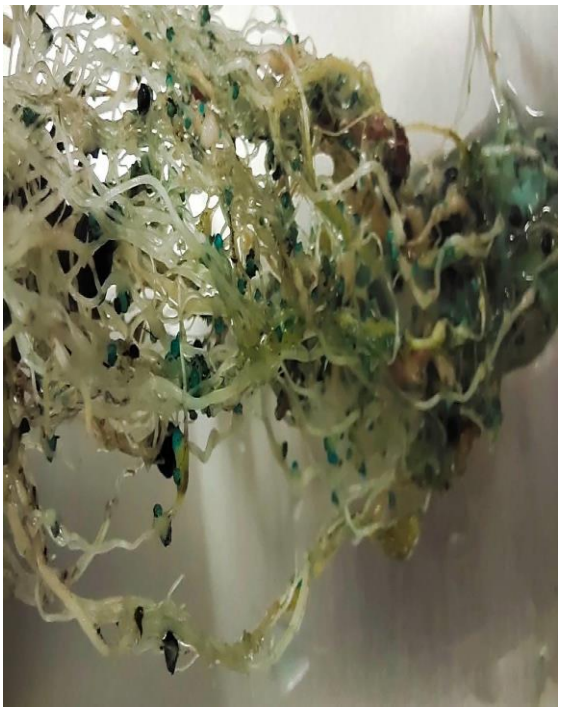
Picture 1. Ryukoku University, Japan

Picture 2. Plant with nematodes symptom

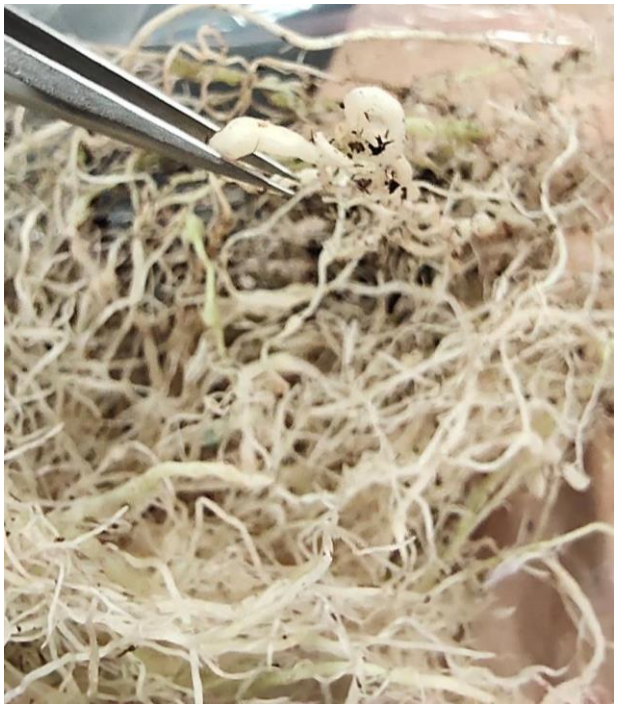
Picture 3. Nematodes collection

Picture 4. Green house

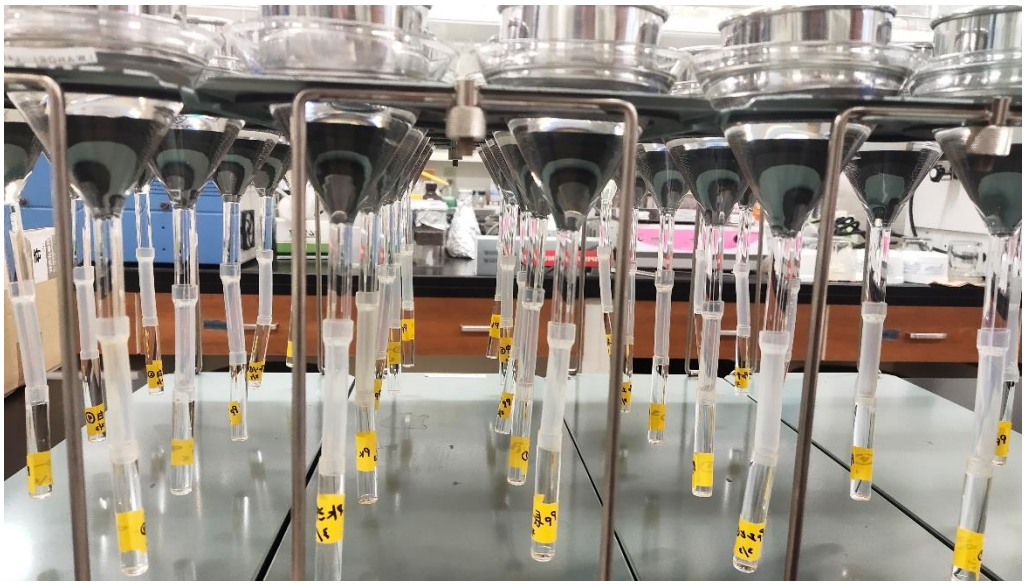
