N2Africa Podcaster no. 8

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Introduction

This issue of the Podcaster is devoted to N2Africa's work in rhizobiology and rhizobial inoculants. While a general lack of technical and scientific capacity in soil microbiology and rhizobiology remains a general concern across the project, you can read about the ongoing initiatives to upgrade facilities and train staff, on actions to procure high quality inoculants and make them available to smallholder farmers, and to work on regulations to ensure that inoculants can be moved readily between countries. Some updates on ongoing research are also presented. Many thanks to Abdullahi Bala for coordinating this thematic issue of the Podcaster!

Ken Giller

N2Africa enhancing laboratory capacity in Africa

Laboratory and greenhouse facilities in African countries are undergoing upgrade and complete makeover – thanks to the substantial investment in state-of-the-art facilities being made by N2Africa to enhance the capacity of NARS to carry out research in rhizobiology and general soil microbiology. The intervention includes the refurbishment of laboratories and purchase or repair of laboratory equipment. New greenhouses are also being built and existing ones renovated.

Objective 3 of the N2Africa project *(Objective 3: Select superior rhizobia strains for enhanced BNF and develop inoculum production capacity in sub-Saharan Africa through collaboration with private sector partners)* focuses on rhizobiology activities and includes the isolation of rhizobia from indigenous populations in African soils from which elite strains are selected for use in the production of rhizobial inoculants. These require the intensive use of laboratory and greenhouse facilities. Activities under this objective had initially been planned to commence using existing facilities at the various NARS partner institutes in the 8 countries where the project is operational. It soon became evident that virtually all the selected institutes had limited capacity to carry out the activities as initially envisaged. The relevant equipment and facilities were completely absent, obsolete, or in a state of disrepair. This situation has severely impacted on the pace of rhizobiology activities, with many of the Objective 3 milestones delayed.

In Kenya, a new greenhouse is under construction for MIRCEN (Microbiological Resources Centre), University of Nairobi. This centre serves as the project's hub for East and Southern Africa (ECA) and some equipment and reagents had earlier been supplied for use during the ECA technicians' training in basic rhizobiology skills which took place in September 2010. Laboratories in Rwanda and the Democratic Republic of Congo (DRC) received assorted reagents, glassware and laboratory equipment, including laminar flow hoods, ovens and electric balances. N2Africa is also processing the renovation of a greenhouse at ISAR (Institut des Sciences Agronomique du Rwanda) and the purchase of rhizobiology equipment, including an autoclave, to support the institute's effort at jump-starting inoculant production, which was disrupted by the 1994 genocide in that country.

Zimbabwe is also benefitting from N2Africa's intervention, with over UD$ 74,000 already committed for the procurement of equipment and reagents to be used at the Soil Productivity Research Laboratory (SPRL), Marondera. As the Rhizobiology hub for Southern Africa, additional funds are available in support of inoculant production at the laboratory. Already, a procurement process has been initiated for the renovation of a greenhouse there. N2Africa and AGRA are partnering to upgrade the laboratory at the Chitedze Research Station in Malawi. The Ministry has refurbished the laboratory, with N2Africa contributing towards the completion of the electrical works. AGRA has approved some funds for the purchase of equipment in the laboratory and N2Africa is providing a counterpart funding for the procurement of core rhizobiology equipment.

A laminar flow cabinet purchased by N2Africa for the Institute of Agricultural Research (IAR), Zaria, Nigeria.

The two partner laboratories in the West African Hub are the Soil Research Institute (SRI), Kumasi, Ghana, and the Institute of Agricultural Research (IAR), Samaru-Zaria, Nigeria. Both institutes have immensely benefitted from the project's laboratory upgrade. SRI Ghana has refurbished a laboratory for rhizobiology activities and have received delivery of assorted equipment worth over UD$ 17,000. The purchased equipment included an incubator, orbital shaker, vacuum pump, hot plate stirrer and water deioniser. In Nigeria, IAR Zaria received the delivery of equipment and reagents valued at more than UD$ 33,000. The consignment included an autoclave, incubator, and a laminar flow cabinet. Receiving the equipment, the Director IAR, Professor Balarabe Tanimu, noted that N2Africa's intervention would not only help to facilitate the execution of the on-going N2Africa project being led by the institute, but also accelerate microbiological research activities. Given IAR's status as the Hub for West Africa, there are plans to invest a further UD$100,000 in the installation of a pilot rhizobial inoculant production facility at the institute.

