Improving legume inoculants and developing strategic alliances for their advancement

Milestones 3.3.2 and 3.4.5

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N2Africa

Putting nitrogen fixation to work for smallholder farmers in Africa

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1 Summary

N2Africa is expected to examine cost effective inoculant production methods including fermentation technologies, carrier selection, inoculant formulation and enhanced shelf life. These achievements were meant to occur in partnership with commercial partners, yet few emerged during the course of the program. Nonetheless, advances were made in terms of recognizing constraints to current production and offering solutions to them. In some cases, we assisted in automating some production steps, and offered alternatives in broth production approaches. Several candidate carriers were examined but few offered the promise of peat, and an excellent, but somewhat remote, peat deposit was discovered. While protocols to quality assessment were designed and adopted among N2Africa partners, they remain questionable as they are neither based upon plant infection nor strain identification. The project also sought to formalize strategic alliances between private sector and research centres for inoculant production and use. To the extent that private and parastatal inoculant production exists, this goal was achieved. A matrix of sectors (government, commercial and international agency) x inoculant production functions (rhizobium collection and evaluation, inoculant product formulation, manufacture, standards, use, regulation and trade) was devised to identify opportunities for better collaboration in the promotion of legume inoculants in Africa. Featuring predominantly within this matrix is the need for national programs to continue their rhizobium culture collections, the call for regulators to establish and enforce quality standards for inoculants to protect customers from inferior products, the responsibility of the public sector to develop new inoculant products and comply with industry standards and the importance of extension agents and farm liaison specialists to include inoculant handling and use in rural development programs. Finally, six recommendations are raised that lead to better inoculants and more effective partnership around this goal in the future.

2 Background

This report combines two related milestones. MS 3.3.2 relates to the status and development of "cost effective inoculant production methods including fermentation technologies, carrier selection, inoculant formulation and enhanced shelf life". It requires that we document and evaluate current inoculant production technologies and propose means for improving their application in Africa. This milestone was originally due by October 2011, but owing to a paucity of, and delays in developing inoculant production among N2Africa countries (MS 3.4.3), its report was delayed until the program's end. Several recent developments have occurred that now make this milestone more timely. The BIOFIX factory in Nakuru, Kenya has operated at commercial scale for almost four years and has had opportunity to re-evaluate and improve its production and quality control methods. A similar process has also occurred within the parastatal production scheme in Zimbabwe. Pilot inoculant production is now underway in Malawi and Rwanda, and smaller, experimental production takes place at the Kalambo Agricultural Center near Bukavu, DR Congo. Ghana and Mozambique rely exclusively upon imported inoculants, and product testing was conducted. Moreover, the Business Incubation Platform at IITA HQ at Ibadan, Nigeria is currently constructing its Nodumax inoculant factory, and planning for that factory required that alternative production approaches be evaluated.

Also included within this report is Milestone 3.4.5 that serves to identify and formalize "strategic alliances between private sector and research centres for inoculant production and use". While initially envisaged as a separate milestone, and indeed part of a separate program Activity (3.4. Expand and upgrade inoculant production capacity in sub-Saharan Africa), it is actually the mechanism through which improvement of legume inoculants becomes systematized and institutionalized. We have expanded the scope of this milestone to include the roles and interactions between the national public and private sectors, and their promotion through international agency (e.g. N2Africa).

To some extent, elements of this milestone were introduced in earlier reports. MS 3.2.1 (Bala et al. 2011) describes the distribution of internationally-recognized industry standard strains to serve as comparison to the candidate elite strains identified by N2Africa through MS 3.1.4 (Woomer at al. 2013). Quality control protocols were established in MS 3.3.1 (Bala et al. 2011) and inoculant standards and grades proposed in 2013 (Woomer, 2013). Background information was provided on inoculant production in MS 3.4.1 (Bala, 2011) that led to meeting and then exceeding inoculant distribution targets set by the program (MS 3.4.3). Each of these Rhizobiology milestone reports helped set the stage for this terminal report that describes how inoculants can be better manufactured, and those responsible for assuring this goal is achieved. This report serves to identify follow up activities for the planned next phase of the N2Africa Program (2014-2018).

3 Inoculant production strategies

Legume inoculant is key to maximizing the BNF of cultivated legumes, especially those cultivated away from their origins. These inoculants contain elite rhizobia bacteria that are coated onto legume seed before planting, or placed into their close proximity (FAO 1984). The inoculants promoted by N2Africa are solid formulation and intended for adhesion to seeds prior to hand planting. Other formulations exist including liquids and granules, each with their own intended uses. Liquids are useful under mechanized systems, and dribbled onto planted rows during or after planting (Tittabutr et al. 2007). Granules allow for greater numbers of inoculant rhizobia to be applied without closely interacting with seed chemicals, particularly fungicides (Lupwayi et al., 2006). Worldwide, about 2000 t of inoculant is produced annually worth US$50 million, sufficient to inoculate 20 million ha of legumes, especially in the soyabean production areas of Argentina, Brazil and USA (Herridge et al. 2002). Africa accounts for a very small portion of inoculant manufacture and use, but inoculants remain central to plans for legume intensification and Integrated Soil Fertility Management within the continent (Sanginga and Woomer 2009). Among the solid formulation, carrier based inoculants, two basic production approaches are available, 1) broth injection of prepackaged carriers and 2) bulk mixing of carrier and broth followed by curing and packaging (Figure 3.1).

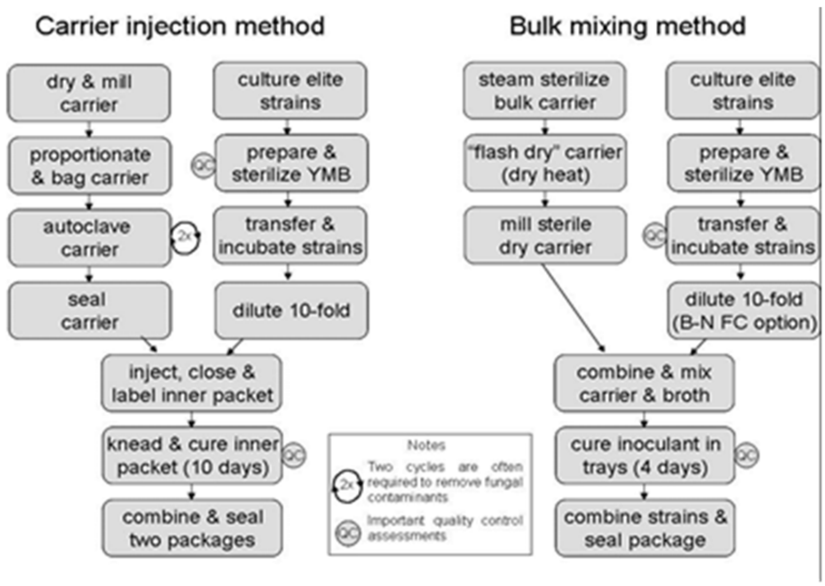

Figure 3.1: Comparison of the carrier injection and bulk mixing inoculant production processes

