

Rhizobiology Master Plan

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(with a contribution for the Tier 1 Countries from Paul L. Woomer)

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N2Africa

Putting nitrogen fixation to work for smallholder farmers in Africa



Summary

N2Africa is committed to ensuring the best legume technologies reach smallholder farmers across sub Saharan Africa (SSA). This **Rhizobiology Master Plan** (MP) is specifically intended for core countries to address the rhizobiology component of *Objective 4: Tailor and adapt legume technologies to close yield gaps and expand the area of legume production within the farm*. It is suggested a single and integrated rhizobiology research plan to instigate a common approach, which will lead to significant improvements on relevant issues such as the consistency in research designs, data collection to feed databases used for meta-analysis, etc., across N2Africa core countries. Other advantages of the MP include assurance of timely delivery of expected project outcomes / outputs.

The rhizobiology MP is built mostly upon lessons learnt from phase I, but also advances achieved in legume technologies across SSA. While previous research efforts have been focused on soybean in general, current evidence suggests that several other grain legumes (*e.g.* cowpea in Ghana and chickpea in Ethiopia) have great response potential to inoculation with rhizobia. Therefore, phase II will focus on bio-prospecting to identify new elite rhizobia strains for four other major grain legumes – common bean, cowpea, faba bean and groundnut. The aim is to isolate elite strains from nodules of each target crop and evaluate for yield increases that can justify recommendation of inoculants to farmers. Thus, any significantly better strain to come, using proper statistical methods to ascertain differences up to 10% at least, that is robust and stable under screen house and field conditions, could be advanced for inoculant production.

Meanwhile, when dealing with promiscuous legumes such as cowpea, that nodulate readily with soil native rhizobia, advances in inoculant technologies depend upon clearly understanding success or lack of success of inoculation. This requires competition studies of background populations of indigenous rhizobia and tracing the inoculant strains in nodules. Standard methods of molecular typing will be used to characterize the role of the rhizobial genotype, from both soil and inoculant sources, in the ($G_L \times G_R$) $\times E \times M$ interaction, and its contribution to yield in farmer's fields.

The rhizobiology MP consists mainly of four activity clusters, each containing a set of activities involving specific tasks. These clusters are structured to ensure a relative flexibility that allows participating countries to adapt their rhizobiology plans to facilities available locally. Two activity clusters namely (1) Biopropecting and (2) Identify elite strains, are expected to be implemented by almost all core countries; Two others, (3) Inoculant formulations and (4) standard operating procedures, will be implemented mostly by the inoculant factory at the central level. In addition, bridges are suggested between the rhizobiology MP and the others, especially with the Agronomy Master Plan, to ensure streamlining across N2Africa interventions.



I. Introduction and justification

The N2Africa Master Plans are documents intended to foster a common approach across all of the Core Countries. The plans are designed to achieve the N2Africa Vision of Success and the Research Framework of the approved project proposal. This means all Master Plans need to ensure timely delivery of the outputs and outcomes.

This Master Plan specifically addresses the Rhizobiology component of:

Objective 4: Tailor and adapt legume technologies to close yield gaps and expand the area of legume production within the farm

Through design of a single, integrated Rhizobiology research plan we aim to ensure consistency in research designs and data collection to allow for meta-analysis across N2Africa core countries.

Strain improvement above existing strains is a process of screening and incremental improvement so any significantly better strain that is robust and stable under laboratory and field conditions can be moved along the development pipeline towards production. Previous research efforts have been sporadic, and coordinated rigorous screening of large numbers of isolates and widespread field trials is therefore necessary. Little attention has been paid to grain legumes other than soybean and common bean, yet recent results from Savanna Agricultural Research Institute (SARI), Ghana in collaboration with EMBRAPA have shown strong inoculation responses with cowpea. N2Africa has already observed 20% increases in yield in inoculation trials with chickpea in Ethiopia. In Phase II, N2Africa will bioprospect and identify new elite strains of rhizobium for the other major grain legumes – common bean, cowpea, faba bean and groundnut.