This investment in facilities has no doubt given rhizobiology activities a shot in the arm, resulting in a flurry of activities across the various countries. MPNs and strain isolations have commenced in many countries; some have gone far in isolate characterisation and strain evaluation. It is evident that the surge in rhizobiology activities is closely linked to the arrival of these new facilities.

Abdulahi Bala

N2AFRICA Rhizobiology Activities at ISAR, Rwanda: June 2011

The ISAR Microbiology Laboratory leads N2Africa rhizobiology activities in Rwanda and liaises with related actions in DR Congo and Rwanda. The team at ISAR is responsible for both Agronomy (Objective 2) and Rhizobiology (Objective 3) activities in Rwanda. The Microbiology Laboratory has cultured 80 isolates from bean and soyabean. Twenty-nine of these isolates were characterized and classified by Congo Red morphotype, BTB reaction and Gram Srain. To date, bio-prospecting has focused solely upon common bean (*Phaseolus vulgaris*) and soyabean (*Glycine max*), but 11 other genera and related species in Rwanda were sampled by the University of Nairobi MIRCEN team, reducing this possible additional shortcoming. Seven hundred (700) packets of bean inoculant containing 80 g each were recently prepared (56 kg total) for use by project research and dissemination activities in the next growing season. The Soil Microbiology Laboratory of ISAR in Rubona had a strong presence in Rhizobiology in Africa in the past, but one that was slow to become restored following civil unrest in the country. The laboratory occupies a well designed building and has assembled a team of ambitious young scientists who must now demonstrate their ability to perform the full spectrum of microbiology skills. This team is led by Ms. Mathilde Uwizerwa, a recent M.Sc. graduate in Soil Science and consists of three other trained members, Felix Nzeyimana (Agronomist), Claire Uwitonze (Lab and Field Technician) and Olivier Niyomugabo (Lab Technician).


ISAR Soil Microbiology Laboratory

Mathilde Uwizerwa and Paul L.Woomer

Legume Inoculant Technology and Quality Control Procedures Workshop

A workshop on Legume Inoculant Technology and Quality Control (QC) procedures was held at the Microbial Resources Centre Laboratory (MIRCEN) in the Department of Land Resource Management and Agricultural Technology (L.A.R.M.A.T.), University of Nairobi (UoN; Kabete Campus) from July 4th to 22nd, 2011. The training was co-funded by the N2Africa Project and SIMLESA (Sustainable Intensification of Maize-Legume Cropping Systems for Food Security in Eastern and Southern Africa) with the focus to provide participants with the latest ideas and techniques in manufacturing Rhizobium inoculants and in QC testing procedures required in achieving high quality legume inoculants.

Thirteen attendees comprising of specialists coming from N2Africa and SIMLESA partners in six countries (Ethiopia, Kenya, Malawi, Nigeria, Zambia and Zimbabwe), University of Nairobi Students and MIRCEN Laboratory technical staff, participated at this workshop. Its structure was a practical and hands-on experience where they learnt to deal with rhizobia cultures, recognize contaminants, maintain rhizobia Mother Cultures, produce batch fermentation cultures, inject inoculant bags and perform QC tests. Participants also had the opportunity to learn serological techniques (Precipitation & Agglutination and the ELISA test) frequently used in the identification of rhizobia species. This was demonstrated by Chief Technologist

Workshop participants (from top left): Victor Mukwa (Zambia), Priscah Echessa (MEA Limited-Kenya), Demola Akanni (Nigeria), James Ndiritu (MIRCEN Technologist), Dr Kenton Dashiell (N2AFRICA Project Leader; CIAT-TSBF), Nabintu Ndusha (UoN Student), Alfred Rumongi Tabaro (UoN Student), Amanuel Asrat (Ethiopia), Maureen Waswa (UoN Student), Dr Anabel Vivas-Marfisi (Australia), Cathrine Mushangwe (Zimbabwe), Prudence Mapiki (Zambia), Professor Nancy Karanja (UoN) and Lloyd Liwimbi (Malawi).

