MASTER CLASS IN RHIZOBIAL TECHNOLOGY

‘The isolation, identification and utilization of root nodule bacteria (rhizobia) in promoting sustainable agricultural productivity’

Ravi Tiwari and S. A. Kulasooriya

1st to 13th December, 2012, Institute of Fundamental Studies
Kandy, Sri Lanka

THE CRAWFORD FUND
For a Food Secure World

Murdoch UNIVERSITY

Australian Government
Australian Centre for International Agricultural Research

Summary

Participants from different countries of Africa and Asia joined together with research trainers from the Centre for Rhizobium Studies, Murdoch University and IFS, Kandy to get hands-on training for use of rhizobia in agriculture in a Master Class held 1st -13th December 2012 in Sri Lanka. The training included all aspects of rhizobial work, starting from isolation and characterization of root nodule bacteria from the field to delivery of elite strains as inoculants to farmers. The Master Class provided a unique opportunity for presenters and participants to share knowledge in rhizobiology. This was a very valuable program for researchers dealing with improving soil fertility and supporting sustainable crop production with the minimum use of costly and environmentally unfavorable chemical fertilizers. This Master Class not only provided attendees with skills in legume inoculant technology but also enabled exchange of information between participants and trainers to develop and foster new relationships and networks, with the ultimate aim of improving the production of legume inoculants in Africa and Asia.

Group photo of Master Class participants, trainers and support staff

Venue and dates:
The Master Class was held at the Institute of Fundamental Studies, Kandy, Sri Lanka from 1 December to 13 December 2012.

Funding:
Crawford Fund, ACIAR and Kirkhouse Trust with in-kind contributions from IFS, Kandy and CRS, Murdoch University.

Trainers:
Ravi Tiwari, Julie Ardley, Anabel Vivas Marfisi, Regina Carr and Liza Parkinson (CRS, Murdoch University); Anand Kulasooriya and Gamini Seneviratne (IFS, Kandy)

Participants:
Fifteen people, from India, Kenya, Malawi, South Africa, Sri Lanka, Thailand, Vietnam, Zambia and Zimbabwe, attended the Master Class. See Annex 1 for details of participants.

Acknowledgements
Jade Lim, Valerie Findlay (Murdoch University) Renuka Ratnayake (IFS) and Marchien Van Oostende (Crawford Fund) for organization and administrative support; IFS support team for organizing accommodation and daily transport; John Howieson and Graham O'Hara (CRS), Jen McComb (coordinator Western Australian branch of Crawford Fund), Dennis Blight (Crawford Fund) for overall planning support; and Eric Craswell (Crawford Fund) for planning and sharing his insights and enthusiasm at the Master Class.

Introduction

Having engaged in high input agriculture - adopting the “green revolution” of the 1960s - most developing countries today find themselves in a quagmire in continuing to support and subsidize their farming communities to sustain this model of crop production. The continuous, indiscriminate application of chemical fertilizer and other agro-chemicals has largely contributed to the elimination of beneficial soil microorganisms and the farmers are struggling to sustain high productivity on virtually ‘dead soils’. This is evident in most tropical countries, where crop productivity has either stagnated or even declined despite the addition of chemical fertilizer. Furthermore, addition of chemical fertilizer to such degraded soils, depleted of microorganisms and associated organic matter and therefore unable to retain the added nutrients, results in large quantities of chemicals being washed into waterways, leading to environmental pollution. Nutrient loading by such leachates results in eutrophication of large water bodies (tanks, reservoirs, ponds and lakes), often producing algal blooms that are harmful to animals and humans. Therefore, both on economic and ecological grounds, the time has come to review the continuation of the “green revolution” model and to seek alternatives of environmentally benign, economically sustainable sytems of agriculture.

Foremost among such alternatives is Biological Nitrogen Fixation (BNF) which has the potetnial to reduce and/or replace the continuous use of nitrogen fertilizer, which is very often the nutrient limiting the productivity of several annual crops. As there are many leguminous crops that in symbiosis with root nodule bacteria are capable of BNF, this area of study has become critical for their utilization in agriculture.

This Master Class on ‘The isolation, identification and utilization of root nodule bacteria (rhizobia) in promoting sustainable agricultural productivity’ was most timely and appropriate, particularly to the participants from developing countries of tropical Asia and Africa, where large populations of rural, resource-poor farmers are engaged in crop production under trying circumstances.