II. Underlying principles

To date most N2Africa Rhizobiology research has been conducted on soybean, which is relatively specific in its requirement for rhizobia and responds strongly to inoculation. Our current evidence suggests that common bean responds to inoculation in some, but not all soils. Given the relatively cheap price of inoculants it is probably worth recommending inoculation of common bean. Although several companies are selling rhizobium inoculants for groundnut we have no evidence to confirm this is worthwhile and we do not advise inoculation of groundnut. Recent evidence suggests that new strains from Brazil can give strong inoculation responses on cowpea in Ghana. This work is still at the experimental stage and needs more widespread confirmation.

Particularly when dealing with promiscuous legumes such as cowpea, that nodulate readily with rhizobia already present in the soil, yield responses to inoculants are found only when the elite inoculant strain is substantially more effective in N₂-fixation than the indigenous strains, and when the elite inoculant strain can be established in the nodules of the grain legume. This is essentially a 'numbers game' and high quality inoculants that can deliver large numbers of the inoculant strain on the seed are needed to ensure success. Understanding success of inoculation under such circumstances relies on studies of background populations of indigenous rhizobia and tracing the success of nodulation by the inoculant strains though 'competition studies' using molecular typing. Standard methods are available for this and will be deployed to understand success, or lack of success of inoculation.

This leads to the main aims of the Rhizobiology research, namely to provide the necessary inoculant technologies to close the yield gaps on farmers' fields by achieving the following:

Aims

- To understand the role of G_R (both background populations of rhizobia and inoculant strains) in the $(G_L \times G_R) \times E \times M$ interaction and the contribution to productivity and yield
- To isolate elite strains for common bean, cowpea, faba bean and groundnut and evaluate whether yields can be increased substantially and consistently to justify recommendation of inoculants to farmers



Approach

- No further bioprospecting will be done with soybean as elite strains have already been identified through prior research.
- Bioprospecting will be focused on common bean, cowpea, faba bean and groundnut. It seems that further research on strains for chickpea may be needed.
- Bioprospecting will be done by isolating only from nodules of the target hosts, either from sampling nodules in the field or using the target legume as a trap host growing soils collected from diverse ecologies.
- While ranking strains can be routinely used for initial characterization and evaluation of isolates from nodules, final screening of rhizobia for the identification of candidate elite strains should use proper statistical analysis of differences to test for significant host response.
- For testing of inoculants it is essential to separate the effects of the rhizobium strain and the inoculant formulation. Where commercial inoculants are available and close collaboration established, this may be achieved through the substitution of current industry standards with candidate elite strains.
- To date no strong and consistent (G_L × G_R) shown to warrant selection of particular strains for particular crop varieties. This means that each crop needs only one inoculant strain to be used in commercial inoculants, but does not preclude the examination of inoculants containing more than one strain.
- Increases in yield of at least 10% above current practice (clearly visible to farmers) are a threshold for identification of better inoculant technologies. This threshold should be reached consistently before N2Africa will actively promote the new technology with smallholder farmers.
- Much of the more detailed Rhizobiology will be conducted through N2Africa PhD research, but this research must address specific cluster tasks described below.

III. Activity clusters within the Rhizobiology Master Plan and relation to other Master Plans

The Rhizobiology Master Plan consists of a set of activities (Figure 1.), which have a set of specific tasks associated with them. The Rhizobiology Master Plan interacts logically with the other Master Plans, in particular with the Agronomy Master Plan through Task 4.1.2. New inoculant strains will be fed into the Dissemination Master Plan as part of the promotion and dissemination of robust inoculant technologies through private inoculant companies.