Nduhiu Gitahi (MSc Immunology) from the Department of Public Health Pharmacology & Toxicology, UoN.

The workshop provided all attendees with new skills that they in turn will be able to share and use in their workplace. It was also an excellent opportunity to interchange information between participants and trainers and to develop and foster new relationships and networks with the ultimate aim of improving the production of legume inoculants in Africa.

Dr. Anabel Vivas-Marfisi (Centre for Rhizobium Studies, Murdoch University, Perth, Australia)

Emerging challenges in cross-border movement of inoculants in sub-Saharan Africa

Since the late 19th century, root nodulating rhizobia (RNB), commonly known as rhizobia, have been known to be responsible for fixing atmospheric nitrogen on legumes. This knowledge soon led to the practice of inoculation, with early adoption achieved by transferring soil from field to field, or soil to seed before planting. However, this was quickly replaced by the use of pure cultures on agar slants, and later on as broths. Inoculant industries were first established in the 1920s and the global practice of inoculation today is valued at more than UD$ 10 billion annually. There is nearly 70 million tonne of soyabean inoculated annually with *Bradyrhizobium japonicum* in the USA, in addition to 34 and 53 million tonne in Argentina and Brazil, respectively. Inoculation activities have been on-going in sub-Saharan Africa since the 1950s, mostly on soyabean and forage legumes. However, adoption of inoculation on a commercial scale has not been appreciable, except in a few countries, such as Zimbabwe and South Africa, where commercial farms had dominated the agricultural sector. With rising fossil fuel costs, continued reliance on nitrogenous fertilisers for crop production in sub-Saharan Africa will lead to high farm production costs which cannot be met by smallholder farmers.

The philosophy of the N2Africa project is to improve system productivity in smallholder farms through enhanced input of biologically fixed N using best-fit technologies. One of such technologies is to use high quality rhizobial inoculants. In our effort to provide farmers with inoculants, we have had to import the products into several countries with varying degrees of success. A close look at the regulatory requirements for inoculant import in all the 8 countries where N2Africa is operational shows that import regulations in the 8 countries are enforced through the issuance of import permits and phytosanitary certificates. The following are the steps to follow in the import/export of rhizobial strains and inoculants:

- The receiver (importer) applies for import permit from the Plant Quarantine Service (PQS) (or the agency empowered with that role) of the importer's country
- The importer sends a copy of the import permit to the person or organisation sending the product (the exporter)
- The exporter presents the import permit to the PQS of the exporting country for a phytosanitary certificate to be raised
- The exporter sends the product along with the phytosanitary certificate.

However, that is where the similarity ends because different countries have varying approaches to the enforcement of the laws governing inoculant imports

The policy environment can be broadly divided into three categories:

The first group comprises those countries, such as Ghana, Nigeria and Mozambique, where inoculation has traditionally not been practised: There is some tentativeness (or even confusion) as to how to enforce inoculant import regulation. The Act governing plant health regulation does not have inoculants specifically identified as products covered by the law and thus is only inferred. And although a regulatory body exists, there may be overlaps in function with other agencies. For instance, the Ghana Plant Quarantine Service has the mandate regarding issues of plant health, but import permits may also be obtained from the Soil Research Institute (SRI). In Nigeria, we were initially able to process our import permits for both inoculants and rhizobial strains from the Plant Quarantine Service (PQS) regional office in Kano. However, there came a sudden instruction that all subsequent applications for import permits be sent to the PQS headquarters in Ibadan and all product inspections are to be done in Ibadan. For now, this is not a problem for us since we could use the services of IITA headquarters in Ibadan to process the permits and physical inspection. However, a serious problem could arise when private sector involvement in inoculant import becomes visible; the entrepreneur that does not have representatives in, or close to, Ibadan will likely face escalating import costs due to the delivery to Ibadan for inspection before getting to its final destination. Inspection by PQS officials at the ports of entry will be much more sensible – rather than shipping the goods to Ibadan. N2Africa has successfully imported inoculants from the UK into both Ghana and Nigeria for the last 2 years using the import-permit system. There also appears to be an informal channel through which inoculants from Zimbabwe find their way into Mozambique along with seeds purchased from SeedCo.