At the opening ceremony the Director-General of Agriculture (Sri Lanka) in his address as the Guest of Honour, stated that the Department has been entrusted with the task of expanding soybean cultivation in Sri Lanka to 75,000 ha by 2015 and the department will depend on the IFS to provide inoculants for these extensive cultivations. The Secretary to the Ministry of Technology and Research (Sri Lanka) in her address as the Chief Guest complimented the IFS for this type of work, which has a direct benefit to agriculture. She said that as the Chairperson of the Procurement Committee for fertilizer imports to Sri Lanka, she would take the contribution of inoculant use into consideration in future planning of such imports.

This Master Class was a hands-on course that aimed to take participants through the steps of isolating rhizobia from a target legume or soil, identifying the isolates by molecular methods, authenticating them as root nodule bacteria and evaluating their effectiveness for N_2-fixation and finally to preparing high quality inoculant from elite rhizobial strains

to deliver them to farmers. The Master Class had both theoretical and practical components and was delivered in the form of four modules.

Module 1 Collection and isolation of rhizobia

The first exercise was field collection of root nodules on intact legume roots and soil samples for trapping of rhizobia. The participants learnt how to accurately label collected material and desiccate root nodules to prevent biological decay. Field Record Sheets were used to record data on site location, soil pH and properties, land use and rainfall – vital information for matching rhizobia to the target edaphic and climatic characteristics.

Collecting soil, inspecting plants for root nodules

Participants then learnt aseptic techniques for isolation of rhizobia from the collected nodules and desiccated nodules supplied by the CRS trainers. All participants worked with their own nodules for learning the techniques for nodule sterilization and aseptic isolation of nodule occupants.

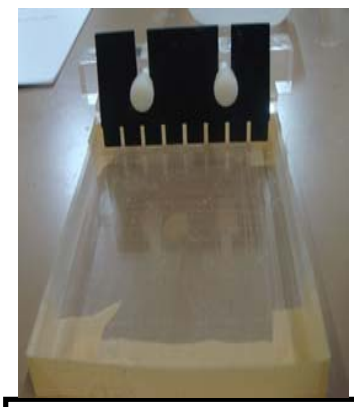
Nodule sterilization and isolation of bacteria

Part of the theory component of the course involved making the trainees aware of the diversity of rhizobial species and the importance of observing the isolation plates daily for both phenotyping isolates and obtaining well-grown, isolated single colonies. Once single colonies had appeared, these were subcultured to obtain pure cultures of their isolates – the first step in obtaining rhizobia for use on a target legume.

Module 2 Identification of isolates using molecular methods

Once pure cultures were obtained the next challenge was to identify the isolated bacteria. This module covered molecular approaches used in identification and characterization of bacteria isolated from root nodules.

The participants performed a variety of PCR methods on selected rhizobial isolates. Two different approaches for preparing templates for PCR were used: participants learnt methods for preparing both whole cells and for extracting genomic DNA, for use as template in PCR reactions. They then performed agarose gel electrophoresis to separate and visualize their PCR products.



Preparation of agarose gel and loading PCR samples

These PCR techniques were used in grouping the isolates by comparing PCR banding patterns. Selected isolates from each group were then further analyzed by DNA sequencing of 16S rDNA gene, a standard gene used in microbiology for identification of bacteria. Participants learnt the methods to amplify 16S rDNA from their rhizobial isolates for DNA sequencing.

A bioinformatics session was run where participants learnt how to assemble DNA sequences and to use a DNA searching software tool (BLAST and FASTA) for strain identification. Various techniques that participants learnt in this module included cell preparation and DNA extraction for PCR template, preparation and running of PCR reactions and sequencing reactions, assembly of DNA sequences for analysis by BLAST and FASTA searches.

Module 3. Authentication, Effectiveness, MPN and Nodule Trapping

The areas covered in this module included authentication and effectiveness of rhizobial isolates using host plants. Specific attention was given to observing strict sterile conditions. Module 3 involved participants doing a lot of preparatory work of media, stocks, equipment, plant seeds, inoculum etc; different types of bacterial growth media,

plant growth media and many of the stock solutions. Participants had many opportunities to practice serial dilutions, an important technique used for counting of bacteria using Most Probable Number (MPN) and Miles and Misra methods.

This module was a very hands-on approach with participants being involved in all aspects of the preparation of glasshouse experiments, including seed germination in sterile conditions, planting of seeds in pots, and then inoculation with a rhizobial culture. They were also shown a variety of glasshouse techniques that could be used to answer different experimental questions.

