Advanced technical skills in rhizobiology

East and Central African, West African and South African Hub

Milestone reference number 5.1.1

Saidou Koala, Patrick Ngokho, Paul Woomer, Nancy Karanja, Fredrick Baijukya, Kenton Dashiell, Joseph Machua, Teressah Wafullah, George Mwenda and Stanley Kisamuli

Abdullahi Bala, Robert Abaidoo, Ado Yusuf, Martin Jemo, Steven Kilani, James Oyedipe, Bola Oke and Paul Woomer

Mazvita Murwira, Akinson Tumbure, Collis Mukungurutse and Judith de Wolf

Submission date: 20 October 2011

N2Africa

Putting nitrogen fixation to work for smallholder farmers in Africa

N2Africa is a project funded by The Bill & Melinda Gates Foundation by a grant to Plant Production Systems, Wageningen University who lead the project together with CIAT-TSBF, IITA, and many partners in the Democratic Republic of Congo, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda and Zimbabwe.

Email: n2africa.office@wur.nl
Internet: www.N2Africa.org

Authors of this report and contact details

Name: Dr. Saidou Koala Partner acronym: CIAT-TSBF
Address: P.O. Box 823-00621, Nairobi, Kenya.
Email: s.koala@cgiar.org

Name: Dr. Paul Woomer Partner acronym: CIAT-TSBF
Address: P.O. Box 823-00621, Nairobi, Kenya.
Email: plwoomer@gmail.com

Name: Prof. Nancy Karanja Partner acronym: University of Nairobi
Address: P.O. Box 29053-00625, Nairobi, Kenya.
Email: Nancy.Karanja@cgiar.org

Name: Dr. Kenton Dashiell Partner acronym: CIAT-TSBF
Address: P.O. Box 823-00621, Nairobi, Kenya.
Email: K.Dashiell@cgiar.org

Name: Abdullahi Bala Partner acronym: IITA
Address: PMB 312, Sabo Bakin Zuwo, Tarauni, Kano, Nigeria
E-mail: A.Bala@cgiar.org

Name: Robert Abaidoo Partner acronym: IITA
Address: PMB 5320 OYO ROAD, Ibadan, Nigeria
E-mail: r.abaidoo@cgiar.org

Name: Patrick O. Ngokho Partner acronym: CIAT-TSBF
Address: P.O. Box 823-00621, Nairobi, Kenya.
Email: p.ngokho@cgiar.org

Name: Mazvita S. Murwira Partner acronym: SPRL
Address: SPRL, P Bag 3757, Marondera, Zimbabwe
E-mail: murwiram@yahoo.com

Name: Akinson Tumbure Partner acronym: SPRL
Address: SPRL, P Bag 3757, Marondera, Zimbabwe
E-mail: atumbure@yahoo.co.uk

Name: Collis S. Mukungurutse Partner acronym: SPRL
Address: SPRL, P Bag 3757, Marondera, Zimbabwe
E-mail: collismuk@gmail.com

Name: Judith J. de Wolf Partner acronym: CIAT
Address: CIAT, P.O. Box MP 228, Mt. Pleasant, Harare, Zimbabwe
E-mail: Judith.DeWolf@cgiar.org

If you want to cite a report that originally was meant for use within the project only, please make sure you are allowed to disseminate or cite this report. If so, please cite as follows:

Koala, S., Ngokho, P., Woomer, P.L., Karanja, N., Baijukya, F., Dashiell, K., Machua, J., Wafullah, T., Mwenda, G., Kisamuli, S., Bala, A., Abaidoo, R., Yusuf, A., Jemo, M., Kilani, S., Oyedipe, J., Oke, B.,l Woomer, P., Murwira M.S., Tumbure A., Mukungurutse C.S. and de Wolf J.J., 2011. Advanced Technical Skills in Rhizobiology in East and Central African, West African, South African Hub, N2Africa Southern Africa Hub, www.N2Africa.org, 55 pp.

Disclaimer:

This publication has been funded by the Bill & Melinda Gates Foundation through a grant to Wageningen University entitled “Putting nitrogen fixation to work for smallholder farmers in Africa”. Its content does not represent the official position of Bill & Melinda Gates Foundation, Wageningen University or any of the other partner organisations within the project and is entirely the responsibility of the authors.

Table of contents

East and Central African Hub – Kenya

Summary

Improved technical capacities in rhizobiology are required to design, evaluate and commercialize legume inoculants in East and Central Africa. A training course was held for the purpose of training key laboratory personnel and MSc students to gain the knowledge and skills to enhance inoculant production in their respective countries. The workshop attracted twelve participants (50% women) from Democratic Republic of Congo, Kenya and Rwanda. In this workshop, various discussions and material presentations were given by experts and practitioners in rhizobiology, microbiology and biotechnology.

The approach chosen was one of mixing theoretical, interactive and practical sessions throughout the training. The participants were able to: discuss various views on rhizobiology, inoculant production and quality control, lab-based PCR methods, nitrogen fixation quantification, and laboratory, greenhouse, and field techniques, exchange experiences and lessons between participants, and learn from inoculant industry practitioners about production system efficiency and product sustainability. Materials provided to participants included a training resource manual, PowerPoint presentations, literature and sample technical papers on biological nitrogen fixation (BNF) both in printed and electronic copies. These materials were used for lectures and discussed with respect to each practical session. Visits were made to the Kenya Forestry Research Institute, Muguga and the CIAT-TSBF laboratories in Nairobi.

All participants agreed that the workshop achieved its stated objectives and that they would be able to carry out the skills learnt during the course. At the end of the training participants discussed and developed country specific action plans which shall facilitate rhizobiology activities in their respective countries (See Annex 3)

Introduction

Background Information

Bacterial associations with certain plant families, primarily legumes species, make the largest single contribution to biological nitrogen fixation in the biosphere. The N fixing rhizobia-legume system contributes around 50–600 kg N/ha/year while rhizosphere associations and free living bacteria supply 5–25 and 0.1–25 kg N/ha/year, respectively. An important component of the project "Putting nitrogen fixation to work for smallholder farmers in Africa" is to select superior rhizobia strains for enhanced biological nitrogen fixation (BNF) and develop inoculum production capacity in sub-Saharan Africa, with public and private sector partners (Project Objective 3). It is expected that the N2Africa project will raise average grain yields by 954 kg/ha in four legumes (groundnut, cowpea, soybean, and common bean), increase average biological nitrogen fixation (BNF) by 46 kg/ha, and increase average household income by $465, directly benefiting 225,000 households (1,800,000 individuals) in eight countries in sub-Saharan Africa (DRC, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Zimbabwe)

The aim of this training was to improve the microbial skills relevant to inoculant and legume production techniques of technical staff from the partner institutions and MSc students in Democratic Republic of Congo, Kenya and Rwanda. Activities at the training included practicing in the laboratory, visiting an inoculant production plant and attending lectures. The training was designed by Dr. Paul Woomer and Prof Nancy Karanja with consultation from other N2Africa team members. The instructors of the training were drawn from N2Africa staff, MIRCEN of the University of Nairobi and other partner organizations with expertise in microbial skills and inoculant production.

Course Objectives

This training was held to train N2Africa laboratory technicians and MSc. students to advance their technical skills in rhizobiology. Specific objectives were to:

- Equip participants with knowledge and skills in inoculant production, quality control and field inoculation of grain legumes.
- Give the facilitators and trainees the opportunity to share varied lessons and experiences, related to methods and tools used at an inoculum production plant and how inoculums are used at farm level.
- Provide each participant with a full set of activities and materials which include: basic rhizobiology, strain selection and inoculant production and use.
- Evaluate and improve the rhizobiology training materials and plan the outline for a training programme to be delivered in the Southern and West Africa hubs.
- Discuss and develop a draft action plan for project objective 3 activities in the respective countries

Participants and Resource Persons

Twelve participants (50% women) from the Democratic Republic of Congo, Kenya and Rwanda were in the course. The participants were selected with preference given to those who have an interest and potential to support the development of inoculant production in their country.. The resource persons were from the N2Africa team and its key partners including Prof Nancy Karanja, Dr. Paul Woomer, Dr Fredrick Baijukya, Dr Kenton Dashiell and Dr Saidou Koala. University of Nairobi-MIRCEN staffs were responsible for preparation and facilitation of laboratory, greenhouse and field practical sessions. Other invited resource persons were Mr. Joseph Machua, Senior Research Officer at the Kenya Forestry Research

Institute (KEFRI) and Mrs. Teressah Wafullah, an AKTP Associate at MEA Limited. The list of participants and resource persons is in Annex 2.

Organization and Structure of the Course

The programme (Annex 1) consisted of lectures, practical and group discussion. Every lecture was followed by a related hands-on practical and the facilitators maintained a continuous dialogue with participants. This enabled participants to better understand the concepts being given and to apply these to the work they will be doing in their countries. The lecture modules addressed the following subjects; basic rhizobiology, BNF in small scale agriculture, the legume-rhizobia symbiosis, rhizobia inoculants and inoculation, strain characterization, identification, authentication and selection, and quality control of inoculants. Each lecture would start from basics to help bring all participants to the same understanding of concepts and to a shared level of knowledge by the end of the training activity.

The practical session helped participants to better understand the lecture material presented. In general, laboratory practicals were conducted during morning sessions and field work performed during afternoons as a means of balancing course contents and reducing microbial contamination. Demonstration and practical materials were inoculated between 21 and 28 days prior to their use during the course.

Towards the end of the course, participants met in country groups for developing a rhizobiology plan (Objective 3) for each country. A hard copy folder and an electronic version of all the presentations and other useful reference materials were distributed to the participants.

Course Proceedings

Day One: An opening ceremony with short remarks from one of the leading scientists in Africa in the BNF field, Prof. Shellemiah Okoth Keya of the University of Nairobi, was carried out. The expected output from the training was outlined after a brief overview of the N2Africa project was given by the project leader and the objectives of this course were given by the objective 5 leader. Next was a lecture introducing nitrogen fixation and taxonomy of Rhizobium by Prof. Nancy Karanja. It was noted that in order for N to be used for growth it must be "fixed" (combined) in the form of ammonium (NH_4) or nitrate ($N0_3$) ions. To break N_2 apart so that its atoms can combine with other atoms requires the input of substantial amounts of energy. There are three processes which are responsible for most of the nitrogen fixation in the biosphere: industrial fixation, atmospheric fixation and biological fixation. In industry, the N fixation needs very high temperature and pressure. In nature, the ability to fix nitrogen is found in certain bacteria. Some live in a symbiotic relationship with plants of the legume family (eg. soybean, alfalfa). Some establish symbiotic relationship with plants other than legumes (eg. alders). Some nitrogen fixing bacteria live free in the soil. Biological nitrogen fixation requires a complex set of enzymes and a huge expenditure of ATP. The lecture also mentioned about nitrogenase, the enzyme that converts N_2 to NH_3, its function and the genetics of its synthesis and activation.