The second category has countries that had in the past had robust inoculant production outfits but which, for various reasons, have now collapsed. This is the case with Malawi and Rwanda. While the import permit-phytosanitary certificate system seems to work, there appears to be some restriction by subjecting applications for import permits to vetting by the research institutes that had earlier been producing inoculants. In the case of Malawi, inoculants are explicitly mentioned as one of the products that are covered by the Plant Protection Act of 1969. For inoculant import to be approved in Malawi, a sample of the product must first be sent for analysis to the Chitedze Research Station. Given that the laboratory there is currently not functional, it is hard to imagine the nature of quality check that takes place to decide whether to approve the product for import or not. The indication here is a tendency for import protection measures in favour of a local product but, since the local product is of poor quality t, import restriction are likely to be relaxed.

Kenya and Zimbabwe constitute the third category of countries where importing inoculants has so far been almost impossible. This is in spite of the fact that the inoculants were to be used for research activities and the quantities for which permits were sought were small. Ironically, MEA Fertiliser Ltd., the inoculant manufacturer from Kenya, has been aggressively marketing its products in neighbouring countries. Similarly, the Soil Productivity Research Laboratory (SPRL), which is the sole manufacturer of inoculants in Zimbabwe, has been selling in neighbouring Mozambique. So far, the non-issuance of permits to N2Africa in Kenya has been due to the current lacuna in the law as a result of the on-going review of the phytosanitary laws by the Kenyan Plant Health Inspection Service (KEPHIS) and the Kenyan Bureau of Standards. The review process has benefitted from inputs from N2Africa's Prof Nancy Karanja and Dr Paul Woomer especially on provisions for inoculant quality standards. It is, however, worrisome that inoculant imports are being kept on hold because of an on-going review. The normal practise would have been to continue using the extant legal and regulatory provisions pending the completion of the review and the enactment of a new law.

What our experiences have so far shown is that we will be faced with varying degrees of obstacles as we continue with our efforts to provide high quality inoculants to African farmers. We need to be proactive in ensuring that the necessary paperwork is done timely and in the right manner to ensure that inoculant imports are not held up for long periods at the ports of entry.

Abdullahi Bala

N2Africa consultant visits the inoculant factory at Marondera, Zimbabwe

Dr Bruce Knight of the legume Technology, UK, was in Zimbabwe on 26th June-2nd July 2011 to work with staff of the inoculant production plant at the Soil Productivity and Research Laboratory (SPRL), Marondera. Legume Technology Ltd is the producer of LegumeFix inoculants for various legume crops and Bruce was engaged by N2Africa as a consultant for his tremendous experience in the inoculant manufacturing industry. His task was to familiarise himself with the technical aspect of production and recommend changes in production process, equipment and other resources that will result in a more efficient and cost-effective process of production and a high quality product.

Working with Ms Mazvita Murwira of SPRL, Bruce looked at the major production steps and made some useful observations and far-reaching recommendations which, if implemented, could bring about a tremendous cut in production costs and a substantial enhancement in the shelf-life of the inoculants from the current 6 months to as much as 2 years. Some of the major highlights of the recommendations include the following:

- *Improve the particle size of the inoculant carrier by milling*: Currently, SPRL uses bagasse, a sugarcane by-product, as the inoculant carrier. The raw bagasse arrives the factory made up of a mixture of fibrous materials and finer particles with a wide range in particle size. This raw material is passed through a sieve to give a finer grade material. However, the sieved material was observed to still contain coarse particles which will require milling to finer grades. Milling will result

in a more consistent particle size, higher moisture and nutrient retention and less wastage of bagasse because less of the material is retained on the sieve and thrown out.
- *Introduce a full- or semi-automation of the sachet filling step*: The current practice is that the processed carrier is weighed into the sachets manually. Automating this step will hasten the process, reduce man hours and result in a more consistent weight.
- *Adopt a 2 cycle heat sterilising process with a longer dwell time at 121°C*: The existing practice is to sterilise the sachets using an autoclave at 121°C for about 15 minutes. This may not achieve full sterility. An additional cycle and longer periods of sterilisation will ensure a more sterile product and hence result in a longer shelf life.
- *Train staff on how to use the freeze dryer in the laboratory*: At present there is no member of staff trained on how to use this equipment and this means that the rhizobial strains in SPRL's culture collection are being eroded with no practice in place to maintain the stocks.