Picture 5. Growth chamber



Picture 6. Staining egg masses

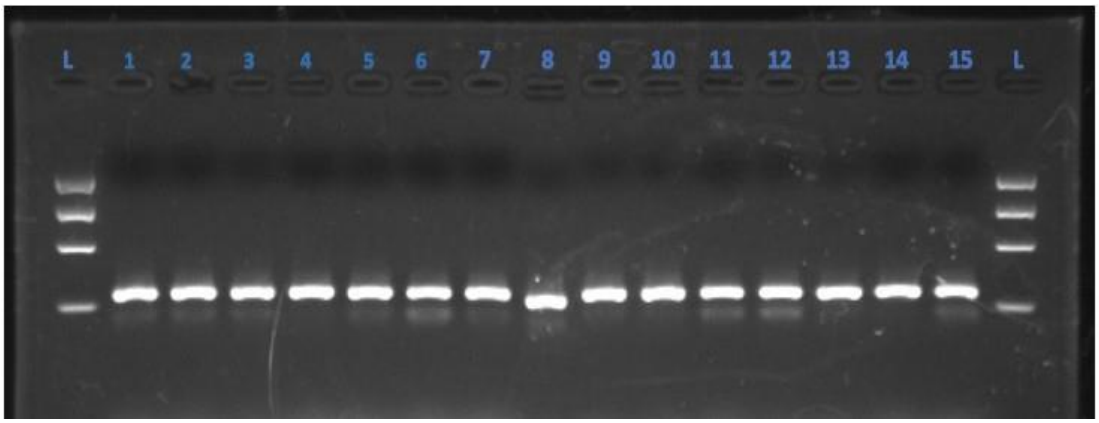


Picture 7. Galls

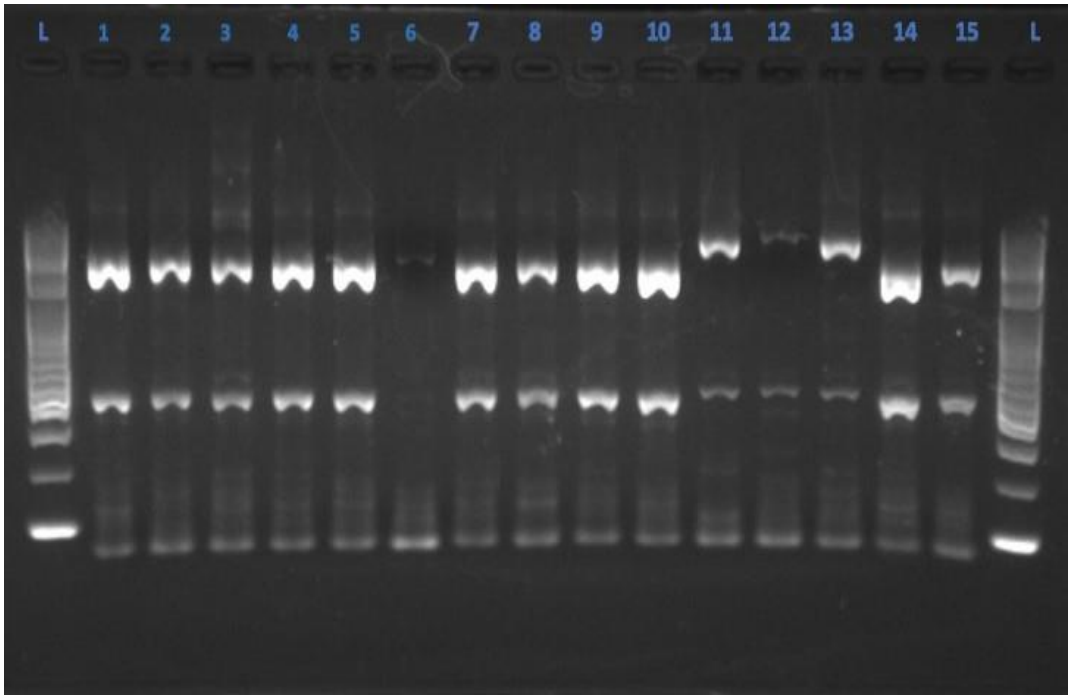


Picture 8. Baermann funnel method

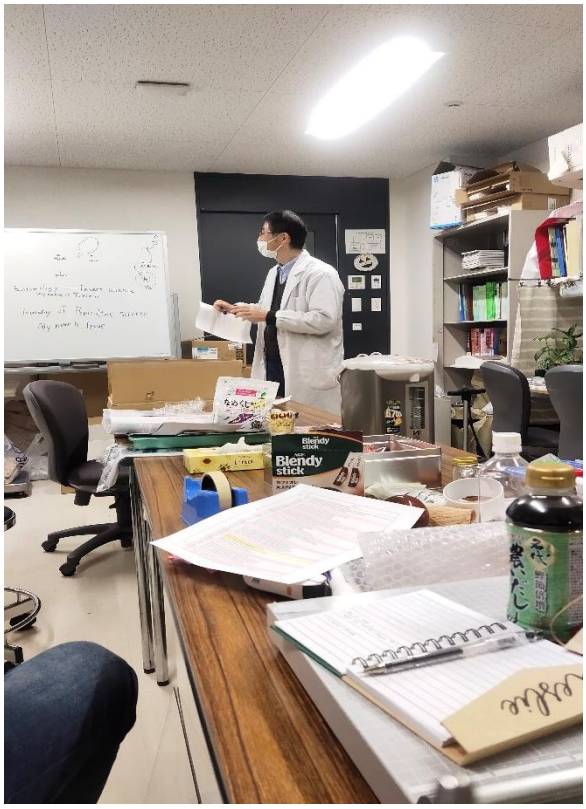