The second component of the lecture was the taxonomy of rhizobia. Only about 15% of the 19,700 species of legumes have been evaluated for nodulation. Rhizobia are gram positive bacteria with a rod shape. They are the most studied symbiotic N_2-fixing bacteria and are now subdivided into several genera. The first rhizobial species was identified in 1889 and most new species were put in the Rhizobium genus until more advanced methods of analysis placed the species in to new genera. The molecular genetics tool using 16S ribosomal RNA uncovered many new species. An introductory lecture on nitrogen and legumes was delivered by Dr Paul Woomer.

In the first day of laboratory work, participants were reminded of the general laboratory safety rules were presented and discussed. After splitting into six working groups of participants prepared growth media, inoculated broth cultures and isolated rhizobia.

Day Two: A Lecture on culturing rhizobia, strain characterization and identification was presented. This was followed by a practical on serial dilutions, quantifying rhizobia by plate count and plant infection. In the afternoon, the participants visited the KEFRI laboratories to observe rhizobia growth on indicator media, gram staining, culture storage and a PCR demonstration. The participants were fascinated by the rhizobia identification technique using the DNA molecular markers.

Day Three: The lecture was on Rhizobia, symbiosis, inoculants and inoculation and production and marketing of inoculants. The practical focussed on seed inoculation techniques and carrier material selection and processing. Characteristics of a good carrier for liquid inoculant production should be: Non toxic, low in cost, readily available, could be used under normal fermentation conditions, nearly neutral pH or easily adjusted, amenable to nutrient supplements, rapid release of rhizobia in the soil, supports rhizobial growth and survival, and is manageable in the mixing and packaging operation. The most suitable carrier for Rhizobium production is peat but peat is not always available, and can be exhausted. Therefore, alternative carriers must be explored.

After the practical session, the participants visited Ondiri peat marsh where they had the opportunity to observe peat in the field. However, they were reminded that the site is a protected water catchment and so one must get permission from the relevant government authority before harvesting peat for research work.

Day Four: Lectures on the fourth day focused on Rhizobium strain authentication and selection and product testing. In the afternoon the participants had a practical on greenhouse management, Leonard jars, growth pouches and preparation of soil for potting. They noted that the plastic Leonard jars are more convenient in terms of weight and space. They were also advised that they can use other materials such as sawdust to fill the Leonard jars in case sand is not readily available. The participants were provided with guidelines on site selection and how to design a good field experiment or demonstration. Participants shared experiences with each other and with the trainers during lecture presentations and in the field.

Day Five: Participants inspected and purified nodule isolates and were shown agglutination and immunodiffusion procedures, preparation of antigens and injecting a rabbit. This session was preceded by a lecture on rhizobia strain identification. The afternoon session focussed on maximizing BNF and response to inoculation.

Day Six: The participants visited the inoculant production plant at MEA limited in Nakuru that enabled them to learn about quality control mechanism in inoculant production, how to prepare the stickers from gum arabica, packaging and distribution of the inoculant.

Day Seven: Free day

Day Eight: Mid-course review was conducted with particular emphasis on the strengths and shortcomings of the course in the first week for improvement in the following week. This session was facilitated by Dr Kenton Dashiell. The key issues raised are highlighted in the section on recommendations. Thereafter the participants observed colony morphology, inspected and purified nodule isolates.

The team visited CIAT-TSBF in Gigiri to observe and learn how inoculant products are tested in the greenhouse and laboratory. The preliminary results from the Commercial Products Project (COMPRO) indicate that some products are more effective than others.

Day Nine: The day started with a group discussion on aligning lab capacity and technicians' skills to N2Africa project activities and milestones before a morning practical on reading plate counts and estimating cell counts with optical density. A lecture on most probable number (MPN) by dilution extinction method was presented prior to MPN set up with growth pouches, building racks, planning MPN, aseptic irrigation, inoculating the pouch and reading the result.

Day Ten: Prof Nancy Karanja delivered a lecture on quality control of legume inoculants. She appreciated the current sophisticated and advanced techniques used in culture storage and quality maintenance. However, she also emphasized on the need to embrace some of the technologies that are readily accessible and affordable by the smallholder farmers. In the afternoon there was a lecture and practical on rhizobia exploration where the participants learnt how to set up a Rhizobia exploration that included collection, isolation, purification, authentication and characterization.

Day Eleven: Participants discussed in groups according to their countries. The discussion was guided by Dr Paul Woomer and Prof Nancy Karanja and focussed on developing action plans for in country rhizobiology activities. After the discussions the participants were asked to prepare a draft action plan for their respective countries. As part of the course practical, the participants read plate count of inoculants and inoculated seed (culture 3). They also gained experience in the MPNES and Inoculation Requirements software utilities.

Day Twelve: Participants presented rhizobiology (project objective 3) action plans for each country. Input was given by the facilitators and other participants, including looking at the feasibility of the plan (Annex 3).

Course Review and Conclusion

There was a post-evaluation of the workshop that involved individual participants filling in a form that that was designed to assess the views of the participants with regard to the workshop modules, presentations, activities and organization. Participants were also asked to suggest how the workshop could be improved. Analysis of views of participants, resource persons and workshop organizers based on the evaluation form and face to face discussions on specific issues lead to the following conclusions;

Methodology: Participants were unanimous in saying that the learning by doing interactive approach adopted as well as the sharing of experiences and ideas between participants and facilitators was effective.

Course Objective: Overall it is possible to state that the methodological approach adopted was regarded as a success and that most participants did achieve a shared understanding of inoculant and legume production skills at the end of the training. The assessment of the participants training feedback (Annex 4) shows that the objectives proposed from the outset were attained and they would be able to carry out the gained skills at their workplace, become a facilitator and guide colleagues in their institutions in rhizobiology activities. The main elements highlighted to have contributed to the achievement of the training objectives were: Practical sessions, individual face-to-face sessions with the training facilitators and group discussions.

Knowledge Transfer: Based on the evaluation form, participants agreed that the workshop has improved their level of knowledge on rhizobiology and further suggest that this training to be continued on a regular basis.

Course Content: All participants gave a high rating on the course content and effectiveness of delivery of the topics. However, they felt that modules on laboratory and data management should be added.

Facilitation: The Workshop employed different facilitation methods that continuously engaged participants to give input or to share experiences. Competencies of the resource persons/trainers were rated to be excellent. Probably, an expert that is not working in the N2Africa project such as J. Howieson would have strengthened the course and provided a broader perspective.

Timing: Careful planning and timing allows for a two-week course. However, the general feeling was that the time allocated to cover the content of the course effectively was too short. In the process, most topics were hurriedly covered. Moreover, the participants felt that more time should have been allocated for the laboratory practical and possibly starting in the afternoon hours so that the morning hours are devoted to lectures. Furthermore, participants were forced to work with undersized root nodules because practical materials were inoculated too late.

Logistics: The participants felt that the logistics were well coordinated except that the distance between the training venue and the KARI Retreat Centre (where they were lodger) was too long. This caused morning sessions to start later then planned. They also complained of lack of internet service at the KARI Retreat Centre.

Recommendations

- The practical sessions enabled the participants to cope with the amount of information being delivered. In this regard future trainings should take into account that lectures should be no longer than one hour and theoretical sessions should always be followed by a practical exercise. Moreover, practical sessions should be in the afternoon so that morning hours are devoted to lectures.
- The training venue should be within or near the hotel where participants are accommodated to reduce time spent travelling between the training venue and hotel.
- Furthermore, it is also critical to maintain continuous communication with participants so participants are constantly reminded to reflect on the project and individual work, and to support participants in evolving into higher levels of understanding.
- The language of presentation and discussion was not ideal for a few French speaking participants that could not communicate effectively in English and hence needed translation. This slowed down the presentation and discussion a little bit at some point but trainees with knowledge in French always intervened making sure that the course progressed smoothly. In addition, Kiswahili was used to overcome weakness in communication to the Francophone participants. Arrangement for translation from English-French and vice versa for participants speaking different languages is necessary in future trainings in case participants come from both Anglophone and Francophone speaking countries. Preferably, TSBF staff fluent in French should take a greater role in day-to-day instruction.
- Further training on inoculant preservation, laboratory and data management is recommended.

Post Training Follow-ups

As important as the training is, what is more important is that it is applied, at the country and institutional level based on project needs. To ensure that the participants are in a position to carry out the skills gained in the training they are requested to consult with their respective Heads of Organizations and the N2Africa hub leaders to ensure that country project activities and follow-ups will be carried out. Participants will be expected to;

- pass the knowledge and skills gained from the training to other staff in their institution.
- have keen interest and be available to participate in the activities of the N2Africa project particularly on rhizobiology and contribute to the implementation of the project objective 3 work plan.
- maintain communication with other participants, the training facilitators and N2Africa project staff and its partners on the progress of carrying out the skills gained in the training;
- play an active role in providing inputs and feedback on the project implementation, especially for objective 3.;

Acknowledgements

We wish to thank George Mwenda and Stanley Kisamuli, for organizing and facilitating the practical sessions of the course and providing excellent support during the two weeks of the course. We are grateful to the Dean Faculty of Agriculture and the Chairman Department of Land Resource Management and Agricultural Technology (LARMAT) of the University of

Nairobi for providing us with the opportunity to use their premises and facilities to conduct the training.