3.1 Carrier packaging and injection

This production method requires pre-packaged sterile carriers, a broth injection delivery system, and the use of plastic bags with gas exchange. It results from up-scaled laboratory procedures in that individual inoculant packages are handled through several processing steps (Figure 3.1). It is the easier approach to initiate, but the more difficult to automate. Carrier injection has several workstations devoted to 1) carrier bulk storage and preparation, 2) production of starter cultures 3) larger-scale fermentation of broth cultures, 4) broth injection into sterilized carrier, 5) mixing of broth and carrier, either manually or by tumbling, 6) curing of packets in a warm room and 7) combining inner "cured" bags packaging. Its main advantages are greater purity, smaller more controlled batches and ease in entry into other biofertilizer products. Its disadvantages include requirement for inner and outer bag, repeated handling of each package, and that the product size (weight) is pre-determined from the onset of carrier bagging. The reliance upon manual processing results in making this production approach difficult to automate, but inner and outer bagging permits the addition of other items, such as detailed instructions or proportionately bagged adhesive, prior to sealing. All of the inoculants produced by N2Africa and its partners, whether commercial, pilot or experiment, rely upon this basic production approach.

Carrier preparation involves obtaining bulk material, its cleaning, drying and milling to about 250 μm, packaging into plastic containers with gas exchange properties, and their sterilization prior to injection. This workspace is "dirty", should be physically removed from the microbiology facilities and be well ventilated. The fermentation area room includes several fermentors, injection hood and a large "massaging" table for mixing carrier and rhizobial broth. Prior to injection, broth may be mixed with liquid media at least 10:1 as a means to increase production as this better positions rhizobia to increase during the earlier stage of curing. Injected, mixed packets are then taken for curing, sealing and packing. The curing area is maintained at 28 ºC, consists of numerous shelves and must be sufficient to contain at least 10 days of production. Product is then boxed and best stored under cool conditions (e.g. 13 to 16 ºC. This design permits ready flow of materials to finished product as raw carrier material enters through the back of the factory and is processed and sterilized well away from microbiological activities, and finished product exits through the other end. This approach relies upon proven technologies and equipment from several decades ago, and tends to substitute labour for more advanced machinery, which in turn reduces its productive capacity.

3.2 Bulk mixing and packaging

An alternative approach is available that handles materials in bulk, dries and grinds carrier at a larger scale and requires less on hand labour (Figure 3.1). These processes were developed in the US mid-west to accommodate massive seasonal demand for soyabean inoculants. Briefly, air dried carrier is flash dried at 600 ºC in a rotary drier, and then ground to about 250 µm. It is placed in a rotary mixer and combined with rhizobial broth, and then cured in large trays. Afterward, it is single bagged for sale.

This approach offers several advantages over injection. Most importantly, packages are handled only once and single bagged, allowing for mixed strain inoculants and packaging on demand. It is more labour efficient as workers operate large equipment rather than manipulate individual bags. Greater gas exchange in trays allows for more rapid curing. Its main disadvantage is greater exposure to contamination and the product cannot be marketed as pure. Another disadvantage is that broth cultures may be diluted prior to combination with non-sterile carrier. Also start up is more expensive because rotary dryers, grinders and mixers are larger equipment than utilized in carrier injection. Another risk is that curing in large trays may result in poor aeration, allowing for competitive advantage to contaminants. Covering of trays as a countermeasure to contaminant entry further reduces aeration and increases curing interval. Despite these risks, bulk production of inoculants appears the better option in factory operations targeting large markets. This production approach is readily adapted to automated packaging using commercially available form-fill-and-seal equipment, and its fine powders flow nearly as well as liquid in volumetric allocation.

3.3 A “Modular Approach”

A unique opportunity is assuming a modular approach, with separate operations for carrier preparation, broth preparation and mixing/packing (Figure 3.2). Each of these three operations may be regarded as a business in itself. Carrier preparation is best conducted near its source, or near commercial sterilization services. The best carriers are peat (Herridge et al. 2002), and the best peat occurs in northern climates. One important supplier of finely ground peat is BioAPT from American Peat Technologies, Atkin, Minnesota, USA) that markets its product in one ton sacks for about $960. Rhizobia may also be produced or purchased in bulk as filter concentrates. Basically, the cells produced in a 1000 l fermentor may be concentrated to as little as one litre and then diluted immediately before mixing. Bio-Next of Wichita, Kansas sells units of 10^{15} cells for only $450, a price that factories in Africa would find difficult to match. Only 1 ml of these concentrates is added per litre of diluent prior to injection or mixing. Rhizobial filter concentrates must be shipped under refrigeration, so shipping and importation must be arranged in advance. Bags and adhesives cost about $0.14 per unit and materials may be combined for $0.12 per unit resulting in a production cost of $7490 for 20,000 units, or $038 per 100-g

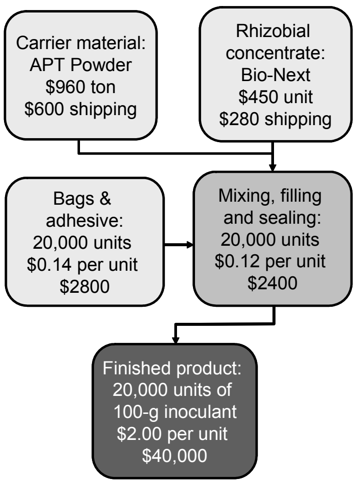

Figure 3.2: Projected materials and processing costs of non-sterile inoculant using the modular product approach

packet worth $2.00 wholesale. This projection is simplistic as it does not include marketing and distribution costs, purchase and depreciation of equipment or return of expired stock, but it does suggest that this is a promising production system for a non-sterile peat-based inoculant.

4 Inoculant quality assurance

Quality assurance is designed to protect legume farmers from inferior products because one cannot judge an inoculant product at the time of purchase. Quality control involves testing inoculants for compliance with industry standards at various times of their production and shelf live. This evaluation includes the numbers of rhizobia, presence of pathogens and the amount within the package (Herridge et al. 2002). Reports on the quality of inoculants produced throughout the tropics (Thompson 1984; Singleton et al. 1997) indicate that between 50% to 90% failed to meet industry standards. Furthermore, the numbers of rhizobia in inoculants are often inversely related to the numbers of contaminants. Indeed, there is need for establishment and compliance of inoculant quality standards of inoculants produced by the private or public sectors, and enforcement of standards should operate independently. Among the countries where N2Africa is active, however, these standards either do not exist or are voluntary, although Ghana and Kenya are in the process of building these standards into law. More information on the quality assurance of inoculants within N2Africa appears within Milestone 3.3.3 report (Woomer 2013).