Picture 9. Results of the Baermann method are readily observable



Picture 10. Detection of Meloidogyne spp. with PCR method



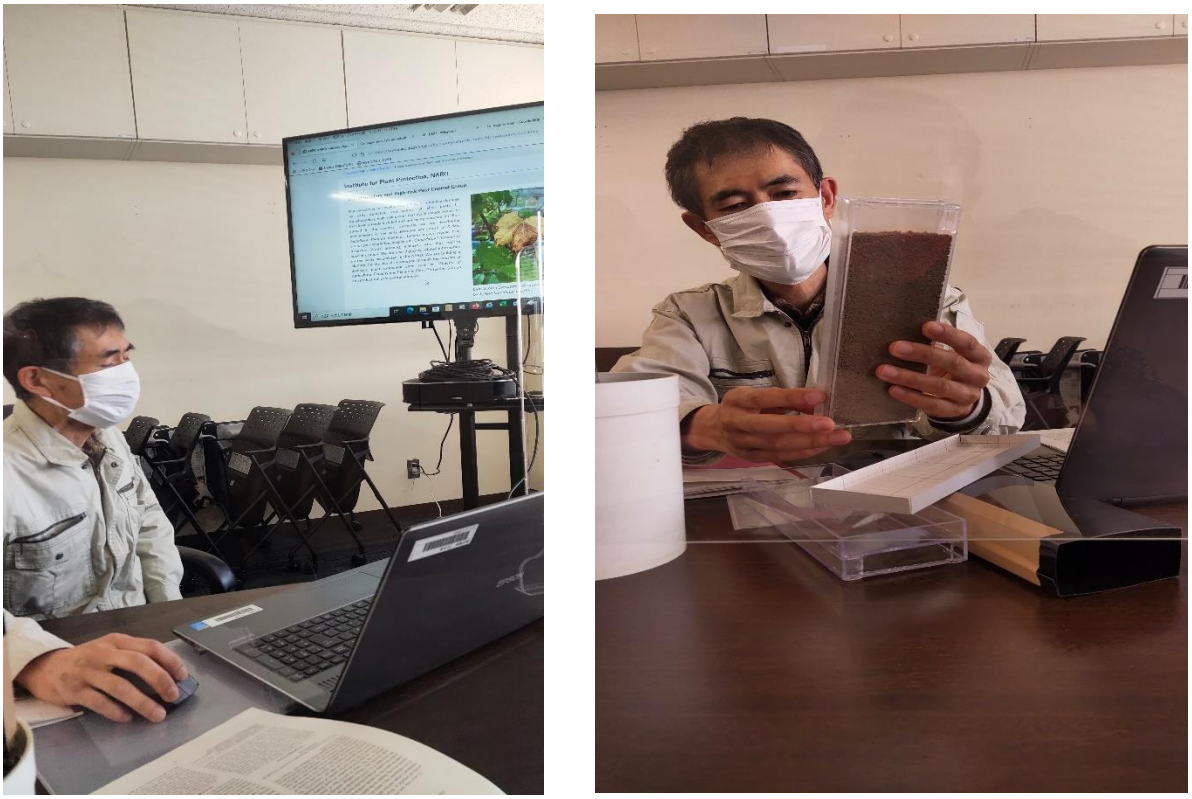
Picture 11. **Detection of Meloidogyne spp. with RFLP method**