Annex 1: Course Schedule

A two week training course conducted in the East and Central Africa Hub of the N2Africa Project

(13-24 September 2010)

Course Schedule

	Topic/Activity	Facilitator
	Sunday 12 September 2010	
	Arrival of participants and transfers to KARI Retreat Centre-Muguga	
	Day One: Monday 13 September 2010	
	Registration of participants	J. Odongo
	Introduction of participants	N. Karanja
Morning Lecture	Overview of N2Africa project	K. Dashiell
	Objective of the training and Objective 3 technical milestones, skill set for project technicians and graduate students.	S. Koala
	Key note address and official opening	Principal-CAVS/Dean Faculty of Agriculture
	Overview of the training process and activities	P. Ngokho
	BNF in African agriculture	S. Keya
	Basic rhizobiology, isolating, characterizing and maintaining rhizobia in the laboratory	N. Karanja
	Tea/Coffee Break	
Morning Practical	Laboratory intro, workstation and partner assignments, media preparation, set up glass fermenters, inoculate broth cultures	N. Karanja & MIRCEN Team
	Lunch Break	
Afternoon Lecture	Nitrogen and legumes	P. Woomer
	Tea/Coffee Break	
Afternoon Practical	Legume identification, nodule exploration, recovery and preservation, rhizobium isolation (culture 1), streaking technique, surface sterilizing & pre-germinating seed	P. Woomer & MIRCEN Team

	Day Two: Tuesday 14 September 2010	
Morning Lecture	Culturing rhizobia, growth requirements and carbon sources, strain characterization & identification	J. Machua
	Tea/Coffee Break	
Morning Practical	Serial dilutions, quantifying rhizobia by plate counts (culture 2) and plant infection (MPN 1)	S. Kisamuli & G. Mwenda
	Lunch Break	
Afternoon Lecture	Rhizobia, symbiosis & BNF	P. Woomer
	Tea/Coffee Break	
Afternoon Practical	Rhizobial growth on indicator media (culture 2 continued), Gram stain, culture storage, PCR demonstration at KEFRI-Muguga	Joseph Machua
	Day Three: Wednesday 15 September 2010	
Morning Lecture	Inoculants & inoculation	P. Woomer
	Tea/Coffee Break	
Morning Practical	Seed inoculation technique (slurry, 2-step & pelleting), plate counts of inoculants and inoculated seed (culture 3)	S. Kasamuli & G.Mwenda
	Lunch Break	
Afternoon Lecture	Producing, marketing and distributing BIOFIX inoculants	T. Wafulah
	Coffee Break	
Afternoon Practical	Carrier material selection and processing, mixing broth and carrier, field trip to nearby Ndera peat marsh	T. Wafulah, S. Kisamuli & G. Mwenda
	Day Four: Thursday 16 September 2010	
Morning Lecture	Rhizobium strain authentication and selection, and product testing in the greenhouse.	Joseph Machua
	Tea/Coffee Break	
Morning Practical	Greenhouse management, Leonard jars, potted field soil	S. Kisamuli & G. Mwenda
	Lunch Break	
Afternoon Lecture	Rhizobium strain selection in the field	F. Baijukya
	Coffee Break	
Afternoon Practical	Field inoculation trials. Experimental design, data collection and analysis	F. Baijukya
	Day Five: Friday 17 September 2010	
Morning Lecture	Rhizobium strain identification	J. Gitahi

	Tea/Coffee Break	
Morning Practical	Inspect and purify nodule isolates as needed (cultures 1&2). Agglutination and immunodiffusion. Visit to Vet antiserum lab and rabbit facilities. Preparation of antigens and injecting animals (demonstration)	S. Kisamuli, J. Gitahi
	Lunch Break	
Afternoon Lecture	Maximizing BNF & response to inoculation	F. Baijukya
	Tea/Coffee Break	
Afternoon Practical	Objective 2 Field Trials and rhizobiology needs. Linking the rhizobium lab to N2Africa field activities	F. Baijukya & N. Karanja
	Cocktail	
	Day Six: Saturday 18 September 2010	
Morning	Field visit to BIOFIX factory (Nakuru)	T. Wafulah
Afternoon	Rift Valley excursion (Lake Naivasha & Hells Gate)	
	Day Seven: Sunday 19 September 2010	
	Free day	
	Day Eight: Monday 20 September 2010	
Morning Lecture	Mid-course review, group discussion and mid-course evaluation. What were the strengths and shortcoming of the course's first week.	K. Dashiell & N. Karanja
	Tea/Coffee Break	
Morning Practical	Observe colony morphology, inspect and purify nodule isolates as needed (culture 1)	S. Kisamuli & G. Mwenda
	Lunch	
Afternoon Lecture	Travel to CIAT-TSBF Headquarters in Gigiri. Inoculant product testing: The COMPRO approach	P. Woomer
	Tea/Coffee Break	
Afternoon Practical	Product testing in the greenhouse and laboratory, comparing available inoculant products	S. Kisamuli & G. Mwenda
	Day Nine: Tuesday 21 September 2010	
Morning Lecture	Aligning lab capacity and technician skills to N2Africa project activities and milestones. Lecture and group discussion.	N. Karanja & P. Woomer
	Tea/Coffee Break	
Morning Practical	Read plate counts (culture 2) and calculating cell densities. Estimating counts with optical density (demonstration)	S. Kisamuli & G. Mwenda

	Lunch	
Afternoon Lecture	Most Probable Number by dilution extinction	P. Woomer
	Tea/Coffee Break	
Afternoon Practical	MPN set up with growth pouches, building racks, planting MPN, preparing –N nutrient solution, aseptic irrigation, selecting for plant uniformity, inoculating the pouch, reading results	P. Woomer, S. Kisamuli & G. Mwenda
	Day Ten: Wednesday 22 September 2010	
Morning Lecture	Quality control of legume inoculants	N. Karanja
	Tea/Coffee Break	
Morning Practical	Inoculant quality testing	S. Kisamuli & G. Mwenda
	Lunch Break	
Afternoon Lecture	Rhizobium exploration: finding better strains	P. Woomer
	Tea/Coffee Break	
Afternoon Practical	Rhizobium exploration set up, isolation, purification, authentication and characterization	P. Woomer, S. Kisamuli & G. Mwenda
	Day Eleven: Thursday23 September 2010	
Morning Lecture	Course review and discussion (1)	N.Karanja & P.Woomer
	Tea/Coffee Break	
Morning Practical	Read plate counts of inoculants and inoculated seed (culture 3). Calculating populations	S. Kasamuli & N. Karanja
	Lunch Break	
Afternoon Lecture	Facilitating grain legume enterprise and mobilizing BNF technologies	P. Woomer
	Tea/Coffee Break	
Afternoon Practical	Computer laboratory, calculating populations using excel, the inoculation requirement utility, MPNES practice	P. Woomer
	Day Twelve: Friday 24 September 2010	
Morning Lecture	Course review and discussion (2)	Team
	Tea/Coffee Break	
Morning Practical	Completion of lab activities, arrangement for distributing cultures and other materials	Team
	Lunch Break	
Afternoon	Group discussion, course evaluation	Team

Comments from participants representative	
Vote of Thanks	P. Ngokho
Closing Remarks	K. Dashiell & N. Karanja
Official Closing and award of certificates	Principal-CAVS/Dean Faculty of Agriculture

Annex 2: List of participants

TRAINING OF TRAINERS IN RHIZOBIOLOGY (ECA)-PARTICIPANTS DETAILS

No	Participant Name	Gender	Nationality	Position	Organization
	Trainees				
1	Maureen Waswa	F	Kenya	Student	Univ of Nairobi
2	James Nderitu	M	Kenya	Lab Technician	Univ of Nairobi
3	MacDonald Wesonga	M	Kenya	CEO	ARDAP
4	Wycliffe Waswa	M	Kenya	Research Technician	TSBF
5	Anne Wekesa	F	Kenya	Technician	KEFRI
6	Uwimana Jeanne	F	Rwanda	Lab Technician	Ministry of Agric & Animal Resources
7	Uwizerwa Mathilde	F	Rwanda	Student	Makerere
8	Rumonge Tabaro Alfred	M	Rwanda	Lab Technician	Ministry of Agric & Animal Resources
9	Nocy Kijana	F	DRC	Scientist	INERA
10	Balume Kayani Isaac	M	DRC	Student	UCB
11	Rukiranuka Bienvenu	M	DRC	Lab Technician	UCB
12	Nabintu Ndusha	F	DRC	Assistant Lecturer	UEA
	Resource Persons/Facilitators/Support Staff				
13	Dr Paul Woomer	M	US	Consultant	CIAT-TSBF
14	Prof Nancy Karanja	F	Kenya	Professor	Univ of Nairobi
15	Prof Shelemiah Keya	M	Kenya	Professor	Univ of Nairobi
16	Joseph Machua	M	Kenya	Senior Research Officer	KEFRI
17	Stanley Kisamuli	M	Kenya	Technician	Univ of Nairobi
18	George Mutegi Mwenda	M	Kenya	PhD Student	Univ of Nairobi
19	Dr Saidou Koala	M	Burkina Faso	AfNET Coordinator	CIAT-TSBF
20	Dr Fredrick Baijukya	M	Tanzania	ECA Hub Coordinator	CIAT-TSBF
21	Teressah Wafullah	F	Kenya	AKTP Associate	MEA
22	John Gitahi Nduhiu	M	Kenya	Technologist	Univ of Nairobi
23	Dr Kenton Dashiell	M	US	Project Leader	CIAT-TSBF
24	Jacqueline Odongo	F	Kenya	Admin Assistant	CIAT-TSBF
25	Mary Nderitu	F	Kenya	Finance Assistant	CIAT-TSBF
26	Patrick O. Ngokho	M	Kenya	Training Specialist	CIAT-TSBF

Annex 3: Objective 3 Preliminary Work Plans

DR Congo: Preliminary Objective 3 work plan

Led by Bienvenu at UCB

Site identification (45 sites) 12 field experiments, 33 legume communities. Collect from Obj 2 field sites and different agro-ecological zones (November)

Collect soils and nodules (Nov-January).

Conduct MPNs (January – April)

Isolate and preliminary characterization of rhizobia (January – June)

Upgrade greenhouse (November)

Test strains in greenhouse (April – September)

Field test strains, best strains sent to Nairobi

Recover carrier material, prepare inoculant

Bintu: UEA, MSc student, isolate and characterize rhizobia

Nocy Kijana: INERA, MSc student, inoculant production, formulation, quality control

Isaac: UCB, technician, MPNs, liaise with Obj 2

Bienvenu: UCB, technician, conduct MPNs, isolate and characterize rhizobia

Problem: three cooperating institutes, UCB agriculture moving into the TSBF building at the new campus

Incoming students require remedial English course (3 months) and can do work with soils and nodules at MIRCEN

Coordination from the TSBF office at Bukavu through Isaac

Kenya

Led by Nancy Karanja at MIRCEN

Site identification (45 sites) 18 field experiments, 27 legume communities. Collect from Obj 2 field sites and different agro-ecological zones (November), coast to lake basin

Upgrade laboratory: UoN MIRCEN, Maseno (no lab planned for Maseno)

Greenhouse at UoN very overdue, no MPNs and strain testing until completed

Many isolates available from BGB project, isolated from sirato

Collect soils and nodules (Nov-January).

Conduct MPNs (January – April)

Isolate and preliminary characterization of rhizobia: UoN (January – June)

Upgrade greenhouse (done)

Test strains in greenhouse (April – September)

Inoculant supply: BIOFIX from MEA, quality control by UoN, continue this arrangement?