The recommendations also looked at various options available in modifying certain production methods and the quality assurance process. One of the exciting observations made by Bruce is the endorsement of the microbiological practice in the laboratory. According to Bruce, ''The Head Technician, Catherine, working at Maronderra has a good grasp of technique and the demonstration - using the starter medicine bottles to inoculate the 1.5L flasks included a QC test of the medicine bottle culture. After 3 days of growth, the CRYMA (Congo Red – yeast extract mannitol agar) plate was clean and showed growth exactly as you would expect from a *Bradyrhizobium* culture''. This is one of the many strengths that N2Africa hopes to build on to get the inoculants produced at SPRL globally competitive. Given that SPRL is the longest established inoculant manufacturer in Africa and has remained in production even under very difficult and trying periods, this is the least the project can do to reward commitment to service, self belief and perseverance.

Mazvita Murwira and Abdullahi Bala

N2Africa goes global in search for high quality inoculants

The N2Africa project sent out invitation to global inoculant manufacturers to tender for the supply of soyabean inoculants for the project's research and development activities in 2011 and to consider establishing partnerships with local agro-dealers in all 8 N2Africa countries. This was done to ensure, that the project provides smallholder farmers with the best quality inoculants available.

Winning bids were selected based on offer of high quality products at the most cost-effective rates. The minimal conditions set for the bids were that the product to be supplied must:

1. Have 10^9 cells/g of viable rhizobia strain USDA110 in a peat carrier when landed at port by the stated date.
2. Contain less than 10^6 contaminant organisms per gram inoculant.
3. Carry labels on package bearing the above information in English as well as identify it as inoculant for soyabean, the manufacturer's details, storage and transport conditions, composition of the carrier, expiry date, and recommended dose (for 1 kg).

Suppliers were also expected to guarantee that in the event that the products failed to meet the quality specifications, in an independent quality assurance (QA) process, the company was to replace the quantity affected at no cost to N2Africa or to the farmers.

Twelve companies were invited to bid and five responded. All the five companies met the minimum requirements specified and so selection came down to which company was able to supply the inoculants at the lowest cost/benefit ratio. At the end of the exercise, ResBioAgro, a company based in Spain, was selected for supplies to Rwanda and

N2Africa's Farm Liaison Officer for Nigeria, Esther Chinedu, off-loads boxes of LegumeFix soyabean inoculants received from Legume Technology Ltd, UK.

DRC, while Biagrosa from Argentina was invited to supply Malawi and Mozambique. Due to import restrictions, it was decided that supplies for Zimbabwe and Kenya should be sourced locally from SPRL Marondera and MEA Fertiliser Ltd. Nakuru, respectively. Supplies to Ghana and Nigeria were not included in the tender process because there was an insufficient time to go through the process before the start of the cropping season in West Africa. Rather, Legume Technology Ltd in the UK was contracted to supply both countries based on previous year's experience of which their products produced outstanding results in the two countries.
Supplies from Legume Technology and ResBioAgro have since been received by the respective countries of destination while the procurement process for supplies by BioAg-

rosa is underway. It is hoped that these business partnerships with foreign manufacturers will go beyond one-off transactions and will rather be the seed for a long-lasting relationship. Our long-term vision is to have manufacturers producing high quality inoculants in those countries, or that different companies are importing and selling inoculants. To facilitate this, N2Africa will assist companies with contacts in the various countries for information on import permits and product registration.

Abdullahi Bala and Ken Dashiell

Inoculation and fertilizer blending affects nodulation of climbing bean and soyabean in Kenya