Picture 12. Learning activities with Prof. Iwahori

Picture 13. Sightseeing plant parasitic nematodes specimen collections at Yokohama Plant Protection Station

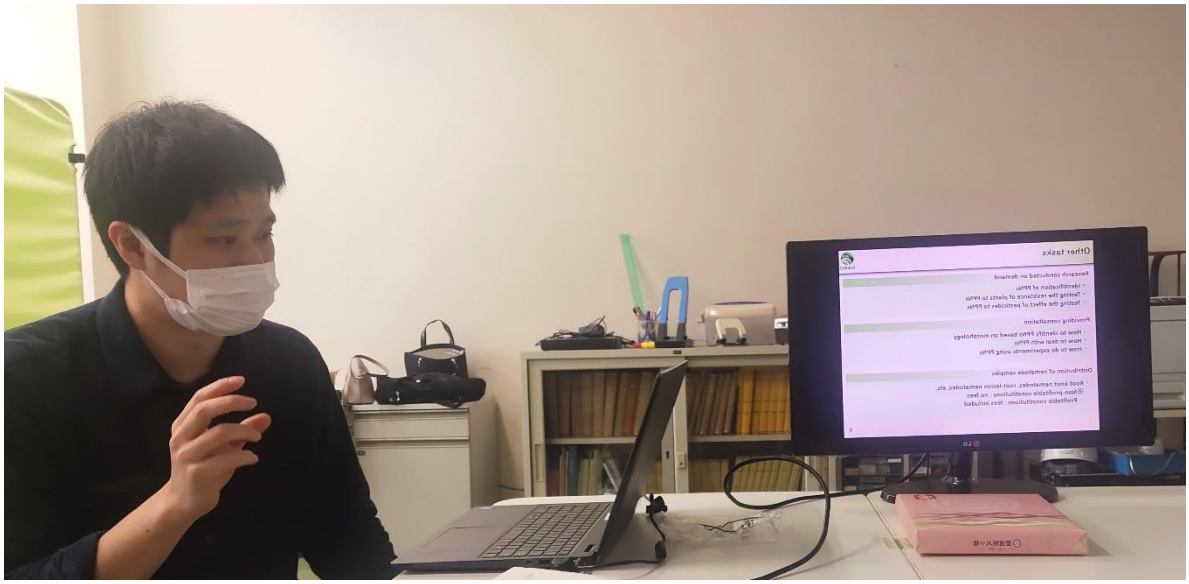
Picture 14. Yokohama Plant Protection Station's green house



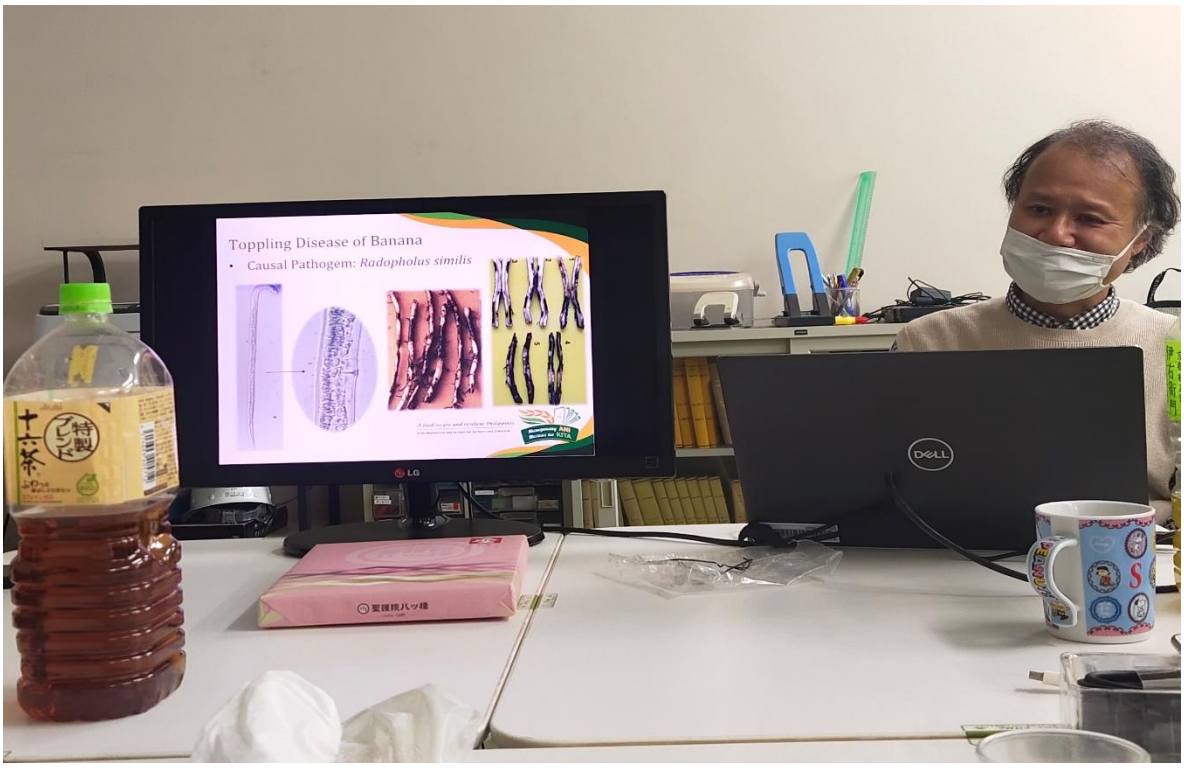
Picture 15. Briefing and orientation with Mr. Okada at Institute for Plant Protection, NARO