Course follow up: more strain ID, PCR fingerprinting, quarterly updates

Maureen: UoN MSc, pending

Wycliffe: TSBF Maseno, liaise with obj 2, MPNs

Macdonald: Moi U MSc, promiscuous SB, starter N, need to inoculate,

James

Anne

George

Rwanda

Led by Matilda at ISAR Rubona

Focus on isolations from bean and soybean

Facility improvement at Rubona a necessity

ID sites (October), 4 EAZ , collecting from north to south, recover nodules

Collection from MPN, start plants prior to soil collection, isolation from resulting nodules

Prepare isolates in a continuous manner, N to S, W to East

Screening in greenhouse at Rubona

Matilde: ISAR, Rubona, exploration, isolation, characterization, bean

Jeanne: ISAR, exploration, isolation, characterization, soybean, soybean

Alfred: ISAR, MSc, student, UoN, inoc formulation, quality control

MPNs still needs to be assigned, Rubona, liaise with Felix

Annex 4: Comments and Feedback Summary

Advancing Technical Skills in Rhizobiology

A two week training course conducted in the East and Central Africa Hub of the N2Africa Project at the College of Agriculture and Veterinary Sciences, University of Nairobi
(13-24 September 2010)

Comments and Feedback Summary

NA=Not applicable, 1=Strongly disagree, 2=Disagree, 3=Neither agree/nor disagree, 4=Agree, 5=Strongly agree

	Percentage (%)					
Item	**NA**	**1**	**2**	**3**	**4**	**5**
Course Content						
Aware of the prerequisite of the course	0	0	8	25	67	0
Had prerequisite knowledge and skills for the course	0	0	0	42	33	25
Well informed about course objectives	0	0	8	25	25	42
This course lived to my expectation	0	0	0	0	50	50
Course content relevant to my job	0	0	0	8	17	75
Course Design						
The course objectives are clear to me.	0	0	0	0	75	25
The course activities stimulated my learning.	0	0	0	0	17	83
The activities in this course gave me sufficient practice and feedback.	0	0	0	0	75	25
The difficulty level of this course is appropriate.	0	0	0	33	67	0
The pace of this course is appropriate.	0	0	0	17	67	16
Course Facilitators						
The instructors were well prepared.	0	0	0	8	25	67
The instructors were helpful.	0	0	0	0	58	42
Course Environment						
The training facility at this site was comfortable.	0	0	0	17	83	0
The training facility at this site provided everything I needed to learn.	0	0	8	0	75	17
Course Result						
I accomplished the objectives of this course.	0	0	0	8	50	42
I will be able to use what I learned in this course.	0	0	0	8	42	50

Suggestions for Improvement
Most participants suggested that the course could be improved by the following;

- Provide better and more information before course
- Increase content covered in course
- Update content covered in course
- Make the course less difficult
- Slow down the pace of the course
- Allot more time for the course

General Comments and Feedback

- Very good chance to exchange experiences among countries on rhizobiology and laboratory infrastructure

- A well organized, structured and presented course, content well balanced and excellent participation
- Participants gender balance a sign of equal opportunities to all
- Practical sessions were very interesting
- Allot more time on DNA isolation practical
- More time should be allocated for practical
- Lectures be conducted in the morning and practical in the afternoon
- MPN could be effectively practiced if the course was over one month
- No internet facilities hence difficulties in communication back home
- Overwhelmed at the pace and standards at which scientists in Kenya have made in the field of rhizobiology
- Establish a communication group (e.g yahoo group) for rhizobiologists and other professionals

West African Hub – Nigeria

A two week training course conducted in the West Africa Hub of the N2Africa Project

Summary

The N2Africa project has the objective of improving productivity and enhancing inputs of nitrogen in smallholder farms, thus leading to increase in household income, improved nutrition and general wellbeing of farm households. To achieve this, the programme has a series of activities that are achieved through a set of milestones. The attainment of these objectives is largely dependent on the knowledge and skills project partners bring to bear in the project. It is in the light of this that the training of technicians during the early phase of the project in basic rhizobiology skills was defined as one of the milestones under Objective 5 (Training) of the project. A two-week training course was held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, targeted at key laboratory personnel and postgraduate students to gain knowledge and skills in the isolation, identification, characterisation and storage of rhizobia, as well as mobilising these organisms for inoculant production and inoculation for enhanced nitrogen fixation. Six participants (40% women) from Ghana and Nigeria attended the training during which various discussions and material presentations were given by experts and practitioners in rhizobiology, microbiology and biotechnology. Training modules were designed to be a mix of theoretical, interactive and practical sessions. Participants were able to hold discussions and exchange experiences on issues regarding rhizobiology, inoculant production and quality control, laboratory-based PCR methods, nitrogen fixation quantification, and laboratory, greenhouse, and field techniques. Materials provided to participants included a training resource manual, PowerPoint presentations, relevant literature and sample technical papers on biological nitrogen fixation (BNF) both in printed and electronic copies. These materials were used for lectures and discussed with respect to each practical session. The participants were all of the opinion that the training was properly aligned with the skill set needed for project activities, were agreed that the workshop achieved its stated objectives and that they would be able to carry out the skills learnt during the course.

Introduction

Background Information

Elemental nitrogen accounts for almost 80% of atmospheric composition. Paradoxically, N is the most limiting of all nutrient elements in agriculture. Bacteria that possess the enzyme nitrogenase can convert elemental N in the air to forms that are readily taken and assimilated by plants. The association between a group of specialised bacteria, known as rhizobia, with members of the plant family Leguminosae makes the single largest contribution to biological nitrogen fixation in the biosphere. This system contributes around 50–600 kg N/ha/year. An important component of the project, "Putting nitrogen fixation to work for smallholder farmers in Africa" (N2Africa) is to select superior rhizobia strains for enhanced biological nitrogen fixation (BNF) and develop inoculum production capacity in sub-Saharan Africa, with public and private sector partners (Project Objective 3). It is expected that the project will raise average grain yields by 954 kg/ha in four legumes (groundnut, cowpea, soybean, and common bean), increase average BNF by 46 kg/ha, and increase average household income by $465, directly benefiting 225,000 households (1,800,000 individuals) in eight countries in sub-Saharan Africa (Democratic Republic of Congo, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Zimbabwe).
To achieve these objectives, skilled technical staff are required and Objective 5 (Develop and strengthen capacity for BNF research, technology development, and application) of the N2Africa project provides for training on rhizobiology, inoculant production and quality control, laboratory-based PCR methods, nitrogen fixation quantification, and laboratory, greenhouse, and field techniques. The aim of this training was to improve the skills of the participants in the mobilisation, manipulation and processing of microbes, especially rhizobia, in relevant inoculant and legume production techniques and enterprise. Six technical staff and postgraduate students from partner institutions in Ghana and Nigeria attended the training. Activities at the training included lectures, interaction sessions, greenhouse and field work. Resource persons for the training were drawn from staff of N2Africa, IITA and partner organizations with expertise in microbial skills.

Course Objectives

The course was held to provide training to N2Africa laboratory technicians and postgraduate students an opportunity to advance their technical skills in rhizobiology. The specific objectives were to:
 Equip participants with knowledge and skills in inoculant production, quality control and field inoculation of grain legumes.
 Give the facilitators and trainees the opportunity to share varied lessons and experiences, related to methods and tools used in rhizobiology, and their deployment for inoculum production and farm-level inoculation practices.
 Provide each participant with a full set of activities and materials which include: basic rhizobiology, strain selection and inoculant production and use.

Participants and Resource Persons

Six participants (40% women) from Ghana and Nigeria attended the course. The participants were nominated by their respective institutions which are collaborating with N2Africa. The resource persons were drawn from the N2Africa team and associated partners including Robert Abaidoo, Abdullahi Bala, Ado Yusuf, Martin Jemo, Steven Kilani, James Oyedipe and Bola Oke. The list of participants and resource persons is in Annex 1.

Organization and Structure of the Course

The programme (Annex 2) consisted of lectures, practical and group discussion. Every lecture was followed by a related hands-on practical which enabled participants to better understand the concepts being given and to apply these to the work they will be doing in their countries. The lecture modules addressed the following subjects; basic rhizobiology, BNF in small scale agriculture, the legume-rhizobia symbiosis, rhizobia inoculants and inoculation, strain characterization, identification, authentication and selection, inoculant quality control and quantification of nitrogen. In general, laboratory practicals were conducted during morning sessions and field work performed during afternoons as a means of balancing course contents and reducing microbial contamination. Trainees were provided with training materials in both in hard copy electronic versions.

Course Proceedings

Day One: The course was opened by Robert Abaidoo on behalf of Dr Paula Bramel, IITA's Deputy Director General (R4D). He explained the essence of the course and urged the trainees to properly utilise the opportunity. Abdullahi gave an explanation on the structure of the course and some of the expectations of the course. This was followed by a lecture on basic rhizobiology, isolating, characterizing and maintaining rhizobia in the laboratory by Robert Abaidoo. The trainees then went into the laboratory where the laboratory technologist, Steve Kilani, briefed the trainees on laboratory practices and safety procedures. They were also introduced to the equipment in the laboratory and were led by Mrs Bola Oke in the preparation, autoclaving and pouring of yeast extra mannitol agar plates containing Congo Red and bromothymol blue.
Abdullahi Bala presented the afternoon lecture that covered 'Nitrogen, legumes and BNF in African agriculture' after which he and Steve Kilani led the trainees to the field for demonstration and activities on legume prospecting and identification, nodule exploration, recovery and preservation and rhizosphere soil collection. Both soil samples and nodules were brought back to the laboratory. Nodules were recovered from herbaceous (*Mucuna pruriens* and *Pueraria phaseoloides*), grain (cowpea and soybean) and tree (*Leucaena leucocephala* and *Pentaclethra* sp.) legumes. Trainees examined nodule morphology, noting the differences between the various legume species.

Day Two: A Lecture on culturing rhizobia, growth requirements and carbon sources, strain characterization & identification was presented by Robert Abaidoo. This was followed by a practical led by Abdullahi Bala on practising streaking on agar plates and isolation of rhizobia on YEM agar. Trainees were made to isolate nodules and streak on plate both inside- and outside of laminar flow cabinet to demonstrate the importance of cleanliness in the laboratory. In the afternoon, Abdullahi Bala presented a lecture on 'Rhizobia, symbiosis & BNF' This was followed by practical on basic principles of spectroscopy, observation of rhizobial growth on indicator media, Gram staining, and culture storage. Trainees also did seed surface sterilisation and pre-germination.

Day Three: The morning lecture on inoculants and inoculation was presented by Ado Yusuf and was followed by a practical on seed inoculation. The afternoon lecture dwelt on maximizing BNF & response to inoculation and was delivered by Robert Abaidoo. This was followed by a practical led by Abdullahi Bala on serial dilutions, quantifying rhizobia by plate counts using pour plate, drop plate and streak plate methods. Demonstrations were made for mixing broth with inoculant carriers.