Various techniques learnt in this module included-(1) MPN: an indirect method to count root nodule bacteria in a soil sample; (2) soil trapping to isolate rhizobia from a soil sample; (3) Miles and Misra and spread plate methods to count the number of bacteria in a soil sample or a bacterial culture.

Seed germination, inoculation and planting

The method for harvesting glass house grown plants was demonstrated using inoculated pots previously prepared by the IFS staff. The expected outcomes from glasshouse experiments were discussed in class and participants learnt how to use MPN tables to calculate bacterial population numbers in a soil.

Module 3 was interlinked with the other modules of the master Class and provided participants with a thorough understanding of best practice glass house techniques.

MODULE 4: Legume Inoculant Technology and Quality Control (QC) procedures.

This module aimed to train rhizobiologists in the latest techniques in manufacturing legume inoculants with a strong focus on quality control (QC) procedures that are essential in manufacturing high quality inoculant products. The content of the training was a practical and hands-on experience where the participants had to produce batch fermentation cultures, inject inoculant bags and perform QC tests.

The main objective of this module was to learn to produce a small scale amount of legume inoculant. The module structure was in its entirety a laboratory practical where participants were involved in most of the steps used in the manufacture of legume inoculants. This involved the preparation and sterilization of carrier material, inoculation of starter cultures for transfer into a flask fermentation system, injection of sterilized

carrier with rhizobia cells and incubation or curing of inoculated bags at 28 ^{O}C for 7 to 14 days before storage at 4 ^{O}C.

A component of this module was Quality Control and its importance was emphasized throughout the Master Class. A good quality inoculant is defined by: a high level of rhizobia cells with over 1 x 10^{9} rhizobia cells per gram of inoculant for a solid carrier (peat, filter mud); able to demonstrate an effective nodulation and nitrogen fixation with their target host; have none or minimal (less than a million) contaminating microbes and should also have an adequate shelf life.

Injection of carrier (coir dust) bags

It was explained to the participants that the importance of having such high numbers of rhizobia cells is that these need to have the ability to compete against the already large but ineffective populations of rhizobia cells in the soil and ensure nodulation and a nitrogen fixation response. Having high numbers will also maximize the ability of rhizobia cells to tolerate stress conditions such as desiccation, seed toxicity, soil pH among others. QC programs therefore deal with the quality of the strains in the inoculants, their numbers and the number of contaminating microorganisms. Manufacturing companies need to go through a strict quality assurance program applied to the entire production process, from rhizobia culture preservation to final legume inoculant, to ensure a high quality product. The practical exercises for this module included QC checks in both the fermentation broths and inoculated carrier. The QC test on broths was by Gram staining and checks on injected carriers by cell counts. It was emphasized to participants that cell counts are an essential component of the quality control process as it determines the quality in terms of viable cells in inoculated carriers.

What did participants think of the course?

Participants' feedback and comments were captured through a post-class survey which was submitted by all 15 participants of the Master Class; see Annex 3. The course was ranked very high on both categories of survey 'the quality of the course' and also on 'knowledge gained'.

Under 'quality of course' 14 out 15 participants strongly agreed that 'the trainers/mentors were knowledgeable and provided lecture/information of a good quality' the remaining one participant also agreed with this point. 14 participants either agreed or strongly agreed that it was sufficient time for running the Master Class, 2 remain neutral. One participant thought that they would have learnt more if the Master Class was run for a longer time. All participants either agreed or strongly agreed for the supply of adequate supporting material, balance between theory and practice, level of English and the understanding of contents.

For survey on 'knowledge gained' also participants' comments were very positive. Fourteen out of fifteen participants either strongly agreed that the training increased their knowledge of international trend/activities and their capacity to conduct research. All participants either agreed or strongly agreed with all criteria in this section except one. Three participants were neutral on learning new or improved ways to communicate with networks within their field of expertise. We agree, more work needs to be done on this issue and it needs further attention in future. Some other comments from participants have been captured in Annex 4.

IFS trainers and support staff did an excellent job in hosting the Master Class. Here we would like to mention some comments from Dr Eric Craswell (Coordinator Crawford Fund Master Class program) regarding IFS's hosting of the Master Class:

'*I cannot think of a more helpful and accommodating host agency for any of my Master Classes with the Crawford Fund'*

'The IFS proved itself to be an excellent host agency, with everything organized effectively and without fuss, from airport greetings to meals to printing of certificates.'