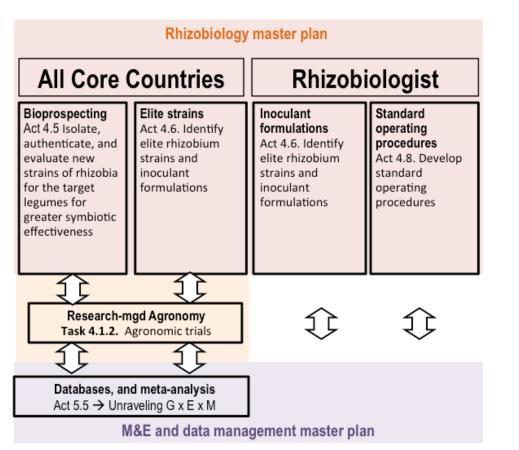


Figure 1: Content of the Rhizobiology, agronomy, and M&E and data management master plans with specific reference to the activities as per the Results Framework of N2Africa Phase II. The different activity clusters are delineated in boxes.

Detailed research under Activity 4.7. "Evaluate competitiveness and survival of introduced rhizobium strains as affected by M x E" will be conducted by PhD and/or MSc students by sampling nodules from inoculant field trials that have been established under Task 4.1.2 and from the long-term trials Task 4.4.2.

IV. Cluster contents

V.1. Bioprospecting cluster

This cluster aims to isolate and evaluate rhizobium strains under Activity 4.5. Isolate, authenticate, and evaluate new strains of rhizobia for the target legumes for greater symbiotic effectiveness. It will consist of three main tasks:

Task 4.5.1 Review of Phase I Rhizobiology data and secondary literature (Year 1)

This task will synthesize the current state of knowledge on the rhizobial strains for each of the key N2Africa legumes. It will bring together experimental data from Phase I, the bridging grant, and published data from the literature. The University of Nairobi MIRCEN laboratory holds several of these isolates identified during Phase I. The N2Africa Rhizobium database lists over 800 isolates collected during Phase I, but the actual strains are held within individual laboratories.

Task 4.5.2 Sample nodules and isolate, authenticate, and evaluate new strains of rhizobia (Year 1-2)

Several countries have already accumulated large numbers of rhizobial isolates during Phase I or in Ethiopia through earlier research by partner organisations. N2Africa has allocated three PhDs to studies of rhizobia on common bean and cowpea. Aliyu Abullahi (Phase I PhD student) is working at Murdoch



University on groundnut rhizobia. Research to identify elite strains for common bean, cowpea, faba bean and groundnut does not need to be conducted in all countries. Where emphasis is placed will depend on the existing skills and capacity of the laboratories.

Where laboratories are already functioning well, samples should be collected from the nodules of the target host in uninoculated fields across a wide range of agro-ecologies. Nodules can be dried, in tubes with silica gel and cotton preferentially, and stored for later isolation of strains. Isolations will be made using standard protocols and all isolates must be authenticated on their relevant host (following Koch's postulates) in growth pouches, Leonard Jars or whatever locally-adapted method is available. Authenticated isolates will be allocated a reference number and added to the N2Africa database.

Effectiveness screening

Routine screening for effectiveness in the glasshouse can then be conducted. It is essential that tests on promising isolates are repeated at least twice before the strains are advanced for further testing. A proposal would be to test 20-40 rhizobial isolates in duplicate in each batch, retaining the best 5-10 isolates for further testing.

Essential treatments in all experiments: Uninoculated control, +N treatment, standard inoculant strain in each batch (e.g. USDA110, USDA 532c).

Stress tolerance screening

Where environmental stresses such as excessive salinity, drought, soil acidy and water logging are likely to constrain nitrogen fixation, there will be need to screen further effective strains for their ability to maintain performance under local conditions.

In that case, experimental treatments may include both standard stress tolerant and candidate strains, tested at increasing stress levels.

Task 4.5.3 Characterization of rhizobia (Year 2-4)

Subsets of rhizobial isolates will be advanced for molecular characterization. The precise methods and the sampling schemes need to be decided depending on more detailed discussions around specific research aims (e.g. characterising elite strains, soil population diversity studies).