Day Four: Lectures on the fourth day focused on rhizobium strain authentication and selection and product testing followed by inspection of rhizobial isolates and a practical on serial dilution and plant infection test. In the afternoon, the participants had a practical on greenhouse management, Leonard jars, growth pouches and preparation of soil for potting

Day Five: The morning lecture and practical were led by Ado Yusuf and were on the principles of field experimentation. Afternoon lecture and practical were on innovation in inoculant production, strain selection strategies, and alternative delivery system.

Day Six: Days 6 was free and participants visited Ibadan.

Day Seven: Free day

Day Eight: Mid-course review was conducted with particular emphasis on the strengths and shortcomings of the course in the first week for improvement in the following week. This session was facilitated by Robert Abaidoo and Abdullahi Bala. Participants expressed

satisfaction with logistics provided but were not happy that conference bags were not provided. Although they were impressed with the quality of lecture delivery, they nonetheless suggested that more time be allocated for practical sessions.
The interaction was thereafter followed by a lecture on methods of nitrogen fixation, delivered by Robert Abaidoo. All of the afternoon was spent in the laboratory performing measurement of nitrogen using ureide method.

Day Nine: Lecture on quality control by Robert Abaidoo was followed by plate counts of rhizobial inoculants in the laboratory. In the afternoon, Abdullahi Bala gave a lecture on Rhizobium exploration and then the participants were in the lab to complete the ureide assay. This was followed by a practical on cell counts using optical density.

Day Ten: The morning session started with discussion on the alignment of skills acquired with the activities of the project. All participants were of the view they could carry out all of the activities covered. However, none of the laboratories had the full complement of all of the equipment required to carry out all of the activities. Participants later went to the Bioscience Centre for practical demonstration on PCR.
The first part of the afternoon was also spent on demonstration in PCR-based methods. Participants later went to the greenhouse to make observation on nodulation. This was followed by calculations for MPNs.

Day Eleven: Participants spent the morning session learning about data collection and management. This was led by Martin Jemo. The later part of the morning was spent in the laboratory, with participants looking at the plates for isolated nodules and the estimating cell populations by plate counts.
The afternoon session was used for the closing ceremony. Dr Robert Asiedu, IITA's Director Tuber and Root Improvement Programme (TRIP), presented certificates to participants on behalf of Dr Paula Bramel, DDG (R4D). Dr Asiedu commended N2Africa for incorporating a capacity building programme that covers technician training early in the life of the project. He congratulated the participants for successfully completing the course and urged to maintain and seek to expand the network of new professional colleagues they formed during the training.

3 Course Review and Conclusion
During the closing ceremony, Mr Patrick Ofori made a presentation on behalf of other trainees on their assessment of the course. This and the outcome of the mid-course review can be summarised as follows:

Logistics: The trainees commended the level of organisation of the programme. They were particularly happy that flight arrangements and transportation to Ibadan were made without a hitch. They were also of the opinion that their being accommodated within walking distance to the venue of the training helped tremendously in ensuring that lectures and practical always started on time. However, they felt that trainees should have been provided with conference bags and were also of the opinion that the perdiem provided was too little.

Methodology: Participants were in agreement that learning was made more effective by getting them to try out the various techniques by themselves.
Course Objective: The participants were unanimous in stating that they felt the course objective was generally achieved, although they felt that it could have been better if they had the opportunity to see practically some of the materials mentioned in the course, such as filter mud, storage beads for culture storage and industrial inoculant fermenters.
Knowledge Transfer: All the participants were of the opinion that the course has tremendously enhanced their knowledge of and skills in rhizobiology, and were confident that they could bring their newly acquired skills to bear in advancing the objectives of the project.
Course Content: The participants were of the view that the course content was relevant to the objective of the training and the method of delivery by the resource persons was effective.

They particularly liked the interactive nature of the lectures especially in the afternoons because it made them to remain alert.

Timing: Participants were of the opinion that more time should be allocated to practical sessions, rather than having up to 1 hour sessions of lectures.

4 Acknowledgements

We acknowledge with thanks the wonderful technical support provided by the trio of Mr Steven Kilani, Mrs Bola Oke and Mr James Oyedipe during the training. We also wish to thank Dr Robert Asiedu and Dr Paula Bramel for their support.

Annex 1: Participants' List

S/N	Participant's name	Gender	Country	Contact address
1	BABA, Khadijatu Kubura	Female	Ghana	c/o Mr. Saibu Zakiyu, NCCE, P. O. Box 53, Damongo, Northern Region, Ghana
2	OFORI, Patrick	Male	Ghana	CSIR Soil Research Institute, Academy Post PMB, Kwadaso – Kumasi, Ghana
3	OWOSENI, Bello	Male	Nigeria	Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria
4	SAIDU, Bosso Talatu	Female	Nigeria	Department of Microbiology, Niger State Polytechnic, Zungeru, Nigeria
5	FARUK, Umar	Male	Nigeria	Department of Soil Science, Bayero University, Kano, PMB 3011, Kano, Nigeria
6	KOLEOLA, Abidemi Adedayo	Male	Nigeria	Department of Soil Science, Federal University of Technology, Minna, PMB 65, Minna, Nigeria

Annex 2: Course program

Advancing Technical Skills in Rhizobiology

A two week training course conducted in the West Africa Hub of the N2Africa Project

Course Schedule

	Topic/Activity	Facilitator
	Sunday 28 November 2010	
	Arrival of participants and transfers to IITA Ibadan	
	Day One: Monday 29 November 2010	
8.00-8.15am	Registration of participants	B. Adeyemo
8.15-8.30am	Introduction of participants	A. Bala
8.30-8.45am	Overview of N2Africa project & objective of the training	R. Abaidoo
8.45-9.00am	Key note address and official opening	Dr P. Bramel, DDG R4D, IITA
9.30-10.00am	***Tea/Coffee Break***	
Morning Lecture	Basic rhizobiology, isolating, characterizing and maintaining rhizobia in the laboratory	R. Abaidoo
Morning Practical	Laboratory intro, workstation and partner assignments, media preparation, set up glass fermenters, inoculate broth cultures	S. Kilani
	Lunch Break	
Afternoon Lecture	Nitrogen, legumes and BNF in African agriculture	A. Bala
	Tea/Coffee Break	
Afternoon Practical	Legume identification, nodule exploration, recovery and preservation, rhizobium isolation (culture 1), streaking technique, surface sterilizing & pre-germinating seed	A. Bala, S. Killani & B. Oke

	Day Two: Tuesday 30 November 2010	
Morning Lecture	Culturing rhizobia, growth requirements and carbon sources, strain characterization & identification	R. Abaidoo
	Tea/Coffee Break	
Morning Practical	Serial dilutions, quantifying rhizobia by plate counts (culture 2) and plant infection (MPN 1)	R. Abaidoo, S. Killani & B. Oke
	Lunch Break	
Afternoon Lecture	Rhizobia, symbiosis & BNF	A. Bala
	Tea/Coffee Break	
Afternoon Practical	Rhizobial growth on indicator media (culture 2 continued), Gram stain, culture storage, PCR demonstration at Bioscience Centre	A. Bala, S. Killani & B. Oke
	Day Three: Wednesday 1 December 2010	
Morning Lecture	Inoculants & inoculation	A. Bala & A. Yusuf
	Tea/Coffee Break	
Morning Practical	Seed inoculation technique (slurry, 2-step & pelleting), plate counts of inoculants and inoculated seed (culture 3)	A. Bala, A. Yusuf, S. Killani & B. Oke
	Lunch Break	
Afternoon Lecture	Maximizing BNF & response to inoculation	R. Abaidoo & A. Yusuf
	Coffee Break	
Afternoon Practical	Carrier material selection and processing, mixing broth and carrier	A. Bala, S. Killani & B. Oke
	Day Four: Thursday 2 December 2010	
Morning Lecture	Rhizobium strain authentication and selection, and product testing in the greenhouse.	A. Bala
	Tea/Coffee Break	
Morning Practical	Greenhouse management, Leonard jars, potted field soil	S. Kilani and B. Oke

	Lunch Break	
Afternoon Lecture	Rhizobium strain selection in the field	A. Yusuf
	Coffee Break	
Afternoon Practical	Field inoculation trials. Experimental design, data collection and analysis	A. Yusuf, S. Killani & B. Oke
	Day Five: Friday 3 December 2010	
Morning Lecture	Measurement of biological nitrogen fixation	R. Abaidoo
	Tea/Coffee Break	
Morning Practical	Estimation of BNF using the ureide method	J. Oyedipe
	Lunch Break	
Afternoon Lecture	Innovation in inoculant production, strain selection strategies, alternative delivery systems	A. Bala
	Tea/Coffee Break	
Afternoon Practical	Alternative inoculant production (diluted broth, liquid formulation, granular formulation, others) using broth culture 1	S. Killani & B. Oke
	Cocktail	
	Day Six: Saturday 4 December 2010	
	Free day	
	Day Seven: Sunday 5 December 2010	
	Free day	
	Day Eight: Monday 6 December 2010	
Morning Lecture	Mid-course review, group discussion and mid-course evaluation. What were the strengths and shortcoming of the course's first week.	R. Abaidoo & A. Bala
	Tea/Coffee Break	
Morning Practical	Observe colony morphology, inspect and purify nodule isolates as needed (culture 1)	S. Killani & B. Oke

	Lunch	
Afternoon Lecture	Most Probable Number by dilution extinction	R. Abaidoo
	Tea/Coffee Break	
Afternoon Practical	MPN set up with growth pouches, building racks, planting MPN, preparing –N nutrient solution, aseptic irrigation, selecting for plant uniformity, inoculating the pouch, reading results	M. Jemo, S. Killani & B. Oke
	Day Nine: Tuesday 7 December 2010	
Morning Lecture	Quality control of legume inoculants	R. Abaidoo
	Tea/Coffee Break	
Morning Practical	Inoculant quality testing	M. Jemo, S. Killani & B. Oke
	Lunch	
Afternoon Lecture	Rhizobium exploration: finding better strains	A. Bala
	Tea/Coffee Break	
Afternoon Practical	Rhizobium exploration set up, isolation, purification, authentication and characterization	A. Bala, S. Killani & B. Oke
	Day Ten: Wednesday 8 December 2010	
Morning Lecture	Aligning lab capacity and technician skills to N2Africa project activities and milestones. Lecture and group discussion.	R. Abaidoo
	Tea/Coffee Break	
Morning Practical	Read plate counts (culture 2) and calculating cell densities. Estimating counts with optical density	M. Jemo, S. Killani & B. Oke
	Lunch Break	
	Day Eleven: Thursday 9 December 2010	
Morning Lecture	Course review and discussion	R. Abaidoo & A. Bala
	Tea/Coffee Break	