Picture 16. Sightseeing laboratory of nematode at Institute for Plant Protection, NARO

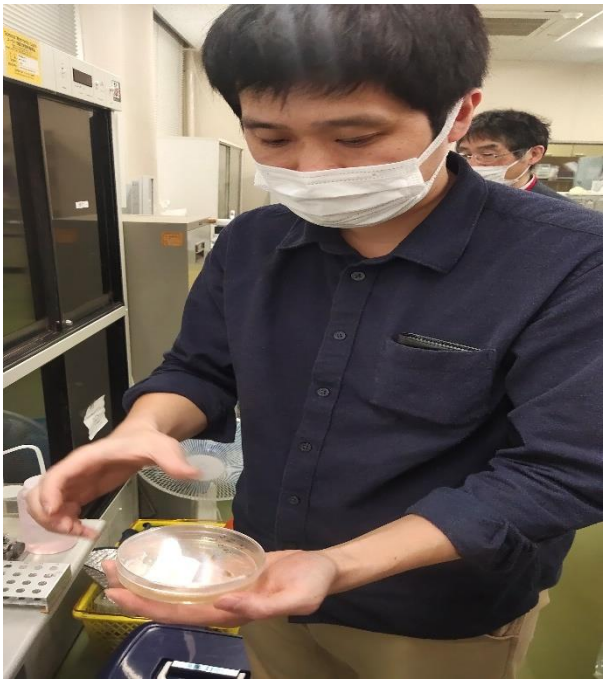
Picture 17. Institute for Plant Protection's green house



Picture 18. Briefing and orientation with Mr. Murata Gaku at Kyushu Okinawa Agricultural Research Center, NARO



Picture 19. Briefing and orientation with Mr. Yoshida Mutsuhiro at Kyushu Okinawa Agricultural Research Center, NARO



Picture 20. Sightseeing plant parasitic nematodes specimen collections at Kyushu Okinawa Agricultural Research Center, NARO

Picture 21. Kyushu Okinawa Agricultural Research Center's green house

Picture 22. Propagation of *Meloidogyne incognita* by hydroponic method

Picture 23. Model of Meloidogyne infection in Arabidopsis

Picture 24. Briefing and orientation with Dr. Mina Ohtsu at Nara Institute of Science and Technology

Picture 25. Plants collection that had been inoculated with Meloidogyne sp., and plant models using agar media.

Picture 26. Plant parasitic nematodes specimen collections at Forestry and Fishery Product Research Institute

Picture 27. Visit to strawberry farm

Picture 28. Visit to sweet potato farm

Picture 29. Visit to Kirishima factory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JAIF Japan-ASEAN Cooperation

Ryukoku University and ASEAN Plant Health Cooperation Network of the ASEANET

with funding support from Japan-ASEAN Integration Fund

CERTIFICATE OF COMPLETION

This certificate is awarded to

Happy Cahya Nugrahana

for having successfully completed the

Attachment Program on Diagnostics of

Plant Parasitic Nematodes in Japan

from 7th February to 4th April 2023

Given this 3rd day of April 2023 at the Department of Agriculture, Ryukoku University

1-5 Yokotani, Seta Oe-cho, Otsu, Shiga, Japan

Hideaki Iwahori

Training Coordinator

Professor, Ryukoku University

Picture 30. Certificate