Table 4.1: BIOFIX inoculant quality over six month intervals in Kenya[1]

Recovery location	batch no.	expiry month	Rhizobia (CV) $\times 10^9$ g^{-1} (%)	Contaminants (CV) $\times 10^6$ g^{-1} (%)
Factory curing shelf	four S batches[2]	-6	7.1 (24%)	2.4 (36%)
Stockist refrigerator	07021302S	-1	3.9 (33%)	98 (23%)
Stockist back room	31071202S	+6	2.7 (88%)	53 (27%)
Stockist back room[3]	13031202S	+12	2.6 (14%)	123 (9%)

[1] Inoculants with 6 month expiry date. [2] Mean of four batches (24031202S, 14041202S, 02031202S and 130812023S) reported by MIRCEN in July 2012. [3] Inoculants not returned or disposed as they were part of earlier test marketing activity.

The N2Africa Program was confronted by the lack of existing quality standards of legume inoculants and enforcement. First, options for measuring quality were examined, and the drop plate technique on Congo Red YMA was identified as most expedient (Bala 2011) and training in this and other techniques offered. Next standards were lacking so a widely accepted minimum of 1 x 10^9 adopted. Finally differences in how quality control programs should be conducted between countries that produce inoculants at commercial (Kenya and Zimbabwe) and larger pilot scales (Malawi and Rwanda) and those that rely upon periodic importation (DR Congo, Ghana, Mozambique and Nigeria). Because the quality of any product is the responsibility of the manufacturer, internal quality control practice was emphasized as equally important within MEA Ltd. and SPRL, the producers in Kenya and Zimbabwe, respectively. One challenge that was not entirely met involves the design and implementation of routine quality control procedures that permitted inferior batches to be intercepted before they were released to supply chains. The situation for imported inoculants was different in that with proper planning arriving product can be held for testing, but when it is late there was a hurry to distribute it with other inputs in advance of the rains. While we have developed the microbiological capacity of quality control testing of inoculants in five countries (DR Congo, Ghana, Malawi, Nigeria, and Rwanda), these have not yet become formalized into routine operations. An exception is Kenya where independent testing of BIOFIX is routinely conducted and the results entered into a utility software and distributed among interested parties (Table 4.1).

Results from Most Probable Number (MPN) counts across several countries and hosts suggests that common greenhouse conditions in the tropics were not suitable for reliable quality assessment. In this case, the results of 87 MPNs conducted using soils on two hosts and in three countries were subjected to Stevens (1957) Range of Transition (ROT) that tests the probability of obtaining a given result based upon the number of steps between entirely positive and entirely negative dilutions. Overall, there was an 87% probability that these greenhouse MPNs yield non-acceptable results (Table 4.2). Whether this is due to high temperatures, contamination of technician error cannot be determined but clearly improved technique and conditions must be achieved before inoculant quality may be based upon MPN. his conclusion is contrasted by others insistence that plant infection counts be the basis for inoculant quality assurance (Thompson 1984; Herridge et al. 2002) because it confirms the presence of root nodule bacteria rather than just milky white colonies on YMA plates. Clearly the role of MPNs in quality assurance among emerging inoculant producers remains another unresolved issue.

Table 4.2: The probability of compliance with underlying assumptions of MPNs conducted under greenhouse conditions in three countries repeatedly suggests failed test of technique.[1]

Country	host	n	ROT	Compliance *p*
DR Congo	bean	17	3.9	0.04
Ghana	soyabean	50	2.7	0.13
Kenya	bean	22	2.6	0.22
Overall		89	3.1	0.13

[1] six-step, ten-fold dilution series with three or four units per dilution level, based on Stevens (1957) and Woomer (1994).

There are concerns over the level of regulatory supervision and their developmental consequences. Aligning industry standards with current technical competence in inoculant production reinforces sustainable product supply as manufacturers are not discouraged from production. Once production is in place opportunity exists for steady product improvement Importation poses a hazard if inoculants are not readily inspected and then released. Holding product under unsuitable storage conditions can lead to quality failure and result in loss of the product. Along similar lines, establishing realistic expiry dates for inoculants is important, and quality control information must be assembled along the entire supply chain (Table 4.1). Results from our cooperators suggest that unsold inoculants should not be carried over to the next season as product quality becomes compromised, even when stored under refrigeration. Australia has adopted a system where the expiry period of inoculants is related to their levels of contaminants, and N2Africa should explore the same approach. Finally, based upon tested shelf life and responsible handling by local stockists, a mutually beneficial return policy for expired stock must be established to reduce risks borne by local retailers.

5 Inoculant production among N2Africa countries

5.1 DR Congo

Table 5.1: Chemical analysis of Walungu peat from DR Congo

Description: dark brown peat

Carbon:	49%
pH:	4.5
Nitrogen:	1%
Potassium:	0.9 cmol/kg
Phosphorus:	18 ppm

The Kalambo Agricultural Center near Bukavu produces an experimental inoculant it calls RHIZOFIX, a brand name currently in use by both Becker Underwood and Ortho. Production started in August 2013 and it is made for use with bean or soyabean using strains USDA 2667 or USDA 110, respectively. It is packaged into 20 and 50 g using Walungu peat as a carrier. The peat is reduced to 212 μm in a two-step procedure, then neutralized, bagged and autoclaved. Broth culture is prepared in 500 ml flasks that requires 12-14 days because there is no electrical power beyond working hours. Packets are injected by hand. The 50-g packet is intended for five kg of seed and is sold for about $2.20. The quality target is 1×10^9 cells per gram but quality control procedures are not yet finalized. Sugar is the recommended adhesive. The next steps for product improvement are 1) identifying a better strain for bean, 2) acquiring a larger autoclave e.g. 160 litres) and rotary shaker to scale up production and 3) organizing a field campaign in eastern Kivu to promote product efficacy. We emphasize that inoculant production at Kalambo was initiated to backstop research and its expanded operations are a recent development. The Walungu peat has excellent properties as a carrier (Table 5.1) and opportunity exists for its commercial recovery and processing.

5.2 Ghana

The main inoculants used in Ghana were imported from Legume Technology (UK). This company was established in 2000 and manufactures a range of inoculants for legumes in both the agricultural and the home gardening markets. Legume Fix inoculants were imported as packages of 200 and 700 g intended for lots of 50 to 175 kg of seed. The product proved effective on soyabean but its large package size forced groups of farmers to share inoculant, awkward both in terms of grassroots coordination and developing marketing channels. The company intends to pursue commercial sales in Ghana and several N2Africa countries but presently lacks distribution partners able to negotiate import permission and enter into existing supply chains.