Morning Practical	Read plate counts of inoculants and inoculated seed (culture 3). Calculating populations	A. Bala, S. Killani & B. Oke
	Lunch Break	
Afternoon	Group discussion, course evaluation	Team
	Comments from participants' representative	
	Vote of Thanks	Trainee representative
	Closing Remarks	R. Abaidoo & A. Bala
	Official Closing and award of certificates	Dr P. Bramel, DDG R4D, IITA

Day Twelve: Friday 10 September 2010

Morning	Departures	Team

Southern African Hub - Zimbabwe:

A two week training course conducted in the Southern Africa Hub of the N2Africa Project at the Soil Productivity Research Laboratory, Marondera, Zimbabwe. (05-16 September 2011)

Summary

A two week training course in advanced technical skills in rhizobiology was held at the Soil Productivity Research Lab (SPRL) in Marondera, Zimbabwe for the Southern Africa hub of N2Africa. The Southern Africa hub of N2Africa is comprised of Malawi, Mozambique and Zimbabwe. The course ran from 5 to 16 September, 2011. The objective of this training was to equip technicians and students on the project with technical skills in rhizobiology, which are required to achieve milestones of the 3rd objective of the project. A pre-training course evaluation was carried out to gauge the level of knowledge of the participants. This was done to determine how best to deliver the lectures and to provide a baseline from which to determine whether the participants had gained in knowledge at the end of the training. The course was designed to give the theory and principles in rhizobiology in the form of lectures. This was backed up with practical sessions in the microbiology laboratory and the inoculant factory to give the participants some starter practice in the techniques they were learning. There were also some discussion sessions to review what had been covered. The courses included basic microbiology (isolation, identification, characterisation and storage of rhizobia), strain selection, and inoculant production including quality control involved in culture maintenance and inoculant production inoculant use in agriculture. Facilitation of the lecture and practical sessions was done by resource persons from the SPRL, the University of Zimbabwe, CIAT-Harare and IITA-Nigeria. There were 11 participants in total. Three were drawn from Malawi, 2 from Mozambique and 6 from the host country, Zimbabwe. There was 27% female representation, each country contributing one trainee. All these staff will be instrumental in achieving Objective 3 of the N2Africa project. One trainee is a Masters degree student on N2Africa (from Zimbabwe). At the end of the training course participants were given the course materials on CD.

Introduction

Background information

African agriculture in the smallholder set-up is characterized by low productivity. Nitrogen is the single element most limiting to agriculture. At the same time, it is also a complex element to manage in inorganic form in the soil. Biological nitrogen fixation (BNF) is a strategic way to manage nitrogen and may be used to replenish soils at low cost. The N2Africa project promotes the use of rhizobial inoculant technologies by African smallholder farmers to triple the inputs of free atmospheric nitrogen by biological nitrogen fixation (BNF) in order to improve crop and livestock productivity, human nutrition and farm income, while enhancing soil health. Legumes will be used as a basis for improving cropping systems and household well-being.

Some of the expected outcomes of the project include the selection of efficient rhizobial inoculant strains with enhanced BNF capacities adapted to various environmental stresses; establishment of state-of-the-art laboratories and culture collections of elite strains of rhizobia for target legumes; and establishment of rhizobial inoculant production in countries of West, East and Southern Africa, through partnership with the private sector.

It is expected that the project will raise average grain yields by 954 kg/ha in four legumes (groundnut, cowpea, soybean, and common bean), increase average BNF by 46 kg/ha, and increase average household income by $465, directly benefiting 225,000 households (1,800,000 individuals) in eight countries in sub-Saharan Africa (Democratic Republic of Congo, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Zimbabwe).

It is important for the project to have trained staff with the requisite skills to avail the rhizobial inoculants for the farmers to use to enhance BNF. The skills required for this were identified and a course was designed. Resource persons were identified and a small group of staff from each participating country in Southern Africa was trained so that the milestones of Objective 3 can be achieved in each country. The trainings were done in the 3 hubs of N2Africa separately, the current report covers the training held at the Soil Productivity Research Lab (SPRL) of the Department of Research and Specialist Services (DR&SS) in Marondera, Zimbabwe.

Course objectives

The course was held to provide training to N2Africa laboratory technicians and postgraduate students to advance their technical skills in rhizobiology. The specific objectives were to:

i. Equip participants with knowledge and skills in inoculant production, quality control and field inoculation of grain legumes.
ii. Give the facilitators and trainees the opportunity to share varied lessons and experiences, related to methods and tools used in rhizobiology, and their deployment for inoculum production and farm-level inoculation practices.
iii. Provide each participant with a full set of activities and materials which include: basic rhizobiology, strain selection and inoculant production and use.

Participants and resource persons

Participants were drawn from the institutions in Malawi, Mozambique and Zimbabwe that are involved in the rhizobiology objective. Participants and resource persons and their affiliations are listed in Appendix I and II.

Organization and structure of the course

The course was organized to facilitate progress of rhizobiology, beginning from the very basics of BNF in agriculture by way of lectures. Basic rhizobiology, strain selection, inoculant production and use and quality control were covered in lectures. Practical sessions to enhance understanding and give participants starter practice generally followed the lectures. Course materials were given in a training manual and an electronic copy given at the end of the course on CD. The programme followed is given in Appendix III.

Course proceedings

Day One: The opening ceremony began with a tour of the Soil Productivity Research Lab (SPRL) and Legume Inoculant Factory (LIF) facilities. The Chief Research Technician, Mr. Tapfuma led the tour, explaining the work done at SPRL as guests were taken through the soil microbiology laboratory, soil chemistry laboratory and LIF. The opening ceremony was chaired by the Head of Chemistry and Soil Research Institute (CSRI), Dr. Dhliwayo. Ms. de Wolf gave an overview of the N2Africa project. She explained that the N2Africa project is working in 8 countries namely the Democratic Republic of Congo, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda and Zimbabwe. She also explained the objectives of the project and listed partners involved in implementation of objectives. Ms. Murwira then explained to participants the objectives of the advanced technical skills in rhizobiology training course and its link to objective 3 of the N2Africa project. Mr. Mukungurutse explained how the course was structured and activities that were expected to be done during the course. This was followed by presentations from other stakeholders, with highlighting by each presenter on how they can work together to add value to each others work with N2Africa. After this, Prof. Mpepereki gave a lecture on biological nitrogen fixation (BNF) in African agriculture. During his lecture he gave insights into soya bean promotion and research work that has been done in Zimbabwe. He also pointed out that BNF offers a good chance for improving communal farmer livelihoods and for this to be realised a multi-sectoral approach was needed. The Principal Director of The Department of Research & Specialist Services (DR&SS), Mrs. D. Hikwa then gave a key note address and officially opened the training course. In her address she highlighted the importance of BNF in agriculture and how it contributes to sustainable agriculture. She emphasized that rhizobiology fits very well into the framework of Zimbabwean agriculture and that N2Africa's work is therefore relevant to the country.

Day Two: A lecture on nitrogen and legumes was presented. This lecture focused on the role of legumes in providing nitrogen to the farming systems of famers in Africa. This was followed by a lecture on basic rhizobiology, isolating, characterizing and maintaining rhizobia in the laboratory. In the afternoon a practical on general laboratory safety, media preparation, setting up of glass fermenters and inoculation of broth cultures was conducted. This was followed by another practical on legume identification, nodule exploration, recovery and preservation.

Day Three: A lecture on culturing rhizobia, growth requirements and carbon sources, strain characterization and identification was presented. This was followed by another lecture on Rhizobia, symbiosis, maximizing biological nitrogen fixation and response to inoculation. A practical on serial dilutions, quantifying rhizobia by plate counts and plant infection was done. Another practical on rhizobial growth on indicator media, Gram stains and culture storage was also conducted.

Day Four: A lecture on inoculants and inoculation was presented. This was followed by another lecture which highlighted the key inoculants production stages, marketing and production of inoculants. This was followed by a practical on the streaking technique and another one on surface sterilizing and pre-germinating seed. In the afternoon a practical on carrier material selection and processing, mixing broth and carrier, the diluted broth technique and curing inoculants was conducted.

Day Five: In the morning, a lecture on inoculants and inoculation was done followed by another lecture on linking lab activities to N2Africa field trials and rhizobiology needs. Another

lecture on inoculant production, marketing and distribution was held before a practical was held on injecting rabbits with antigens and extracting antisera. In the afternoon two lectures were held on inoculant product testing in the greenhouse and innovation in inoculant production.

Day Six: An interactive lecture was held at the Inoculant factory on carrier material selection and processing. This was followed by a practical on Greenhouse management, using Leonard jars and potted field soil. Another practical was held on seed inoculation techniques.

Day Seven: On this Sunday, Prof. Abdullahi Bala facilitated a session of preparing the rhizobiology workplans with participants from Malawi and Mozambique. This was followed by a game drive.

Day Eight: A group discussion was held on aligning lab capacity and technician skills to N2Africa project activities and milestones. Zimbabwe presented its work-plan for rhizobiology activities. A lecture was held on most probable number by dilution extinction. A practical was then held on observing colony morphology and purifying nodule isolates. This was followed by a mid-course review. Participants were put in 3 groups and tasked to report on their opinions on progress of the course in terms of content, time management and general welfare strengths and shortcomings. A descriptive summary of issues raised is shown in the indexes.

Day Nine: A lecture on most probable number by dilution extinction was given in the morning. This was followed by a lecture on field inoculation trials. It covered experimental design, data collection and analysis with examples. This was followed by a discussion session to identify those topics which participants felt needed more attention or had not been tackled yet. A practical on observation of colony morphology was done following this.

Day Ten: Wednesday was taken off class for recreation as the participants had worked through the weekend to take advantage of Prof. Bala's presence. Participants were taken on a short Harare tour with shopping stops. The day was concluded with a braai at the Chivero Lakeside.

Day Eleven: A lecture was held on quality control of inoculum followed by another lecture on plant infection by most probable number reading nodulation and calculating populations. Another lecture was held with practice questions done on using MPNES software and excel. A practical was then done on inoculant quality testing.

Day Twelve: This was the closing day of the training course. A post-evaluation of the course was conducted by Judith de Wolf. The evaluation assessed the views of the participants on the course modules, presentations, activities and organization. This was followed by the official closing ceremony and presentation of certificates. A participates representative gave some short comments about the training course and how the participants will use the acquired knowledge. Dr. Dhliwayo gave a vote of thanks which started by thanking the SPRL team and CIAT for organizing the training course and also thanked all the resource persons who presented lectures and practicals. The deputy director of the Research Services Division, Dr. Chikwenhere, officially closed the training course and presented certificates, with Dr. Dhliwayo to all the participants.