V.2. Identify elite strains

This cluster aims to identify new elite rhizobium strains under 4.6. Identify elite rhizobium strains and inoculant formulations for beans, groundnut, and cowpea. It will consist of two main tasks:

Task 4.6.1 Test elite strains identified during Phase I and from other collections over multiple locations (Year 1-2) N.B. Trials will be established under Task 4.1.2 Agronomy Master Plan

This task will be focused on common bean, soybean and cowpea where a number of candidate strains have been identified. During Phase I strains were isolated in Kenya that performed better with common bean than CIAT899 and better than USDA110 with soybean trials (NAK strains). HB429 (an isolate from Ethiopia) showed excellent performance in almost all test locations and should be included in tests. The Savanna Agricultural Research Institute in Tamale, Ghana has reported promising results with cowpea strains isolated in Brazil through a collaborative project with EMBRAPA, and other promising cowpea strains are available in Ethiopia.

Standard treatments: Uninoculated control, +N treatment, recommended strain for each legume e.g. USDA 110, USDA 532c for soybean.

Inoculant formulations: Produced in sterile carriers to guarantee consistency and large numbers of cells on the seed or through substitution of current industry standard with candidate elite strains where commercial inoculants are available and collaboration exists.



Measurements

Standard soil analysis before planting, most probable numbers (MPNs) to count soil rhizobia using relevant host at planting.

Nodule assessments, nitrogen accumulation at physiological maturity, sampling of broad-leaved weeds and shoots for ^{15}N -natural abundance estimation of N₂-fixation.

Grain and stover yield at harvest - %N measured to estimate N accumulation.

Task 4.6.2 Evaluate stability of inoculant strains during repeated subculture and for suitability for inoculant use (Year 1-2)

This task will be conducted at IITA-Ibadan by the N2Africa microbiologist to describe the cultural characteristics and examine the suitability of the strains for repeated culture and stability of effectiveness through glasshouse plant testing.

V.3. Inoculant formulations

This cluster will evaluate alternative inoculant formulations under Activity 4.6. Identify elite rhizobium strains and inoculant formulations for beans, groundnut, and cowpea. It will consist of two main tasks:

Task 4.6.3 Compare formulations of existing inoculant products available on the market in Africa (Year 2)

This task will be conducted by the microbiologist at Ibadan. The aim is to compare the different carriers currently being used by the various producers in Africa (e.g. peat, lignite, bagasse, filter mud) and assess them for their ability to maintain large numbers of rhizobia and to deliver a large number of rhizobial cells onto each seed, depending on the recommended method or adhesive.

Task 4.6.4 Explore alternative formulations for inoculants (Year 3-4)

This task will explore alternative formulations, such as liquids or granules for delivering rhizobia into the field. This could be of particular interest in case elite strains are found which could be used for inoculation with groundnut and common bean.

V.4. Standard Operating Procedures cluster

This cluster will execute Activity 4.8. Develop standard operating procedures for the production, quality control and application of effective rhizobium inoculants. Tier 1 Countries will also participate in this cluster as described in Appendix 1. This cluster consists of two tasks:

Task 4.8.1 Collate and revise standard operating procedures (Year 1)

Working closely with COMPRO, standard operating procedures for the production, quality control and application of effective rhizobium inoculants will be revised and published.

Standard methods for quality control of inoculants were developed and summarised in earlier reports (see Report 19, Bala 2011). The Rhizobiology methods manual will be published in 2014 and will contain detailed methods for all procedures.