Participants also had similar views on the hosts of this Master Class.

Lessons learned

The course was designed as a series of modules that would take the participants from isolating and identifying rhizobia from a target legume through to the practical tests that are applied to determine the isolates' capacity for N_2-fixation and their saprophytic competence and finally, to preparing high-quality rhizobial inoculant for symbiotic nitrogen fixation in agricultural programs. As such, the course aimed to teach a large number of diverse skills (basic microbiology and laboratory skills, glasshouse techniques, soil testing, molecular biology, accessing microbial genome databases, inoculant technology).

As the participants in the course came from a wide range of backgrounds, experience and skills, and were working in both basic and applied science fields, not all of these modules may have been directly relevant for their needs. Perhaps a pre-course survey, in which participants could identify areas in which they need to gain skills, would be useful. However, we feel that all participants have been provided with a set of skills in all the areas covered and, more importantly, have been given the training and access to resources that will allow them to become confident and independent researchers in their various fields.

We also believe that the Master Class gave our participants both theoretical and practical experience in how to design a program of research and how to solve problems that might arise during that research. Our hope and expectation is that this will aid our participants in developing, in their own agricultural systems, the legume-rhizobia symbiotic nitrogen fixation programs that are important to providing sustainable and cost-effective increases in productivity.

One of the best results that came out of this Master Class was the sense of teamwork that developed through the two weeks that it was run. This was apparently the first time that participants from such a variety of countries and cultural backgrounds had taken part together in a Master Class. It was wonderful for us, as trainers, to see how this diverse group of people bonded together as a team and to note the enthusiasm with which they tackled the course. We also greatly appreciated all the help provided by our Sri Lankan hosts and in particular, by the IFS team. Running the Master Class was a very rewarding experience for us all.

ANNEX 1

List of participants

PERSONAL DETAILS	ROLE, AFFILIATION AND RESPONSIBILITY	COUNTRY	ADDRESS/CONTACTS
Charmalie Abayasekara Ph.D	**Senior Lecturer** University of Peradeniya Teaching and research	Sri Lanka	charmaliea@gmail.com
La Tuan Anh M.Sc.	Soils and Fertilizers Research Institute (SFI) Research and development of production technology of new microbial preparations adapted in Vietnamese ecological conditions	Vietnam	latuananhvsv@yahoo.com.vn
Oscar Dlodlo	**MSc.Student** University of Cape Town	South Africa	Oscar.dlodlo@gmil.com
E. M. H. G. S. Ekanayake	Rhizobial inoculant production unit, Institute of Fundamental Studies	Sri Lanka	ranilasara@gmail.com
Gunaratne Amal MSc. Crop Science	**Assistant Manager**, Research and Development Plenty Foods (pvt.) Ltd. Productivity improvement, Technology transfer & training of people	Sri Lanka	hmac_gunarathne@yahoo.com
Mahendra Gunawardane M.Phil Microbiology	**Senior Lecturer I, Head of the Department of Microbiology** Department of Microbiology, University of Kelaniya, Sri Lanka Teaching, research and supervision of research at university	Sri Lanka	mahendra@kln.ac.lk
Tatenda Thelma Kainga	**MPhil Student** University of Zimbabwe Department of Soil Science and Agricultural Engineering	Zimbabwe	thelmakay28@gmil.com tatendakainga@yahoo.com
Victor Kwalombota Mukwa B.Sc. Biological Science	**Agriculture Research Officer** Zambia Agriculture Research Institute Research in biological nitrogen fixation	Zambia	vkmukwa@yahoo.co.uk

PERSONAL DETAILS	ROLE, AFFILIATION AND RESPONSIBILITY	COUNTRY	ADDRESS/CONTACTS
Cathrine Mushangwe Diploma Laboratory Science Technology	**Principal Research Technician** Soil Productivity Research Laboratory (SPRL) of the Chemistry and Soil Research Institute in the Ministry of Agriculture Inoculant production and research	Zimbabwe	ckabade@yahoo.com
Archana Ojha	**PhD Student** North Eastern Hill University, Shillong, Meghalaya, India	India	archanaojhal@gmail.com
Aparna Raturi Ph.D	**Post Doctoral Fellow** JNV University, Jodhpur, India Molecular characterization of diazotrophic and PGPRs	India	aparna_raturi@yahoo.co.in
Akinson Tumbure	**Senior Research Officer** **M.Phil Student** Soil Productivity Research Laboratory Marondera Zimbabwe Rhizobium inoculant technology research	Zimbabwe	atumbure@yahoo.co.uk
Maureen Nekoye Waswa	**MSc Student** University of Nairobi	Kenya	maureewaswa@yahoo.com
Jenjira Wongdee	**MSc Student** Institute of Agricultural Technology, Suranaree University of Technology (SUT)	Thailand	BBG.jane-Na@hotmail.com
Esnart Nyirenda Yohane	**Agricultural Research Scientist** **MSc Student** Department of Agricultural Research Services in the Ministry of Agriculture and Irrigation-Malawi	Malawi	nyirendaesnart@yahoo.com