Course review and conclusions

A mid-course and end of course review and evaluation was done. Participants were asked to give their views with regard to the content of presentations, lecture delivery, practical sessions, time management, general welfare and training materials. For the midcourse review participants were split into three groups and asked to present their opinions and for the end of course evaluation participants were given questionnaires to fill in. Full responses from the questionnaire are given in the Appendix. A brief summary of participants' view is given below:

Methodology: Participants felt that there was a need for field visits for some practicals e.g. where experimental setup in the field was concerned.

Course Objective: From the questionnaire given all participants confirmed that they accomplished the objectives of the course and will be able to use what they learned from the course.

Knowledge Transfer: Participants highlighted that materials provided were sufficient and most of the facilitators were in line with the objectives. Lecture delivery and response to questions was good. Participants also mentioned that one presentation was irrelevant especially for participants from outside of Zimbabwe where legislative laws were presented.

Course Content: Participants said the course content was relevant and is started from basics in a step-by-step fashion that was well organised and the material had a good flow.

Time management: The Mozambique participants were delayed and as a result the organisers moved most lectures and practical sessions that had been scheduled for Monday to Tuesday for them to benefit. This resulted in frequently rescheduling of the final course timetable which did not go well with participants as they felt most sessions were running behind the original schedule. Participants also felt that there was a miscalculation in the amount of time needed to do practical sessions as they needed more time to finish up a practical session. Participants felt that there were too many activities that were cramped in one day. They also highlighted that time was too limited for participants to adequately follow up on some aspects of the content.

Logistics: Participants felt that the transport provider was terrible during the first days and incidental allowances were not provided on time. Foreign participants also felt that there should have been given a choice for dinner or allowances rather than having everything paid for them without consultation. Participants also highlighted that there was minimal interaction between local participants and foreign participants.

Recommendations

- There is need to provide better information to participants before the course. This will enable them to prepare adequately for the course in-order for them to fully benefit by starting on a better level of understanding.
- Training course should run for more than two weeks to enable participants to fully absorb new concepts and give participants more practice to master new skills.
- Field visits where participants communicate with farmers make the course more stimulating and improve participants understanding of application of concepts learned.

- Smaller groups are encouraged during practicals so as to shorten waiting periods between groups and more processes can run at the same time and have groups alternate at each process.
- Both local and foreign participants should be accommodated at the same place to encourage sharing of knowledge and experiences between participants. Also excursions should also include local participants.

Appendix I: Lists of participants

NAME	COUNTRY	STATION
Liwimbi Lloyd	Malawi	Chitedzi Research Station, Malawi
Lundu Yanna	Malawi	Chitedze Research Station, Malawi
Mhango Joseph	Malawi	IITA Malawi, P.O. Box 30258, Lilongwe
Colial Henriques Victor	Mozambique	IITA-Nampula
das Rosas Nancy	Mozambique	IITA-Nampula
Chingwa Mupambi	Zimbabwe	Soil Productivity Research Laboratory
Kainga Tatenda	Zimbabwe	University of Zimbabwe
Manjengwa Anesu	Zimbabwe	Soil Productivity Research Laboratory
Mazengera Togaraseyi	Zimbabwe	Soil Productivity Research Laboratory
Tapfuma Joram	Zimbabwe	Soil Productivity Research Laboratory
Zvomuya Munyaradzi	Zimbabwe	Soil Productivity Research Laboratory

Appendix II: Lists of resource persons

NAME	COUNTRY	STATION
Bala Abdullahi (Professor)	Nigeria	IITA – Nigeria
Chabata Isaac	Zimbabwe	CIAT – Harare
de Wolf Judith	Zimbabwe	CIAT – Harare
Kororo Caroline	Zimbabwe	Soil Productivity Research Laboratory
Mombeyarara Talkmore	Zimbabwe	CIAT – Harare
Mpepereki Sheunesu (Professor)	Zimbabwe	University of Zimbabwe
Mukungurutse Collis S.	Zimbabwe	Soil Productivity Research Laboratory
Murwira Mazvita S.	Zimbabwe	Soil Productivity Research Laboratory
Mushangwe Cathrine	Zimbabwe	Soil Productivity Research Laboratory
Nhongo Enoch	Zimbabwe	Veterinary Department
Sithole-Niang Idah (Professor)	Zimbabwe	University of Zimbabwe
Tumbure Akinson	Zimbabwe	Soil Productivity Research Laboratory

Appendix III: Course Program

	Topic/Activity	Resource person/Facilitator
Sunday 4 September 2011		
	Arrival of participants and transfers to Dzimbabwe Lodges	Chabata Isaac, CIAT- Harare
Day One: Monday 5 September 2011		
0830: 1030 hrs		
	Registration of participants	Caroline J. Kororo, Executive assistant, DR&SS
	Introduction of participants	Dr. David K.C. Dhliwayo, Head, Chemistry and Soil Research Institute
	Overview of N2Africa project	Judith de Wolf, N2Africa Zimbabwe
	Objective of the training and Objective 3 technical milestones, skill set for project technicians and graduate students.	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
	Overview of the training process and activities	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
	BNF in African agriculture	Professor Sheunesu Mpepereki, Soil Microbiology Professor, University of Zimbabwe
	Basic rhizobiology, isolating, characterizing and maintaining rhizobia in the laboratory	Ms. Mazvita Murwira, Principal Research Officer, DR&SS
	Key note address and official opening	Mrs. Danisile Hikwa, Principal Director, DR&SS
Tea/Coffee Break		
1100: 1300 hrs	Laboratory intro, workstation and partner assignments, media preparation, set up glass fermenters, inoculate broth cultures	Mr. Akinson Tumbure, Senior Research Officer, DR&SS
Lunch Break		
1400: 1530 hrs	Nitrogen and legumes (Modules 1 & 2)	Professor Sheunesu Mpepereki, Soil Microbiology Professor, University of Zimbabwe
Tea/Coffee Break		
1600: 1700 hrs	Legume identification, nodule exploration, recovery and preservation, rhizobium isolation (culture 1), streaking technique, surface sterilizing & pre-germinating seed	Professor Sheunesu Mpepereki, Soil Microbiology Professor, University of Zimbabwe
Day Two: Tuesday 6 September 2011		
0830: 1030 hrs	Culturing rhizobia, growth requirements and carbon sources, strain characterization & identification	Ms. Mazvita Murwira, Principal Research Officer, DR&SS/ Mr. Akinson Tumbure, Senior Research Officer, DR&SS
Tea/Coffee Break		
1100: 1300 hrs	Serial dilutions, quantifying rhizobia by plate counts	Mrs. Catherine Mushangwe,

	(culture 2) and plant infection (MPN 1)	Principal Research Technician, DR&SS
Lunch Break		
1400: 1530 hrs	Rhizobia, symbiosis & BNF (Modules 3 & 4); Maximizing BNF & response to inoculation (Module 7)	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
Tea/Coffee Break		
1600: 1700 hrs	Rhizobial growth on indicator media (culture 2 continued), Gram stain, culture storage, Objective 2 Field Trials and rhizobiology needs. Linking the rhizobium lab to N2Africa field activities.	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader Talkmore Mombeyarara, Agronomist, CIAT
Day Three: Wednesday 7 September 2011		
0830: 1030 hrs	Inoculants & inoculation (Modules 5 & 6)	Ms. Mazvita Murwira, Principal Research Officer, DR&SS
Tea/Coffee Break		
1100: 1300 hrs	Seed inoculation technique (slurry, 2-step & pelleting), plate counts of inoculants and inoculated seed (culture 3)	Mr. Joram Tapfuma, Chief Research Technician, DR&SS/ Mrs. Catherine Mushangwe, Principal Research Technician, DR&SS
Lunch Break		
1400: 1530 hrs	Producing, marketing and distributing inoculants	Mr. Akinson Tumbure, Senior Research Officer, DR&SS/ Mr. Chiwawa, Economist, DR&SS/ Mr. Joram Tapfuma, Chief Research Technician, DR&SS
Coffee Break		
1600: 1700 hrs	Carrier material selection and processing, mixing broth and carrier, the diluted broth technique, curing inoculants, (bagasse sieving demonstration)	Ms. Mazvita Murwira, Principal Research Officer, DR&SS/Mr. Akinson Tumbure, Senior Research Officer, DR&SS/ Mr. Joram Tapfuma, Chief Research Technician, DR&SS
Day Four: Thursday 8 September 2011		
0830: 1030 hrs	Rhizobium strain authentication and selection, and product testing in the greenhouse.	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
Tea/Coffee Break		
1100: 1300 hrs	Greenhouse management, Leonard jars, potted field soil	Mr. Collis Mukungurutse, Principal Research Officer, DR&SS
Lunch Break		
1400: 1530 hrs	Rhizobium strain selection in the field	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
Coffee Break		
1600: 1700 hrs	Field inoculation trials. Experimental design, data collection and analysis	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader/ Mr. Collis Mukungurutse, Principal