5.3 Kenya

BIOFIX is a commercial legume inoculant manufactured and distributed by MEA Ltd (Figure 5.1). It is a registered trademark of the University of Nairobi and produced and sold under license from the University of Nairobi. Separate inoculants are sold for soyabean, bean, groundnut, pea, green gram and pigeon pea using internationally recognized rhizobia (e.g. USDA 110 for soyabean, CIAT 899 for bean). Commercial production started in late 2009 and reached 10.5 tons over the past year. Package sizes range from 10 to 150 g with recommended doses of 10 g inoculant per one kg seed. The carrier is filter mud from sugarcane pressing that is milled and autoclaved. Broth is produced in five litre flasks connected in series with filtered air (Figure 5.2) that is auto-injected and rotary mixed, then cured at room temperature for about 10 days. It is packaged in an aluminium foil laminate to protect the product and distributed in cardboard boxes of 150 units. The price of a 100-g packet is about $2.20. Quality standards are $> 1 \times 10^9$ rhizobia and $< 1 \times 10^6$ contaminants, although the latter target is often exceeded. Independent quality assessment is conducted by the Nairobi MIRCEN under agreement with KEPHIS. The adhesive is gum arabic packed separately and included within the package that is diluted to a 10% solution and applied at about 20-30 ml per kg seed. BIOFIX is intended for use in conjunction with Sympal, a fertilizer blend (0-24-16 + Ca, S, Mg, Zn) designed for symbiotic legumes.

Large amounts of BIOFIX inoculants for soyabean are also marketed in Zambia. The planned steps for improvement of BIOFIX by MEA Ltd. include 1) expanded milling capacity of carrier, 2) increasing the effectiveness of carrier sterilization, 3) fully automating product filling and sealing and 4) more widely register the product in Africa countries.

Figure 5.1: Packaging of BIOFIX in temperature-reducing aluminum laminate includes labelling compliant with recommended international standards

Figure 5.2: Arrangement of flasks containing rhizobial broth serviced a common filtered air pump

5.4 Malawi

An inoculant has been produced at pilot scale by the Chitedze Agricultural Center for many years. The product is tentatively labeled "Chitedze Inoculant" and is only intended for use with soyabean. It is packaged in 50 g packets sold for $0.60 each with about 15,000 produced over the past year. The carrier is sugarcane filter mud that is milled with a laboratory grinder, packaged and steam sterilized. Broth is produced using YMB in 5 L glass flasks, and then manually injected and hand kneaded. The final product cured on shelves for up to 6 weeks, then sold from the factory gate. Quality is assessed both in broth culture and by plate counts of cured inoculants. Its target minimum content is 1.0×10^9 rhizobia per g but contaminants are not considered. At the time this report was prepared there were no quality control results available but some users have reported inconsistent results. The recommended adhesive is sugar but is not included in the package.

The producer has several plans for improvement. USDA 110 and N2Africa elite strains will be tested against and possibly substituted for the local strain already in use. Packaging will be improved and a more commercial brand name identified. The product will then be registered with the Malawi Bureau of

Standards and a commercial partner identified for planned privatization. From the technical side, milling must be improved using larger equipment and the carrier must be better sterilized. Because there is only one growing season per year in Malawi, facilities must be obtained for mid-term storage. The laboratory is also formalizing national quality standards and developing means to have imported inoculants evaluated as well.

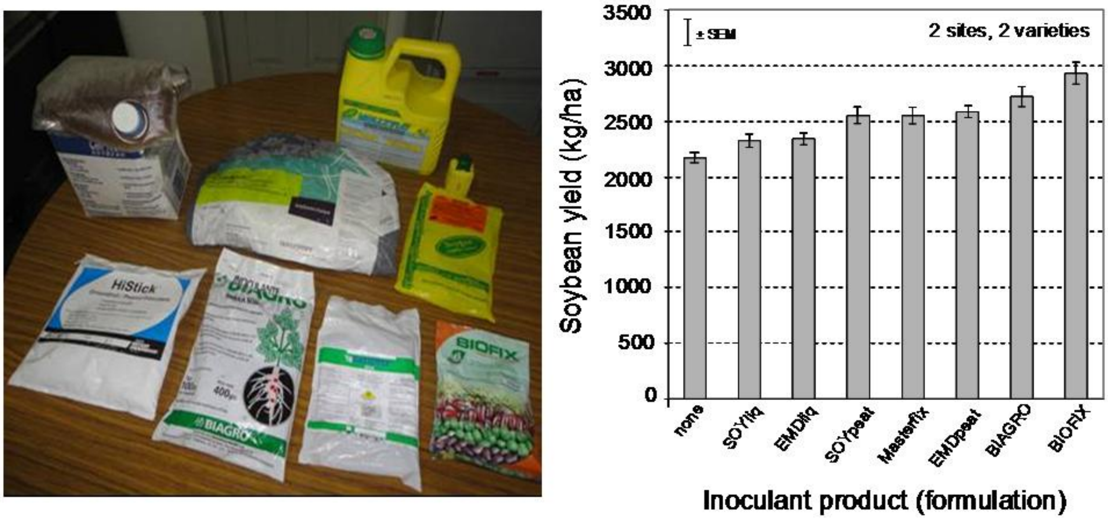

Figure 5.3: A comparison of legume inoculant products imported into Mozambique

5.5 Mozambique

No inoculants are produced in Mozambique, experimental or otherwise, instead it relies upon an assortment of imported products. These inoculants include both liquid and solid formulations that were tested across two sites (Ruace and Sussundenga) and two popular varieties of soyabean (Storm and TGX-1904) (Figure 5.3). Even the non-inoculated management performs well (> 2 t ha^{-1}) but is improved by all the inoculants. Liquid formulations did not perform as well as their solid counterparts, increasing yield by 8% and 18% respectively. The best performing inoculant was BIOFIX from Kenya that increased nodulation by 75% and improved yield by 35%. A weakness in this study is that the contents of the inoculants (e.g. strains and cell concentrations) were not examined and if Mozambique continues to import inoculants it should further develop capacities to evaluate their qualities.

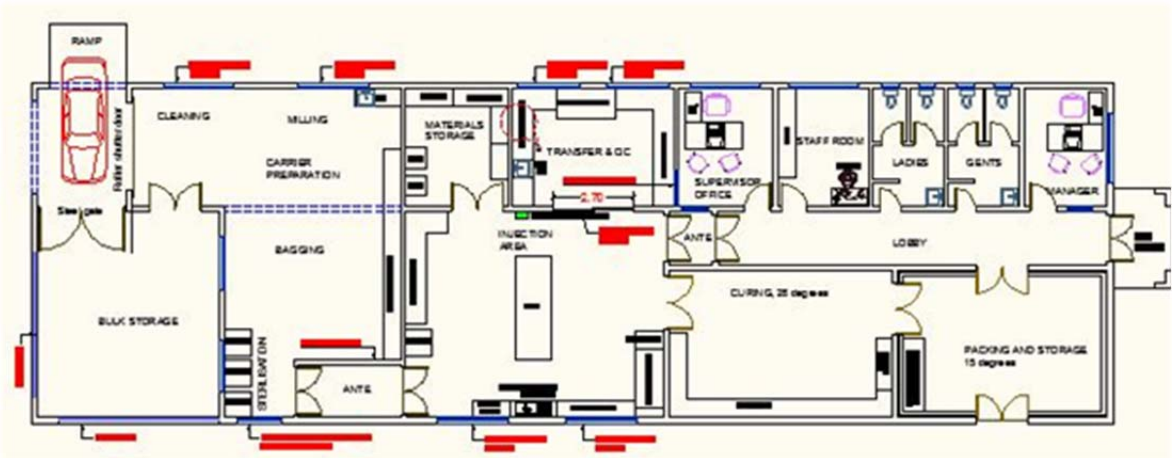

Figure 5.4: Floor plan of the inoculant factory under construction at IITA, Nigeria