Task 4.8.2 Procedures for production and application of inoculants (Year 1-4)

Procedures for industrial production and application of inoculants vary among companies and are not the responsibility of N2Africa. Procedures for production and application of inoculants *for experimental use* will be developed in Year 1. To ensure the best possible delivery of large numbers of rhizobia onto the seed, gamma-sterilized peat sachets containing polymer will be used. As no gamma irradiation facilities are readily available in Core and Tier 1 Countries and different carriers are in use in various countries, Wageningen University is responsible for assembling and distributing these sterile sachets to all country Rhizobiology teams through their Country Coordinators. Capacity to produce inoculants for experimental use will be generated within each country, but for testing across countries these inoculants



containing the elite strains will be made centrally by the microbiologist at IITA-Ibadan using the accepted abovementioned sterile sachets.

Effectiveness of inoculant technology for enhanced legume production is dependent on availability of high quality legume inoculants. N2Africa will work together with the private sector to ensure high quality inoculants are produced and that quality is maintained to the point of use. Where inoculant quality fails to meet required standards, N2Africa will support industry research into inoculant formulation and packaging. The inoculant pilot plant in Nigeria will focus on replicable issues related to formulation, mixing, curing packaging and commercialization, so we will not attempt this in all countries.

Standard inoculant formulations are suitable for most legumes. If elite strains are identified for groundnut, research to identify appropriate inoculation methods will be warranted as the thin seed coat (testa) of groundnut makes it vulnerable to damage. In this case dry inoculation or a method to apply inoculant in the planting furrow has been shown to be advantageous.

Inoculant quality control along the supply chain

N2Africa is committed to ensuring the very best technologies reach the hands of smallholder farmers. Companies producing inoculants assure a certain standard of quality¹ as the inoculant leaves the factory. With a live product, such as a rhizobial inoculant it is essential to ensure the quality of the inoculant at the point of use in the farmer's field. It is likely that problems may arise if the inoculant is exposed to heat, or to too cold conditions, during storage and transport.

Studies will be done to follow the quality of inoculants and see how this changes along the supply chain. A number of different sampling strategies can be proposed depending on how the supply chain is organized.

We assume that the inoculant quality is known at point of manufacture. The starting point for assessing quality along the supply chain could be to test quality at the point of use in farmers' fields. If the quality is found to be good, then there is no need to trace back through the supply chain. If the quality is poor (unacceptable) at the point of use in farmers' fields more detailed investigation is warranted. An approach would be to work backwards up the supply chain from point of sale to point of manufacture, or to sample at all levels at the same time.

Once samples are taken for quality testing they must be transported to a laboratory for testing as quickly as possible so that problems/variability is not introduced. This requires samples to be kept in a cool box but not over-cooled or put in contact with ice as excessively low temperatures can also reduce the numbers of rhizobia surviving.

Some five levels can be identified along the supply chain at which the quality should be checked (Figure 2). The number of samples to be taken will increase down the chain towards the farmers' field to capture the variation in handling and use that will be introduced. The numbers of samples required at each level will depend on the numbers of packets being sold and used.

It's likely that most variability in inoculant quality will occur at the end user level due to unpredictable methods of handling from the acquisition of the sachets up to the effective seed inoculation at planting. However, this portion of variability could be the most difficult to capture. If we assume that most farmer's will be using a single sachet only for inoculation, then 10% of such sachets should provide a reasonable sampling rate to assess inoculant quality under farmer's conditions. Intact sachets can be collected in the field just before inoculation starts, with a clear description on how they were handled since being taken from the retailer (where and how long inoculant was kept, batch references, etc.). Still other situations such as "coated seed" ready for planting, "remnant inoculant" intended for further use, etc., are interesting cases that deserve attention (e.g. >10 samples each, depending on other factors as above).

¹ By quality, we mean the number of rhizobia delivered onto the seed at planting which is often influenced strongly by the number of contaminants in the inoculant when it leaves the factory.



Lower rates of sampling (e.g. 5% for retailers and <5% for whole sellers, \leq 1% for factories), could be applied upwards to actors on the value chain.

It is recommended that ahead of sampling, arrangements are made with the nearest equipped laboratory to handle quality control when local facilities are limited, in order to avoid improper storage of already collected samples.