An assessment of legume root nodulation by two grain legumes in response to inoculation and fertilizer source suggests that nodulation number, frequency of crown nodulation and the effectiveness of symbiosis may be controlled by crop management. Two grain legumes, climbing bean "Kenya Tamu" and soyabean SB19 were established at twelve locations in west Kenya. Seed of both crops were planted with and without BIOFIX rhizobial inoculant (CIAT 899 for bean and USDA 110 for soyabean) after application of single super phosphate (SSP) fertilizer in a 2 x 2 design. In an additional fifth management, Sympal was substituted for SSP and inoculated soyabeans planted. Sympal contains not only phosphorus, calcium and sulfate (as does SSP) but also potassium and magnesium. Plants were carefully uprooted six to eight weeks after emergence, soil removed by gentle shaking and inspected for root nodulation using a 0 to 5 scoring system where 0 = no nodules, 1 = 1 to 5 nodules (rare), 2 = 6 to 10 nodules (few), 3 = 10 to 20 nodules (moderate), 4 = 20 to 50 nodules (abundant) and 5 = >50 nodules (extra abundant). Nodule distribution was also scored as crown (many at upper tap root) or diffuse. Two nodules per plant were cut open and nodule contents described as red (including pink) or other (white, green or grey). Trials were installed, managed and data recorded by farmers receiving training from the N2Africa Project. One trial at Bara in Kisumu experienced crop failure but results from the other eleven trials were entered into a spreadsheet data base and summary statistics calculated. Inoculation did not greatly affect nodulation of bean, but more than doubled the proportion of nodules with red interiors, presumably effective nodules (Table 1). Inoculation of soyabean increased nodule scores, crown root nodulation and the proportion of effective nodules.

Table 1. Nodulation characteristics within different on-farm BNF technologies in west Kenya during the 2011 long rains.

legume[1]	inoculant[2]	fertilizer[3]	sites	nodulation score[4]	crown nodulation	nodules w/ red interior
			n	(0-5 ± SEM)	--frequency (%) --	
bean	none	SSP	11	2.58 ± 0.12	29	22
bean	CIAT 899	SSP	11	3.13 ± 0.09	29	45
soyabean	none	SSP	11	1.08 ± 0.08	7	45
soyabean	USDA 110	SSP	11	2.27 ± 0.09	37	62
soyabean	USDA 110	PKS+	10	2.45 ± 0.13	45	71

[1] climbing bean "Kenya Tamu" or soyabean SB19. [2] Commercially available BIOFIX inoculant for bean and soyabean. [3] SSP = single super phosphate and PKS+ = MEA Sympal blend, both at 100 kg per ha. [4] Based upon observation of 40 plants per site, 430 plants total.

Further modest increases were observed in soyabean when Sympal fertilizer was substituted for SSP (Table 1). These results suggest that nodulation may be managed through inoculation and fertilizers but that these effects may be subtle, as when the proportion of effective vs non-fixing nodules increases. These results also indicate that farmers attending two day training in BNF technologies are well positioned to conduct simple diagnostic on-farm experiments. Yield measurements are being performed from these trials and will be reported at a later date.

Paul L. Woomer, Celister Kaleha, Josphat Ambugua, Alex Maguga, Andrew Kasera, Dick Ongai, Sheila Risiema and Jane Achieng

Samson Foli on working visit to Nigeria

Samson Foli, an MSc student of Wageningen University, The Netherlands, is in Kano, Nigeria on a working visit. He arrived Kano on 25th July and will be in the country up until 30th November. During his stay, Samson will conduct nutrient omission trials in greenhouse and field experiments to try to unravel the cause of non-responsiveness of soyabean to fertilisation especially of phosphorus. The study became necessary following observations of the problem in several N2Africa demonstration plots during the 2010 season. Samson will also carry out detailed farm characterisation of different farm topologies in two communities where the N2Africa is operational. He has since commenced work.

Samson Foli (extreme right) poses for a photograph with farmers in one of the communities where he is carrying out detailed farm characterisation.

Speaking of his experience so far, Samson says he enjoys eating Semovita (an 'Ugali'-like meal made from maize and wheat flour) and egusi soup, a staple in most parts of Nigeria. He is also getting adjusted to the frequent power outages experienced in Kano as in most parts of the country. N2Africa welcomes Samson to Nigeria and wishes him an exciting stay.

Abdullahi Bala

Sharing highlights from the revised Agricultural Development strategy of the Bill& Melinda Gates Foundation

Here we share the revised Agricultural Development Strategy recently sent to N2Africa.

The value of Inoculants

Click here for a useful document from the USA on the use of rhizobial inoculants.

Soyabean best practices checklist in English and Swahili

Click here for checklists in English and Swahili on "Best practices to maintain high yield and grain quality of soyabean".

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