5.6 Nigeria

An inoculant manufacturing plant is under construction at IITA, Ibadan (Figure 5.4) as part of the larger Business Incubation Platform. The purpose of the factory is not only to produce inoculants for sale, but to demonstrate their economic viability. to private sector investors and to provide incentives and training for their future operations. Production will begin in December 2013 under the brand name NoduMax sold in 100-g packets and intended for soyabean. Production targets are 24 tons in the first year increasing thereafter to 30 tons per year. A three-step approach is being followed; 1) development of the core facility, 2) operation and recordkeeping of the core facility and 3) iterative improvement of core facility production, leading to replication and adoption by the private sector. Initially the factory will examine carrier injection, bulk mixing and modular production approaches as well as evaluate a granular product. Several production questions must be resolved including improved options for carrier selection and sterilization, choice of inoculant strains and quick substitution of manual with automated operations. Operations may interface with the commercial gamma irradiator near Abuja. The factory will at first monitor its own product quality with help of the N2Africa Program, and part of its business incubation includes continued product assurance after the factory is replicated ten-fold by the private sector. A preliminary market analysis suggests that Nigeria requires about 300 tons of soyabean inoculant per year for 30,000 tons of seed grown on 500,000 ha (Table 5.2). Inoculation will result in an additional 150,000 tons of soyabeans worth $93 million per year.

Table 5.2: Projection of inoculant requirements and profitability in Nigeria. The Nodumax factory targets 10% of this market

Parameter	value
soyabean yield (t/ha)	1.20
soyabean area (ha)	500000
soyabean production (t)	600000
inoculation response (%)	25
yield increase (t)	150000
soyabean price ($/t)	$620
increased soyabean value ($)	$93,000,000
seed rate/ha (kg)	60
total seed (t)	30000
inoculation rate (kg/kg)	0.01
inoculant required (kg)	300000
100 g packets required	3000000
cost per 100 g packet ($)	2.50
total value of inoculant ($)	$7,500,000
production costs ($)	$2,460,000
manufacturer's profit ($)	$1,860,000
retail mark up ($)	$3,180,000
inoculant benefit:cost	12.4

5.7 Rwanda

The Rwanda Agricultural Bureau (RAB) produces inoculant intended for bean and soyabean at its Ribona station. Production of this pilot product started in 2011 using standard strains (CIAT 899, USDA 110) and a local isolate (UMR 1957). Inoculant is sold in 80 g packets costing $0.80 intended for 7 kg of seed with about 44.500 units sold over the past year (= 3.6 tons). Independent product testing indicates that it falls slightly below international standards (Table 5.3), but an average batch still provides about 1.0 x 10^6 rhizobia per seed when directions are followed (assuming seed of 0.2 g). The laboratory reports that its product regularly exceeds 1 x 10^9 cells. The inoculant relies upon a local peat carrier that is milled (200 mesh), neutralized (to pH 6.5) and autoclaved. The peat is slightly silty and this may account for its low numbers of rhizobia. Broth is cultured in either large flasks or a small fermentor and then manually injected into autoclaved bags of peat, and then cured at room temperature. Sugar is the recommended adhesive. The laboratory has several planned steps for improving its product including 1) accessing better quality peat and better sterilization, 2) semi-automating its injecting and packaging, 3) improving the timing of quality control services, 4) expanding the products shelf life and 5) linking

Table 5.3: Independent quality assessment of RAB inoculants by MIRCEN suggests that the pilot product falls slightly below international standards

Product	Rhizobium	Contaminants
	x 10^9 (CV)	x 10^6 (CV)
Soyabean	0.16 (92%)	4.2 (28%)
Soyabean	0.33 (23%)	2.5 (171%)
Bean	0.87 (133%)	3.3 (71%)
Bean	0.40 (25%)	n.a.
Overall	0.44 x 10^9	3.3 x 10^6

directly to agrodealers. When contacted, the pilot facility expressed no interest in privatizing its operations.

5.8 Zimbabwe

Legume inoculants are produced by the Soil Productivity Research Laboratory (SPRL) at Marondera. The factory started in 1962 to produce small amounts of inoculants for pasture legumes and expanded to grain legumes in 1967. Currently it produces nine inoculants for soyabean, common bean, groundnut, cowpea, *Crotalaria*, *Lucerne* (alfalfa), pea, *Calliandra* and *Leucaena*. The strains in use by the factory are USDA 110 (soyabean), CIAT899 (bean), MAR 1510 (cowpea, groundnut) and others. The product is sold in 100 g sachets for US $5 each. Production for the 2012-2013 growing season was 86,300 units, 93% of which were intended for soyabean.

Inoculant production at the Marondera factory represents a practical adaptation of the carrier injection method. First crude sugarcane bagasse is sieved to remove coarse materials, and the fine powder combined with salts, lime and water in a cement mixer. The moist carrier mixture is weighed into high density polyethylene bags, and heat sealed leaving a 1.5 cm wide vent at a top corner. A drinking straw is inserted into the opening to permit gas exchange during autoclaving (121°C for 30 min.). Prior to sterilization, the bags packs are left overnight at room temperature to allow fungal and bacterial spores in the bagasse to germinate. After autoclaving the bags are carefully sealed and stored until needed, with random samples are tested for sterility on YMA plates.

To reduce production costs, rhizobia are cultured in standard medium that substitute 0.7% sucrose for 1% mannitol. Batches of 1.5 1 are inoculated with 10 ml of a pure broth culture and aerated by bubbling sterilized compressed air for 2-4 days at 28^{o}C. The broth is further diluted five-fold prior to injection in an autoclaved yeast extract-sucrose solution (1.3 g yeast extract and 23 g cane sugar per litre). Then only 15 ml of this mixture is injected into each sterile carrier sachet using an auto-syringe and the needle hole sealed with an adhesive label bearing the batch number, the intended legume and the expiry date. In this way, each diluted flask represents a production batch of 500 units. The inoculated packs are cured at 28°C for 14 days, after which they are visually graded for fungal contamination and 2 sample packs randomly selected from each batch and tested at the laboratory. If the results are satisfactory, they are stored at 4°C until dispatch to commercial distributors.

Rigorous quality control testing is in place. Sampling stages include mother cultures, mature broth, unmixed carrier and the finished product after curing. Each stage is checked for both rhizobia and contaminants with product standards of at least 10^9 rhizobia and less than 10^6 contaminants per g. Production strains are also checked annually for nodulation and N-fixation using Leonard jars in a glasshouse.