ANNEX 2

Training Schedule

Day 1: Sunday, 2nd December 2012 – Kandy

9.00 am	Welcome, brief introduction	
	Familiarization with facilities	
	Background to Day 1 exercises	
10.00 am	EXERCISE 1.1	Media and stock preparation
	EXERCISE 1.2	Nodule and soil collection (field work)
	EXERCISE 1.3	Isolation of rhizobia from nodules
	EXERCISE 3.1	Media and stock preparation
	EXERCISE 3.3	Preparation of inocula
	EXERCISE 4.1	Media and stock preparation
	EXERCISE 4.2	Preparation of fermentation unit

Day 2: Monday, 3rd December 2012 - Kandy

9.00 am	Official opening of Master Class	
11.00 am	Module 2	Identification of isolates using molecular methods
	Appendix B & C	Stocks
	EXERCISE 2.1	Preparation of DNA template for PCR
	EXERCISE 4.3	Inoculation of starter cultures

Day 3: Tuesday, 4th December 2012 - Kandy

9.00 am	EXERCISE 1.3	Isolation of rhizobia from nodules (contd.)
	EXERCISE 3.1	Media and stock preparation (contd.)
2.00 pm	Participant presentations (6-8 participants)	

Day 4: Wednesday, 5th December 2012 - Kandy

9.00 am	Participant presentations (contd.)	
11.00 am	EXERCISE 1.3	Isolation of rhizobia from nodules (contd.)
	EXERCISE 1.4	Maintaining rhizobial collections and databases
	EXERCISE 2.2	Genomic fingerprinting using rep-PCR
	EXERCISE 2.5	Amplification of 16S rRNA gene
	EXERCISE 4.4	Inoculation of fermentation unit

Day 5: Thursday, 6th December 2012 - Kandy

9.00 am	EXERCISE 1.3	Isolation of rhizobia from nodules (contd.)
	EXERCISE 3.2	Preparing seeds for germination
	EXERCISE 3.5	Effectiveness
	EXERCISE 3.6	Authentication
	EXERCISE 3.7	Nodule trapping of rhizobia from soil
	EXERCISE 3.8	Most Probable Number (MPN)
	EXERCISE 4.5	Quality Control
	EXERCISE 4.7	Cell counts: Miles & Misra

Day 6: Friday, 7th December 2012 - Kandy

9.00 am	EXERCISE 1.5	Subculturing of rhizobial isolates
	EXERCISE 2.3	Rep-PCR gel electrophoresis
	EXERCISE 2.6	Gel electrophoresis for PCR product control
	EXERCISE 4.6	Carrier injection
	EXERCISE 4.7	Cell counts: Miles & Misra (contd.)

Day 7: Saturday, 8th December 2012 - Kandy

9.00 am	EXERCISE 1.5	Subculturing of rhizobial isolates (contd.)
	EXERCISE 3.3	Preparation of inocula (contd.)
	EXERCISE 3.4	Total viable counts of rhizobia
	EXERCISE 3.5	Effectiveness (contd.)
	EXERCISE 3.6	Authentication (contd.)
	EXERCISE 3.7	Nodule trapping of rhizobia from soil (contd.)
	EXERCISE 3.8	Most Probable Number (MPN) (contd.)
	EXERCISE 4.7	Cell counts: Miles & Misra (contd.)

