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Pacific Horticultural and Agricultural Market Access (PHAMA) Program
Department of Foreign Affairs and Trade
01-Dec-2015
Doc No. FIJI27

Identification Training Program for Plant Parasitic Nematodes

Plant Parasitic Nematode Identification Training Programme Output

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Client: Department of Foreign Affairs and Trade

ABN: 47 065 634 525

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01-Dec-2015

Job No.: 42444251

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Quality Information

Document Identification Training Program for Plant Parasitic Nematodes

Ref 42444251

Date 01-Dec-2015

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Revision History

Revision	Revision Date	Details	Authorised	
			Name/Position	Signature
1.0	10-May-16		Stephanie Symon Project Manager	

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Executive Summary

Fijian trainee Ms Unaisi Turaganivalu from the Ministry of Primary Industries, Department of Agriculture, Koronivia Research Station (KRS), completed the plant parasitic nematode identification training programme with Dr Zeng Qi Zhao of Landcare Research, Auckland. The training was conducted from the 10th to the 21st of August in Auckland and in Suva from the 23rd to the 28th of November 2015. During these periods, Ms Unaisi Turaganivalu successfully achieved the following objectives: 1) foliage nematode symptom recognition, 2) plant parasitic nematode dissection and extraction, 3) nematode specimen preparation and identification, 4) nematode specimen illustration (line drawing and micro-photography), 5) nematode specimen characterising and measuring, and 6) molecular taxonomy (single nematode DNA extraction, PCR, sequencing and GenBank BLAST search).

Ms Unaisi Turaganivalu arrived on the 9th of August 2015 and departed on the 22nd of August 2015. She was based at Landcare Research, Tamaki Campus in Auckland and stayed at Waipuna Hotel, 58 Waipuna Rd, Mount Wellington, Auckland, 3.7 km from the Landcare Research building. Dr Zeng Qi Zhao came to Suva on the 21st of November 2015 and departed on the 29th of November 2015. He stayed at Tanoa Plaza Hotel, Suva, approximately 20 km from KRS.

1.0 Training Outcomes

1.1 Auckland training (10 to 21 August 2015)

Induction: On the first day in Auckland, Unaisi was given an induction to Landcare Research, including essential health and safety training for the use of Landcare Research buildings and laboratories. Unaisi was issued with a security pass card and given an introduction to the operation of computers, microscopes, the Imaging Facility, and EcoGene lab (molecular identification).

Slide preparation: Unaisi was shown the standard procedures for slide mounting at the Landcare Research slide-preparation room. She had the opportunity to mount 20 permanent slides for the National Nematode Collection New Zealand.

Microscope use: Unaisi was shown the basic procedure for using the stereo and compound microscopes at Landcare Research. She was shown for the first time the effect of Differential Interference Contrast (DIC) for observation of the nematode specimens and how to achieve the best effect for observing nematodes.

Nematode identification, illustration and characterisation: Unaisi was taught how to draw, measure and take digital photographs of the nematode specimens. Unaisi was shown the main distinguishing characteristics between *Radopholus similis* and its closely related species.

Molecular identification: Unaisi was taught the basic knowledge of molecular techniques that are applied in nematode taxonomy. She practiced DNA extraction using a single nematode, PCR preparation, gel imaging and preparing PCR products for sequencing in the Landcare Research EcoGene lab. She also practiced DNA sequence analysis and BLAST search in GenBank (Appendix, Figure 1).

1.2 Suva training at Koronivia Research Station (23-28 November 2015)

Training preparation: On the first day in Suva, Unaisi and Dr Zeng Qi Zhao worked in the lab at Koronivia Research Station (KRS). All facilities for nematodes were checked and prepared, such as nematode extraction tools, dissecting and compound microscopes, slides and cover slides.

Field sampling: Five ginger growers at Veikoba and Muanaweni were visited, with more than 20 soil and ginger rhizome samples collected. Samples were prepared in the lab using the Whitehead and Hemming (1965) method for soil nematode extraction and the Southy (1986) maceration method for ginger rhizome extraction.

Nematode morphological identification: After leaving samples 48 hrs on the extraction tray, nematodes were harvested, heat killed, preservated and temporary specimen for identification prepared. Unaisi demonstrated her skills learnt from the Auckland training in these areas.

Lecture and practice: Unaisi, two staff from Biosecurity Fiji and more than ten students from the University of the South Pacific were lectured at the KRS. General knowledge of nematology was taught and nematode extraction, fishing and making nematode slides were practised in the lab (Appendix A, Figure 2).