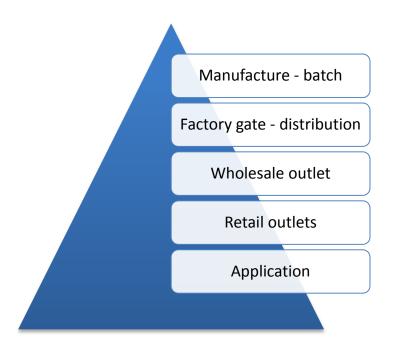


Figure 2. Recommended levels of sampling for assessing the quality of legume inoculants along the supply chain. Note the increasing number of samples to be taken down the chain from the factory towards field application

V.5. Links to long-term experiments in the Agronomy Master Plan

Aspects of rhizobium ecology will be included in the long-term experiments that are planned under the Agronomy Master Plan. Questions to be explored are whether or not repeated inoculation is needed, and the impacts of soil fertility management practices and crop sequences on survival of rhizobia in soil. This can be achieved by measurement of yield and nitrogen fixation responses in relevant treatments and monitoring of populations of compatible rhizobia using MPNs.

V. Approximate timing of cluster implementation

With efficient screening systems in the glasshouse we can move to multi-locational screening in the field within 2 years, and multi-locational screening across rainfall gradients can act as a substitute for multiple seasons. In some regions (e.g. Uganda and Northern Tanzania) there are two cropping seasons each year which also facilitates testing.



Activity	2014	2015	2016	2017	2018
4.5	G1	G2	G3		
4.6	G1	G2	G3		
4.8	G1				
4.1.2		G1	G2	G3	

Uni-modal areas (note: 'G' refers to 'Generation')

Bi-modal areas

Activity	20	14	20	15	2016	20)17	2	018
		G1	G2			 			
	[as relevant]								

VI. References

Bala, A., 2011. Quality assurance (QA) protocols based on African capacities and international existing standards developed, www.N2Africa.org, 16 pp. (Link)



Appendix 1 Tier 1 Rhizobiology Activities

Tier 1 Countries have a much narrower mandate in rhizobiology than the Core Countries that is largely built upon the laboratory upgrading funding the project's previous phase and the need to assure that legume inoculants reaching farmers in the Tier 1 countries meet a minimum standard. Necessary skills are in place as training was provided to microbiology technicians during Phase 1 and necessary facilities are in place (Figure 3). QC protocols are established (Bala, Milestone Report No. 19) and industry standards set (Woomer, Milestone Report 57). N2Africa seeks to establish project-wide standard operating procedures by end of 2015 (Activity 4.8).



Figure 3. A workstation suitable for routine quality assessment of inoculants in place at MIRCEN

The Tasks. Four Phase 2 activities specifically relate to rhizobiology, with three of them assigned as Lower Priority for Tier 1 countries, and one as High Priority. Partners are only required to report upon High Priority Activities but are encouraged to address other activities as they see fit.

Task 4.5. Isolate, authenticate, and evaluate new strains of rhizobia for the target legumes for high symbiotic effectiveness (Lower Priority). Each Tier 1 country assembled a Rhizobium Germplasm Collection during Phase 1 and contributed to the development of the N2Africa Rhizobium Database. Any new information relating to further strain characterization or additional strains collected should be entered into database. Relay any new findings through Tier 1 Rhizobiology Officer

Task 4.6. Identify elite rhizobium strains and inoculant formulations for beans, groundnut, and cowpea (Lower Priority). Main responsibility for this task falls upon the Rhizobiology Officer at IITA. A call was made for all Phase 1 best candidate strains identified by Tier 1 cooperators to be sent to IITA for further evaluation. Promising strains should also be sent to MIRCEN. Again, the N2Africa Rhizobium database will be updated based upon findings from this Task.