		Research Officer, DR&SS
Day Five: Friday 9 September 2011		
0830: 1030 hrs	Rhizobium strain identification	Ms. Mazvita Murwira, Principal Research Officer, DR&SS/ Mr. Akinson Tumbure, Senior Research Officer, DR&SS
Tea/Coffee Break		
1100: 1300 hrs	Inspect and purify nodule isolates as needed (cultures 1&2). Agglutination and immunodiffusion. Visit to Vet antiserum lab and rabbit facilities. Preparation of antigens and injecting animals (demonstration)	Dr. Nhongo, Veterinary Department, Ministry of Agriculture/ Mrs. Catherine Mushangwe, Principal Research Technician, DR&SS
Lunch Break		
1400: 1530 hrs	Innovation in inoculant production, strain selection strategies, alternative delivery systems	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
Tea/Coffee Break		
1600: 1700 hrs	Alternative inoculant production (diluted broth, liquid formulation, granular formulation, others) using broth culture 1	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader / Ms. Mazvita Murwira, Principal Research Officer, DR&SS
Cocktail		
Day Six: Saturday 10 September 2011		
	Day trip?	Ms. Mazvita Murwira, Principal Research Officer, DR&SS
Day Seven: Sunday 11 September 2011		
	Free day	
Day Eight: Monday 12 September August		
0830: 1030 hrs	Mid-course review, group discussion and mid-course evaluation. What were the strengths and shortcomings of the course's first week.	Judith de Wolf, N2Africa Zimbabwe/ Mr. Collis Mukungurutse, Principal Research Officer, DR&SS
Tea/Coffee Break		
1100: 1300 hrs	Observe colony morphology, inspect and purify nodule isolates as needed (culture 1)	Professor Sheunesu Mpepereki, Soil Microbiology Professor, University of Zimbabwe
Lunch		
1400: 1530 hrs	Most Probable Number by dilution extinction	Ms. Mazvita Murwira, Principal Research Officer, DR&SS/ Mr. Akinson Tumbure, Senior Research Officer, DR&SS
1600: 1700 hrs	MPN set up with growth pouches, building racks, planting MPN, preparing –N nutrient solution, aseptic irrigation, selecting for plant uniformity, inoculating the pouch, reading results	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
Tea/Coffee Break		
Day Nine: Tuesday 13 September 2011		
0830: 1030 hrs	Aligning lab capacity and technician skills to N2Africa	Dr. Abdullahi Bala, N2Africa

	project activities and milestones. Lecture and group discussion.	Rhizobiology Objective Leader / Mr. Collis Mukungurutse, Principal Research Officer, DR&SS
Tea/Coffee Break		
1100: 1300 hrs	Read plate counts (culture 2) and calculating cell densities. Estimating counts with optical density (demonstration)	Ms. Mazvita Murwira, Principal Research Officer, DR&SS/ Mr. Akinson Tumbure, Senior Research Officer, DR&SS
Lunch		
1400: 1530 hrs	TRAVEL TO HARARE	
1600: 1700 hrs	PCR demonstration at UZ- Biochemistry	Professor Idah Niang Sithole, Biochemistry Department, University of Zimbabwe/ Ms. Mazvita Murwira, Principal Research Officer, DR&SS
Tea/Coffee Break		
Day Ten: Wednesday 14 September2011		
0830: 1030 hrs	Quality control of legume inoculants	Ms. Mazvita Murwira, Principal Research Officer, DR&SS/ Mrs. Catherine Mushangwe, Principal Research Technician, DR&SS
Tea/Coffee Break		
1100: 1300 hrs	Inoculant quality testing	Ms. Mazvita Murwira, Principal Research Officer, DR&SS/ Mrs. Catherine Mushangwe, Principal Research Technician, DR&SS
Lunch Break		
1400: 1530 hrs	Plant infection by Most Probable Number	Ms. Mazvita Murwira, Principal Research Officer, DR&SS/ Mr. Akinson Tumbure, Senior Research Officer, DR&SS
Tea/Coffee Break		
1600: 1700 hrs	Growth pouch set up. Reading plant infection counts and reading nodulation pattern. Using MPN tables.	Mr. Akinson Tumbure, Senior Research Officer, DR&SS
Day Eleven: Thursday 15 September 2011		
0830: 1030 hrs	*What topics remain?*	Mr. Collis Mukungurutse, Principal Research Officer, DR&SS
Tea/Coffee Break		
1100: 1300 hrs	Read plate counts of inoculants and inoculated seed (culture 3). Calculating populations	Mr. Akinson Tumbure, Senior Research Officer, DR&SS
Lunch Break		
1400: 1530 hrs	Facilitating grain legume enterprise and mobilizing BNF technologies (Modules 8 & 9)	Mr. I. Chabata, N2Africa Zimbabwe Farm Liaison Officer/ Mr. Joram Tapfuma, Chief Research Technician, DR&SS
Tea/Coffee Break		

1600: 1700 hrs	Computer laboratory, calculating populations using excel, the inoculation requirement utility, MPNES practice	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
Day Twelve: Friday 16 September 2011		
0830: 1030 hrs	Course review	Team
Tea/Coffee Break		
1100: 1300 hrs	Completion of lab activities, distribution of course materials	Team
Lunch Break		
1400: 1500 hrs	Group discussion, course evaluation	Team, Judith de Wolf, N2Africa Zimbabwe
	Comments from participants representative	
1530: 1630 hrs	Closing Remarks	Dr. Abdullahi Bala, N2Africa Rhizobiology Objective Leader
	Vote of Thanks	DKC Dhliwayo
	Official Closing and award of certificates	Dr. Cames Mguni, Director, Research Services Division
	Some participants depart	Caroline J. Kororo, Executive assistant, DR&SS
Saturday 17 September 2011		
Remaining participants depart		

Appendix IV: Summary of Evaluation Questionnaire

The eleven participants of the 'advanced Technical skills in Rhizobiology' filled an evaluation questionnaire on the last day of the training. Below a summary of their feedback.

N/A=Not applicable, 1=Strongly disagree, 2=Disagree, 3=Neither agree/nor disagree, 4=Agree, 5=Strongly agree

COURSE CONTENT	N/A	1	2	3	4	5
1. I was aware of the prerequisites for this course.	0	0	0	9%	73%	18%
2. I had the prerequisite knowledge and skills for this course.	0	0	9%	18%	55%	18%
3. I was well informed about the objectives of this course.	0	0	0	18%	36%	45%
4. This course lived up to my expectations.	0	0	9%	9%	45%	36%
5. The content is relevant to my job.	0	0	0	0	27%	73%
COURSE DESIGN						
6. The course objectives are clear to me.	0	0	0	0	36%	64%
7. The course activities stimulated my learning.	0	0	0	9%	45%	45%
8. The activities in this course gave me sufficient practice & feedback.	0	0	0	36%	64%	0
9. The difficulty level of this course is appropriate.	0	0	9%	27%	55%	9%
10. The pace of this course is appropriate.	9%	9%	27%	27%	27%	0
COURSE INSTRUCTOR (FACILITATOR)						
11. The instructors were well prepared.	0	0	0	9%	64%	27%
12. The instructors were helpful.	0	0	0	9%	64%	27%
COURSE ENVIRONMENT						
13. The training facility at this site was comfortable.	0	0	0	9%	73%	18%
14. The training facility at this site provided everything I needed to learn.	0	0	0	9%	64%	27%
COURSE RESULTS						
15. I accomplished the objectives of this course.	0	0	0	0	82%	18%
16. I will be able to use what I learned in this course.	0	0	0	0	36%	64%

	How would you improve this course?	Times mentioned	% of participants that ticked this
A	Provide better information before course	5	45.45
B	Clarify the course objectives	1	9.09
C	Reduce content covered in course	1	9.09
D	Increase content covered in course	2	18.18
E	Update content covered in course	2	18.18
F	Improve the instructional methods	5	45.45
G	Make course activities more stimulating	6	54.55
H	Improve course organization	8	72.73
I	Make the course less difficult	1	9.09
J	Make the course more difficult	0	0
K	Slow down the pace of the course	3	27.27
L	Speed up the pace of the course	3	27.27
M	Allot more time for the course	10	90.91
N	Shorten the time for the course	1	9.09

What other improvements would you recommend in this course?

Some people felt everything was good and there was no need for any improvements. Other suggestions related to the pace of the practicals. In general the practicals were considered very useful, but some felt by working in groups and lack of time not everyone always got a chance to practice and at the same time it caused long waiting times for others. Some felt the whole training should be extended to for example three weeks.

Local participants would have wanted to spend more time together with the foreign participants. It was also felt that facilitator – participant interaction could have been more so that participants could have benefitted more and the selection of facilitators should have been wider and more varied. Some felt the trainees would have benefitted from the full involvement of the objective leader throughout the duration of the course.

What is least valuable about this course?

This question caused only few people to comment. The PCR practical was given as an example of something that will not be used by the participants and therefore it was not necessary to include that in the training. Also it was felt that the information about the host country's legislative bodies and agricultural marketing acts of parliament was somewhat irrelevant for the group of participants. Again the fact that local participants were not also provided with accommodation away from their homes was identified as a weakness of the training.

What is most valuable about this course?

- All processes of identifying the legume crops, collect and isolate nodules, clean and then isolate bacteria from nodules;
- How to prepare the media
- How to infect the plant, collect data form trials in greenhouse and field
- How to make aseptic conditions
- Apply new techniques in lab
- Identify inoculants and rhizobacteria, Isolation and characterization of rhizobia
- Inoculant production and quality control
- The hands-on experience for the technical persons in the participating countries in the N2Africa project in Southern Africa
- Practicals: they gave far better insights to the objectives of the course than the lectures. Participants understood their expected duties and take-home messages from the participatory practical sessions (especially technical staff)
- Course materials and share experiences
- It equipped participants with all the fundamentals in rhizobiology
- It is an eye opener and confidence boaster for the technical staff
- How to culture bacteria, from nodule collections
- The importance of BNF to communal farmers.
- The technical skills acquired and improved will help participants to be flexible when it comes to working back at their stations. Some of the skills were new and advanced showing that participants have learnt something new. It feels great to learn new things.

List of project reports

1. N2Africa Steering Committee Terms of Reference
2. Policy on advanced training grants
3. Rhizobia Strain Isolation and Characterisation Protocol
4. Detailed country-by-country access plan for P and other agro-minerals
5. Workshop Report: Training of Master Trainers on Legume and Inoculant Technologies (Kisumu Hotel, Kisumu, Kenya-24-28 May 2010)
6. Plans for interaction with the Tropical Legumes II project (TLII) and for seed increase on a country-by-country basis
7. Implementation Plan for collaboration between N2Africa and the Soil Health and Market Access Programs of the Alliance for a Green Revolution in Africa (AGRA) plan
8. General approaches and country specific dissemination plans
9. Selected soybeans, common beans, cowpeas and groundnuts varieties with proven high BNF potential and sufficient seed availability in target impact zones of N2Africa Project
10. Project launch and workshop report
11. Advancing technical skills in rhizobiology: training report
12. Characterisation of the impact zones and mandate areas in the N2Africa project
13. Production and use of Rhizobial inoculants in Africa
18. Adaptive research in N2Africa impact zones: Principles, guidelines and implemented research campaigns
19. Quality assurance (QA) protocols based on African capacities and international existing standards developed
20. Collection and maintenance of elite rhizobial strains
21. MSc and PhD status report
22. Production of seed for local distribution by farming communities engaged in the project
23. A report documenting the involvement of women in at least 50% of all farmer-related activities
24. Participatory development of indicators for monitoring and evaluating progress with project activities and their impact
25. Suitable multi-purpose forage and tree legumes for intensive smallholder meat and dairy industries in East and Central Africa N2Africa mandate areas
26. A revised manual for rhizobium methods and standard protocols available on the project website
27. Update on Inoculant production by cooperating laboratories
28. Legume Seed Acquired for Dissemination in the Project Impact Zones
29. Advanced technical skills in rhizobiology: East and Central African, West African and South African Hub

Partners involved in the N2Africa project

Caritas Rwanda

Diobass

Eglise Presbéterienne Rwanda

Resource Projects-Kenya

Université Catholique de Bukavu

University of Zimbabwe