Day 8: Sunday, 9th December 2012

9.00am	FIELD TRIP

Day 9: Monday, 10th December 2012 - Kandy

9.00 am	EXERCISE 1.5	Subculturing of rhizobial isolates (contd.)
	EXERCISE 1.7	Nodule scoring
	EXERCISE 4.7	Cell counts: Miles & Misra (contd.)
	EXERCISE 4.9	Testing of inoculated carriers

Day 10: Tuesday, 11th December 2012 - Kandy

9.00 am	EXERCISE 1.6	Phenotyping of rhizobial isolates
	EXERCISE 3.8	Most Probable Number (MPN) (contd.)
	EXERCISE 4.8	Determination of cell counts from broth
	EXERCISE 4.11	Most Probable Number (MPN) plant test
	EXERCISE 4.12	Seed inoculation

Day 11: Wednesday, 12th December 2012 - Kandy

9.00am	EXERCISE 2.4	Analysis
	EXERCISE 2.7	Assembling sequences
	EXERCISE 2.8	Identification using reference database
	EXERCISE 2.8	Building phylogenetic trees
	EXERCISE 3.4	Total viable counts of rhizobia (contd)
	EXERCISE 3.10	Counting colonies from Miles and Misra plates
	EXERCISE 4.10	Determination of cell counts from carriers

Day 12: Thursday, 13th December 2012 – Kandy

9.00 am	Debriefing
11.00 am	Closing ceremony
12.00 noon	Class finishes

ANNEX 3

Feedback: Course delivery and knowledge acquitted

A Quality of the Course	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Not Relevant
(1) The content of the Master Class was directly related to my field of work at time of completion	8	6	1			
(2) I was provided with adequate supporting material	9	6				
(3) The trainers/ mentors were knowledgeable and provided lectures/information of a good quality	14	1				
(4) The content was easy to understand	9	6				
(5) The level of English used was good	11	4				
(6) There was sufficient time allowed for the Master Class to get a good understanding of the content	5	8	2			
(7) The course was well balanced between theory and practice	7	8				

Other Comments

- The classes were arranged in such a way that it helped gradual build up knowledge and practical experience simultaneously.
- Adequate time had been allocated for hands on experience, rather than over loading with lectures.
- Individual development of experimental skills was done very effectively.
- The organization of the classes was superb.
- A lot of effort put in by the resource persons to pass on knowledge and skills.
- Course gave me some new knowledge.
- It is better to organize course theory and practical parallel.
- If the duration could have longer we would have learnt much more.

B Knowledge Gained:	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Not Relevant
(1) The training increased my knowledge of international trends/activities	14	1				
(2) I increased my capacity to conduct research	14	1				
(3) I better understand issues and principles in my field	11	4				
(4) I acquired new technical skills	12	3				
(5) I acquired new ways to approach work problems	10	5				
(6) I learned techniques for managing and organizing people and projects	4	10				
(7) I learned new or improved ways to communicate with networks within my field of expertise (eg farmers, donors, research organisations, government)	5	7	3			

Other Comments

- This Master Class was really great help for research work.
- I got acquainted with so many approaches which will help in my future to complete my research work and also to serve my nation for food security.
- I managed to learn a lot, from simple techniques (how to do them better) to molecular biology and bioinformatics.
- This master class has helped me a lot to increase my knowledge of rhizobiology such that now I can carry out my research with knowledge and understanding.
- This Master Class is an essential and effective tool to disseminate information and skills that are critical as a resource to many end users directly (participants) and indirectly the communities they serve which may vary from an academic institution, research station, a village, a town, a country to a continent.
- I think field activities should be incorporated in addition to the laboratory work.
- A special thank you to Prof Ravi, Julie, Regina, Liza and Anabel for sharing their knowledge and skills and their patience!

ANNEX 4

Other responses from participants

- It was a very well planned Master class.
- The trainers were friendly that made the trainees to get good understanding of the material delivered during the master class.
- A lot of preparatory work must have done meticulously prior to the commencement of the Master Class.
- The duration of the master class did not allow for most of the practical courses that involved plant growth to run to the end.
- It will be more beneficial to understanding what actually happen in the field level.
- The Master class motivated me to continue research in Rhizobiology, with a renewed awareness of the importance of inoculants for sustainable agriculture.
- It was great to meet many others from different countries involved in research in Rhizobiology, leading to successful application of Rhizobial inoculants in agricultural fields.
- This training was fulfilling and I have learnt a lot. I would like to thank the organizers, sponsors and facilitators it was a fulfilling master class, thank you.
- A big thank you to all the mentors of master class and also a Crawford fund and other funding supporters.
- I take this opportunity to thank all the resource persons, the organizers and the Crawford Fund for enabling me to participate in this useful and very well conducted Master class.
- I would love to attend more workshops of this nature as they work as capacity builders for me as an individual and for my country through the experience I have gained.