Task 4.7. Evaluate competitiveness and survival of introduced rhizobium strains as affected by M x E (Lower Priority). Some information on competition and strain interactions were collected during Phase 1 and from studies by other projects using N2Africa strains. These results should be relayed to the Tier 1 Rhizobiology Officer. Findings relating to competition and strain interactions from Core Countries from Task 4.6 will also be reported to Tier 1 countries by the Tier 1 Rhizobiology Officer

Task 4.8. Develop standard operating procedures (SOP) for the production, quality control (QC) and application of rhizobium inoculants (High Priority). This Task is mandatory among all Tier 1 partners. It is primarily intended to ensure that all inoculants reaching farmers through N2Africa are of sufficient quality to assure benefits from BNF. Partners should familiarize themselves with inoculant

quality testing and industry standards. N2Africa targeted Grade A of > 1 x 10^9 rhizobia and < 1x 10^6 contaminants per gram of inoculant (Table 1). This is determined on a sub-sample of 0.2 to 1% of test batches depending upon their size. Two basic QA strategies are employed, one for producers where testing is part of a larger product quality assurance campaign and another for periodic imports where batches are held until quality testing confirms compliance with industry standards. Emphasis is placed upon rapid assessment in order to intercept inferior inoculants from shelves, rather than

Table 1. Proposed industry standards among N2Africa partners and the project seal of approval (left).

Quality	Grade	Rhizobia	Contaminants
Legume	В	>10^8	<10^7
Product	A	>10^9	<10^6
AA T	AA 🔻	>10^9	nd
by N2Africa	AAA	>10^10	nd



retrospective improvement through hindsight (as often occurred in Phase 1). A general procedure follows:

- 1. A ten-fold dilution series to 10⁻⁷ is prepared from the inoculants. The lowest 10:1 dilution is extracted from 10 g of inoculant in 90 ml of diluent using a wrist action shaker and the following ten-fold dilutions agitated with a vortex mixer.
- 2. Three drops of 20 µL from the highest three dilutions is plated onto Congo Red Yeast Extract Mannitol Agar using the drop plate technique with three replicates for each dilution.
- All plates were incubated at 28°C for 3-7 days. Only the number of colonies growing in the range of 5 to 55 colonies are counted and colony forming units g⁻¹ were back calculated. Presence of contaminants is also recorded. Population sizes are calculated and reported using the N2Africa QC Utility.
- 4. QC findings should be reported through the Tier 1 Rhizobiology Leader and uploaded onto the N2Africa intranet.

The Inoculant QC Utility constructed in MS Excel and developed at MIRCEN is available to all as a mechanism to calculate results and share and store QC findings. Calculations are based on three "plate replicates" and two samples per batch. It calculates rhizobia at 10⁻⁷ and contaminants at 10⁻⁵ if present at that level. It provides CV of both operator and within batch error. User input requires adjustments for results falling within too numerous to count or and too few to count dilutions and also allows for correction of drop factor. Results are compiled into a short report summary (Table 2). The QC Utility is available from the N2Africa intranet. Table 2. Report from the N2Africa QC Utility.

Batch 1	mean	CV		
Rhizobium x 10^9				
Sample 1	3.83	27		
Sample 2	7.00	14		
Batch mean	5.42	41		
Contaminants x 10^6				
Sample 1	3.33	173		
Sample 2	6.67	115		
Batch mean	5.00	47		
Entered by: S. Kisamuli (13-7-13, MIRCEN)				

A strategy to launch standardized QC tests across all N2Africa countries is underway. Country Coordinators must get involved to identify lab supervisors and technicians operating in their respective countries. The availability of workstations and sampling frameworks must be confirmed and a modest allocation made to these activities from country budgets. The status of that strategy is described in Table 2 and completing this process that leads to competencies in inoculant quality assessment is the highest priority among Tier 1 countries. This Task is to be performed on all inoculants whether locally produced or imported. It should be consistent with any national biofertilizer regulations, or to assist in their formulation. Partners producing inoculants are also encouraged to formalize their production guidelines and submit them to the Tier 1 Rhizobiology Leader.