Sugar is the recommended adhesive, but this is not included within the product. Inoculants are sold by distributors approved by the Ministry of Lands and Agriculture. These are primarily farmer's cooperatives and seed companies with branches throughout the country. Provincial and district agricultural offices also act as distributors. Boxes of inoculant are dispatched overnight by road, ensuring that they are kept cool until received by the selling agents. Plans for improvement of this process include automating the packing of bagasse as this labour-intensive operation is presently expensive and rate limiting.

6 Improving inoculant production

Several options are available to improve legume inoculants, whether their production operate at the commercial, pilot or experimental scales. Indeed, all stages of the production process are subject to improvement, with the key being to address rate-limiting or contamination-prone operations in a stepwise fashion.

1. The choice of carrier material greatly influences how it is processed and the quality of the final inoculant product. Acceptable carriers must have high surface area, be easier to hydrate and available in large quantities at a low price. Search for carriers has not proven particularly successful. Rice husks are very difficult to process. Coconut husk (coir) and biochar offer poor habitat for rhizobia (Figure 6.1: Comparison of biochar, coir and North American peat as carriers of rhizobia (I. Balume, MSc Thesis)). Sugar cane bagasse, and filter mud from sugar mills are in use, but appear inferior to peat. But peat is rare in the tropics, and the best deposits are poorly remote (Walungu in eastern DRC) or legally protected (Kikuyu, Kenya).

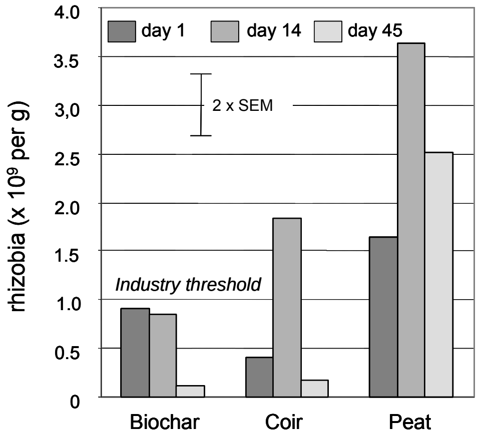

Figure 6.1: Comparison of biochar, coir and North American peat as carriers of rhizobia (I. Balume, MSc Thesis)

2. Sterilization of carrier as also proved difficult. Reliance upon even large capacity autoclaves is technically feasible but difficult to scale up as over-packed chambers yield poor results. Options widely available in developed countries, electron beam and gamma irradiation, are not available in Sub-Saharan Africa with the exception of a facility near Abuja, Nigeria. Even the simple use of flash drying using a rotating drum (e.g. alfalfa or wood chip desiccator), a widely acknowledged "trade secret" was not fully appreciated in Africa until recently (Tom Wacak, personal communication). More work must be done in this area, for example inoculant producers may contract carrier providers using facilities located in close proximity to key sources that process and bag materials ready for injection or mixing. When to seal carrier packages in conjunction with autoclaving is also an issue. Pre-sealing limits contact with pressurized steam while after-sealing is messy, and autoclaved plastics are more difficult to handle.
3. Broth preparation relies upon up-scaled laboratory approaches where rhizobia are raised in large, aerated flasks, each producing four to eight litres of injectable broth (e.g. MEA Ltd., Kenya). This approach allows for adjustable production on demand and reduces the consequences of "batch" failure (since each batch is smaller), but increases dependence on autoclaves and technician labour, and greatly complicates quality inspection (because more batches must be monitored). An alternative is the installation of smaller industrial fermenters with inbuilt heating elements that are able to produce 25 to 100 litres of broth in a single run (in use at RAB, Rwanda). Other operations that increase the efficiency of broth production is its ten-fold dilution immediately before injection and/or the substitution of analytical reagent-grade mannitol with lower-cost and more readily available glycerol or sucrose. The technical requirements and economic advantages of these innovations must be carefully examined before substituted for proven, simpler approaches.
4. Injection of the rhizobial broth into the sterile carrier and their mixing may also be improved. The current approach is for one technician to inject broth into bagged carrier and then immediately pass it to several labourers who place an ID sticker over the entry hole and massage each packet until broth and carrier are well mixed. This operation may be streamlined using auto-injectors and mechanical mixers or other more advanced broth delivery systems (Prem Warrior, personal communication). The choice of carrier materials greatly influences mixing requirement as some of the (best) carriers are hygrophobic (harder to wet). Overheating carrier during flash drying also renders material more difficult to wet. One option is to pre-wet materials prior to autoclaving.

5. Curing is an essential production step that offers opportunity for refinement. While curing, rhizobia increase in number as they colonize the moistened carrier, and then are slowly "hardened" as the inoculant dries to powder through its plastic bag that is permeable to water vapour. This process takes 10 to 14 days in small plastic bags depending on temperature and aeration and requires ample space to accommodate production volume over that interval. Packing too many curing packets onto insufficient shelf area delays the process and risks inferior product. Options for improvement include temperature and humidity controls to 28^{o}C and 70%, respectively. With bulk mixing, product is cured in large trays that reduce curing to only four days, but poses a greater risk of late contamination. Finally, placing curing racks on wheels allows them to move between workstations and then be tether packed" into a curing room.
6. Automated packaging and sealing is feasible but not yet in practice. Form-Fill-and-Seal equipment widely used in local food, cosmetic and seed industries have not yet been applied to commercial inoculant production by N2Africa partners. Fine, properly cured inoculant is a powder that "flows" in a manner similar to liquids and can be inexpensively dispensed using volumetric pistons (Heinz Hoben, personal communication). A widely available packaging device (Uplex FFS) costs about $25,000 and can pack and seal 40 to 50 units of 50 to 100 g per minute (BrazAfric Enterprises Ltd., Kenya).

The options for improving basic factory operations are preliminary, and somewhat generic, but as factory operations fall under examination, new and better ways of sterilizing, mixing and packing will certainly result in a better and less expensive product.

7 Strategic Alliances for Advancement of Inoculants

It is important that strategic alliances be formalized between public and private sectors in order for inoculant production capacity in Sub-Saharan Africa to improve and expand. The role of N2Africa and other international agencies with expertise in applied and developmental research is also important. In the Milestone 3.3.3 report (Woomer, 2013), a matrix table was introduced that described the relationships among the public sector, private business and international agents in the production, quality assurance and improvement of legume inoculants. This analysis forms the basis of Milestone 3.4.5 and is thus expanded within this report (Table 7.1). Briefly, eight functional elements are listed relating to rhizobium collection and evaluation, and to inoculant product formulation, manufacture, standards, use, regulation and trade. Then, the roles of three sectors, the public sector, the private sector and international agency within these elements, and their key interactions are described within N2Africa Program activities.