Nematode culture: Unaisi refreshed her nematode culturing skills. Three nematodes that tentatively identified as *R. similis* were inoculated onto carrots. The nematode tentatively identified as *R. similis* was isolated from an abandoned patch of ginger from Muanaweni. Only 5 nematodes were found from the sample extracted and three females were inoculated onto carrots as one part of the training course designed for Una and two (1 male and a female) were brought with Dr Zeng Qi Zhao back to Auckland for molecular identifications. As a result, the molecular ID showed that the nematode is not *R. similis* based on the 18s sequences Blastn result. It is one species that is close to *Ditylenchus*. Moreover, the nematode culture in the KRS lab was checked by Una after a two month time inoculation and it was confirmed that no signal nematode was found from the carrots. This means that the nematode culture was not successful.

Nematode databasing: Building up Fiji's nematode database and collection was discussed with Unaisi. An excel datasheet for the Fiji nematode collection was implemented and 20 nematode permanent slides were registered.

2.0 Recommendations

Differential Interference Contrast (DIC) microscopy needs to be implemented in the KRS lab. Without a DIC function the details of important characteristics for most nematodes cannot be observed clearly under the 100 x objective lens with oil.

The dissecting microscopes need to be replaced in the KRS lab. There are several dissecting microscopes in the lab but they are all in poor condition.

Basic molecular facilities are suggested for KRS lab. Due to the difficulty of using morphology for nematode taxonomy, molecular techniques have been used worldwide in nematology. Therefore, building up a molecular lab is suggested for KRS, such as PCR machine, high speed centrifuge, digital gel image system, gel electrophoresis apparatus, etc.

Follow-up training for Unaisi is suggested. Three weeks of training on both morphology and molecular nematode taxonomy was not sufficient. Although Unaisi has some nematology background, she is new to nematode taxonomy. Therefore, more practice and training are needed for her to enhance her knowledge of nematology.

Appendix A

Appendix A

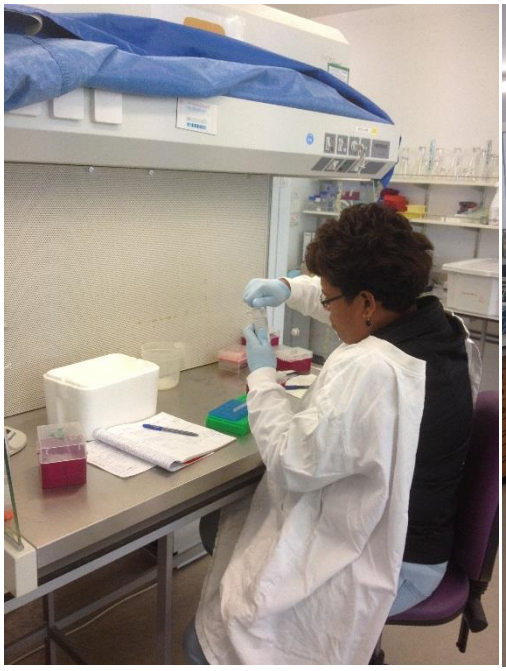
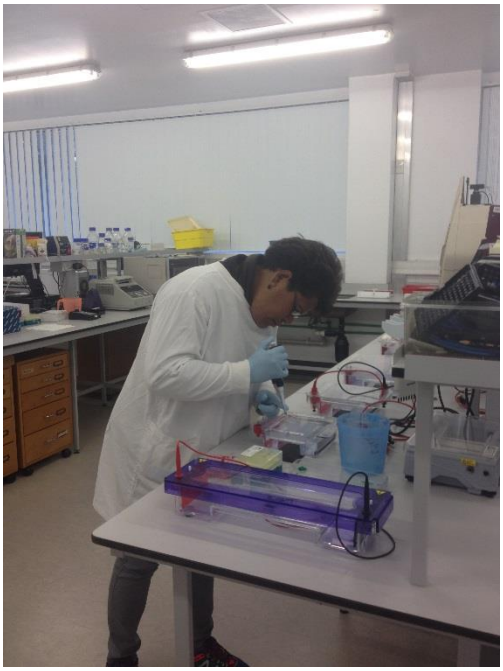


Figure 1: Ms Unaisi Turaganivalu in the Landcare Research EcoGene lab



PCR preparation and gel loading

Figure 2: Ms Unaisi Turaganivalu, university students and staff from Biosecurity Fiji at Koronivia Research Station

Nematology training class and practice