Table 7.1: Relationships among the public sector, private business and international agents in the production, quality assurance and improvement of legume inoculants

Function	Public Sector	Private Sector	International agency
Rhizobium			
collection & curation	Maintain national culture collections.	Access industry standards, maintain mother cultures.	Bio-prospect, initiate & coordinate national culture collections.
strain evaluation	Conduct routine strain evaluation, identify candidate elite strains.	Compare new elite strains to industry standards.	Identify, characterize and exchange elite strains.
Inoculant			
formulation	Support pilot facilities & access to product components.	Develop new formulations and products.	Assist in streamlining production costs and methods.
manufacture	Compile and release commodity statistics.	Produce inoculants in profitable, cost effective manner.	Evaluate and exchange different production approaches.
standards	Establish standards for labelling and contents.	Develop processes and competencies to comply with standards.	Compare standards & provide guidelines for compliance.
use	Conduct extension campaigns on inoculation.	Establish branded demonstrations & participate in shows.	Develop and translate extension materials on inoculant use.
regulation	Monitor product quality and report compliance.	Label product properly & conduct quality assurance.	Design protocols for product testing.
trade	Reduce obstacles to cross-border trade.	Develop distribution networks and product advertising.	Provide market information and policy support.

7.1 Rhizobial curation

Most N2Africa participants have initiated or greatly expanded rhizobial culture collections but the level of their institutionalization and maintenance beyond program lifetime is uncertain. Indeed, even the curation of these entire culture collections is questionable given that most lack the attributes of unique or elite strains (see MS 3.1.3, Woomer et al. 2013). What is most important is that these elite strains, those out-performing industry standard strains under African conditions, reach their intended targets, particularly established and nascent inoculant producers, and that these strains be available into the future. Commercial interests tend to maintain mother and working cultures, and to be reliant upon research for their choice and provision of cultures. Continuity in the supply of elite strains is an important role of international partners, and it is imperative that the program's unique and elite strains be assembled, more completely documented and entered into long term storage with provision to supply them to future interested parties. International partners serve to initiate and coordinate national culture collections and to assist in the exchange of strains. International institutes may also maintain specialized collections corresponding to their mandate crops or biodiversity preservation of the microsymbionts of rare or threatened hosts.

7.2 Strain evaluation and recommendation

Culture collections are routinely being tested, with new entries undergoing routine tests, and new tests being applied to better document the larger collection. These efforts should lead to strain recommendations for specific legumes, their varieties and even habitats that are then directed toward commercial interests. Inoculant producers will also test strains, but along different criteria aimed at product improvement. Evaluating strains' abilities to utilize lower cost carbon sources with faster growth rates (Hungria et al. 2001) may lead to reduced production costs. Identifying rhizobia resistant to commonly applied seed chemicals is another pathway to product improvement (Campo et al. 2009). Selecting for strains with weak saprophytic competence strengthens product demand (and protects indigenous soil biota). International agencies can further evaluate elite and unusual strains using state-of-the-art molecular techniques less available to national partners, International partners can also assist others in keeping abreast of changes in rhizobial taxonomy as this can obscure the identities of elite strains. One major crosscutting issue is the rhizobia associated with promiscuous and specifically nodulating soyabean, as they clearly belong to different sub-groupings (Abaidoo et al. 2000) but inoculant producers in Africa have not yet developed separate products for them.

7.3 Inoculant formulation and manufacture

Clearly it is for the private sector to lead in inoculant formulation and manufacture. Commercial interests are promoted through proprietary information and trade secrets so that they may remain competitive. At some point many "secrets" become common knowledge such as the use of alfalfa desiccators to flash dry and partially sterilize carriers, or the addition of adhesives (PVP, mineral oil) to powdered inoculants. Nonetheless, there are important roles for other parties in this process. Each new inoculant formulation is led by research including the reintroduction of liquid formulation inoculants (see Tittabutr et al. 2007) and their effective delivery (need reference), development of inoculant granules (Lupwayi et al. 2006), and the breakthrough approach of broth dilution prior to injection (Somasegaran, 1985). Within the African developmental context, the public sector may initiate pilot-scale inoculant production as has occurred in Malawi and Rwanda, although historical reservations exist over the ability of laboratory pilot operations to commercialize (Herridge et al. 2002). Certainly the licensing of BIOFIX from the University of Nairobi to MEA Ltd. and its subsequent success sets an impressive precedent (Wafullah 2013). International agencies can assist by evaluating different production approaches and conducting research that streamlines production costs and methods. It is also important to compile and distribute statistics on both national and international production and trade of inoculants at the national and international levels.

7.4 Inoculant use

While the formulation and manufacture of inoculants is led by the private sector, promotion and instruction on their use is shared between the private and public sectors. Commercial inoculant producers develop advertising campaigns around their products, and advance this information through input supply networks, but this approach is insufficient to reach poorer and more remote households. Agricultural extension, where it is effective, has an important role in advancing knowledge on the availability and use of legume inoculants as well. A similar role falls clearly within the mandate of international development organizations and their grassroots operations. N2Africa clearly demonstrated through its outreach activities and partnership arrangements that international agency also has an important role to play in developing extension information (Figure 7.1), initiating training of trainers, providing incentives for conducting technology demonstrations and field days, and arranging media attention around grain legume enterprise and BNF technologies. For example, the N2Africa extension publication "Biological Nitrogen Fixation and Grain Legume Enterprise" (Woomer 2010) first prepared in English for the ECA Impact Zone was later adapted to Southern and West Africa, and translated into five additional languages, an activity only possible through international agency. How responsibilities toward responsible inoculant product use are divided varies with country and setting, particularly the commercialization level of BNF technologies, but change ultimately occurs at the grassroots level, so focus must be placed upon farmers as technology adopters and farm input customers.

Figure 7.1: Pictorial guidelines on inoculation using the slurry technique developed for Lead Farmer training and used in wider extension programs

7.5 Inoculant standards, regulation and trade

This report deals primarily with technical issues relating to inoculant production and progress made by the N2Africa Program in this area. A recent milestone report (MS 3.3.3, Woomer 2013) describes inoculant standards worldwide and recommends similar quality targets for N2Africa partners. Two important milestone reports are in progress that examine the regulation and trade of rhizobia and inoculants. Milestone 3.5.1 presents a policy review of legume and inoculant regulation and cross-border trade, and Milestone 3.5.2 examines procedures for exchange of, and access to rhizobial strains across Africa, and readers are referred to these reports for more policy and trade information. Notwithstanding, a brief discussion on the relationships between inoculant standards and regulation as separate but related functions is in order.

It is for governments to establish standards for the labelling and contents of legume inoculants, the private sector to develop processes and competencies to comply with those standards and for international agencies to compare and advise upon those standards. Whether these standards are built into law, or established by regulatory bodies is subject to national conditions. Regulation may be regarded as a separate issue, as this serves to assure compliance with standards and ultimately to protect customers from inferior products. Whether product testing be complete (every batch produced or imported tested and results reported), periodic or spot-checked is again an issue of local concern (Thompson 1984), but producers and importers must be aware that failure to comply with standards will result in loss of goods and possible penalties. In this regards it is for government to monitor product quality and report compliance, the commercial sector to comply with labelling and product standards, and to extend genuine quality assurance and for international agents to design and test tools and protocols for product testing (Olsen et al. 1996).

8 Conclusions and recommendations

N2Africa sought to jumpstart inoculant production among its eight partner countries but had limited success during its four year operation. Commercial inoculant production in Kenya coincided with the launch of N2Africa and collaboration between the two has resulted in product improvement. N2Africa has invested in renewing equipment and supporting the long-established inoculant production plant in Zimbabwe which provided a reliable source of inoculants for BNF technology outreach in that country. Two partners in Malawi and Rwanda found opportunity to leverage program training and laboratory upgrading into development of pilot scale inoculant production and, with guidance from N2Africa, will hopefully transition their operations into self-sufficient commercial enterprise. A major, but late emerging development is the planned Nodumax inoculant factory, part of the new IITA Business Incubation Platform, that is expected to commence production in December 3013.

The scale of inoculant production was also examined. Current production is based upon scaled up laboratory approaches where carrier is bagged, sterilized, injected and cured. This production method can produce quality inoculants but relies heavily upon repeated manual operations and is difficult to automate. Two alternatives are presented, one that relies upon bulk mixing and curing in trays, and another that examines the combination of modular enterprises. Ultimately, commercial inoculant production must become more automated if it is to reach the large number of customers engaged in legume enterprise. Several opportunities for improving and expanding inoculant production are offered including 1) improved carrier material, 2) more effective sterilization procedures, 3) larger scale broth production and better broth delivery systems, 4) automated mixing of injected broth and carrier, 5) more efficient and rapid curing systems, and 6) opportunities for automated packaging and sealing based on currently available equipment from food processing, cosmetics and seed processing industries.

This report also outlines the roles of public, private and international organizations in the advancement of inoculants in Africa. A matrix table was introduced that described the relationships among these three sectors across eight functional elements relating to rhizobium collection and evaluation, and to inoculant product formulation, manufacture, standards, use, regulation and trade. This approach resulted in 24 distinct elements that received elaboration within the context of N2Africa, and its international facilitation, and our public and commercial partners. Featuring predominantly within this matrix is the need for national programs to continue their rhizobium culture collections, the call for regulators to establish and enforce quality standards for inoculants to protect customers from inferior products, the responsibility of the public sector to develop new inoculant products and comply with industry standards and the importance of extension agents and farm liaison specialists to include inoculant handling and use in rural development programs.

Recommendations. Based upon the considerations in this report, the following recommendations are raised:

1. Do not impose quality standards that are beyond the technical capacities of aspiring manufacturers so as to preclude investment in inoculant production but at the same time protect retailers and their customers from inferior products. Once in place, inoculant producers should be offered incentives to steadily improve product quality and cost efficiency.
2. Movement is possible toward more automated operations, particularly in the areas of carrier bagging, carrier and broth mixing, and product sealing. Manual operations are not inferior per se, but may represent rate limiting or potential contaminating steps. Relatively inexpensive equipment used in other industries may be applied to inoculant production.
3. Several advances in inoculant product efficacy and delivery are available. Gum arabic, an African product, appears to be the best adhesive. Granules can deliver larger numbers of inoculant rhizobia that overcome failed responses by bean and groundnut. Several of the N2Africa candidate elite strains identified under MS 3.1.4 appear to outperform industry standards CIAT 899 (for bean) and USDA 110 (for soyabean). Seed coating technologies that combine inoculants, pesticides and fertilizers were not explored by N2Africa but warrant attention.
4. Carrier selection and their sterilization remain unresolved issues. Some carriers that are readily available organic resources were tested and rejected. Peat from Africa was found difficult to

access. The possibility of importing peat from northern countries was examined late in the program, and while appearing cost effective, their milling, neutralization and sterilization remain uncertain. At the same time, liquid formulation inoculants designed for mechanical planting operations do not appear suitable for use at the level of small-scale farming.

5. The quality of quality control must improve. It is one thing to adopt standards, and another to meet and enforce them. Quality control starts with the manufacturer, and greater in-house precautions circumvent product decline. Presently no inspection is made at the strain level using well established serological or molecular methods. Similarly, quality inspection appears fixed at the factory level, with too little attention on quality decline during distribution and marketing.
6. It is perhaps idealistic to assert that all inoculants should not contain contaminants, rather contaminants should be kept within lower thresholds. Even the level of $< 1 \times 10^6$ contaminants per gram appears difficult to achieve, and fresh inoculants containing $> 1 \times 10^9$ rhizobia typically perform well in presence of contaminants. At the same time, pure inoculants are likely to outperform contaminated ones, and to have longer shelf lives, so manufacturer's attaining this product level will have an inherent competitive advantage. The Australian system of assigning shelf live based upon contaminant level appears applicable under African conditions.

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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 soyabeans, 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
30. Memoranda of Understanding are formalized with key partners along the legume value chains in the impact zones
31. Existing rhizobiology laboratories upgraded
32. N2Africa Baseline report
33. N2Africa Annual country reports 2011

34. Facilitating large-scale dissemination of Biological Nitrogen Fixation
35. Dissemination tools produced
36. Linking legume farmers to markets
37. The role of AGRA and other partners in the project defined and co-funding/financing options for scale-up of inoculum (banks, AGRA, industry) identified
38. Progress Towards Achieving the Vision of Success of N2Africa
39. Quantifying the impact of the N2Africa project on Biological Nitrogen Fixation
40. Training agro-dealers in accessing, managing and distributing information on inoculant use
41. Opportunities for N2Africa in Ethiopia
42. N2Africa Project Progress Report Month 30
43. Review & Planning meeting Zimbabwe
44. Howard G. Buffett Foundation – N2Africa June 2012 Interim Report
45. Number of Extension Events Organized per Season per Country
46. N2Africa narrative reports Month 30
47. Background information on agronomy, farming systems and ongoing projects on grain legumes in Uganda
48. Opportunities for N2Africa in Tanzania
49. Background information on agronomy, farming systems and ongoing projects on grain legumes in Ethiopia
50. Special Events on the Role of Legumes in Household Nutrition and Value-Added Processing
51. Value chain analyses of grain legumes in N2Africa: Kenya, Rwanda, eastern DRC, Ghana, Nigeria, Mozambique, Malawi and Zimbabwe
52. Background information on agronomy, farming systems and ongoing projects on grain legumes in Tanzania
53. Nutritional benefits of legume consumption at household level in rural sub-Saharan Africa: Literature study
54. N2Africa Project Progress Report Month 42
55. Market Analysis of Inoculant Production and Use
56. Grain legumes and fodder legume materials with high Biological Nitrogen Fixation Potential identified in N2Africa impact zones
57. A N2Africa universal logo representing inoculant quality assurance
58. M&E Workstream report
59. Improving legume inoculants and developing strategic alliances for their advancement

Partners involved in the N2Africa project

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Caritas Rwanda

Diobass

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