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Biological Nitrogen Fixation in SSA

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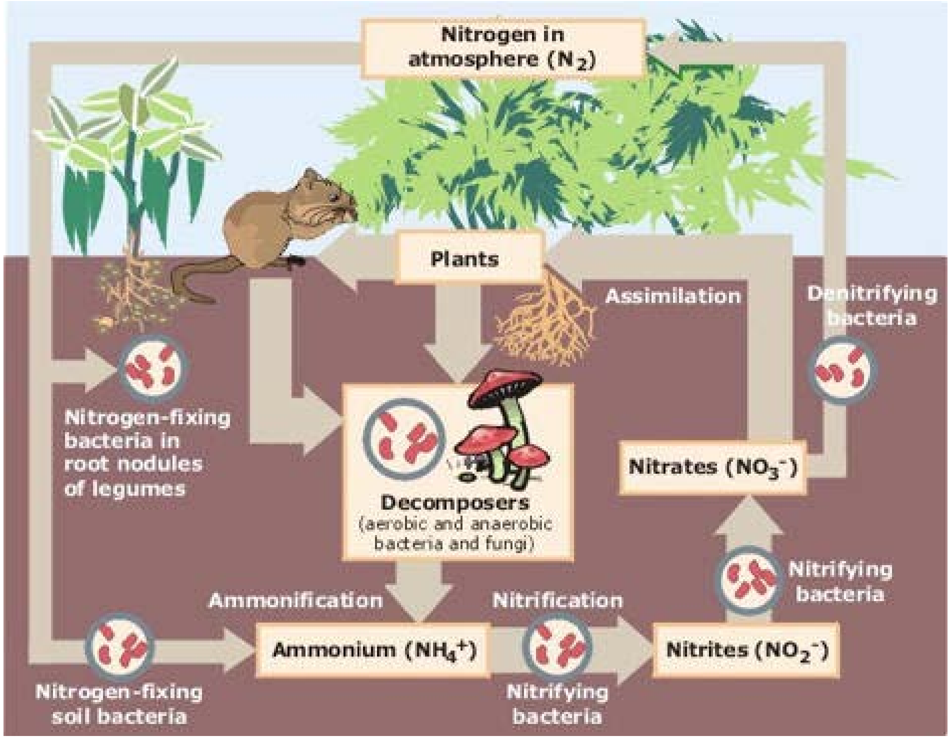
Lack of nitrogen (N) is often cited as the most limiting factor in agriculture. Nitrogen's role in synthesizing amino acids and other metabolic processes makes it essential for plant survival, but although N composes nearly 80% of the atmosphere, plants are unable to use this form of the element (N_2) because of the strong triple bonds between the two atoms. Plants instead must use N compounds such as ammonium (NH_4^+) and nitrate (NO_3^-) available in the soil or applied as inorganic fertilizer or manure (see Figure 1). However, most soils used for more than a few years in cultivation are nitrogen deficient (Roy, Finck, Blair, & Tandon, 2006).

Figure 1. The Nitrogen Cycle



Source: EPA, available at http://www.uwsp.edu/geO/faculty/ritter/geog101/textbook/earth_system/biogeochemical_cycles.html.

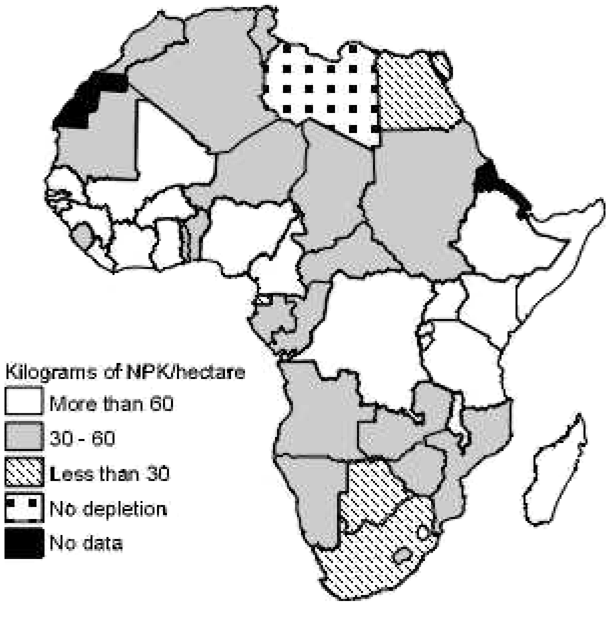
Nitrogen deficiency is especially problematic in the soils of Sub-Saharan Africa (SSA). Population pressure has reduced (or in many cases eliminated) fallow periods, limited soil fertility regeneration, and increased cultivation of marginal soils (Drechsel, Gyiele, Kunze, & Cofie, 2001). Monocropping, low fertilizer application, and inadequate soil conservation have also contributed to yearly net N losses through soil erosion and leaching (Dreschsel et al., 2001; Henao & Baanante, 1999b). A 1993 estimate from 38 African countries (still cited as the most comprehensive measure of African soil depletion) found an average annual depletion rate of 22 kg/ha and a predicted rise to 26 kg/ha by 2000 (Stoorvogel, Smaling, & Janssen, 1993). This estimate seems to have come to fruition when comparing 1999 estimates in Table 1. Nitrogen loss varies by country with Burundi, Guinea Bissau, Ethiopia, Malawi, Nigeria, Rwanda, and Uganda experiencing the highest rates of loss (Henao & Baanante, 1999b). See Table 1 and Figure 2 for examples of nutrient loss across the continent.

Table 1. Annual nutrient depletion in agricultural soils in countries of Southern Africa

Country	Nitrogen depletion (kg ha^{-1} year^{-1})	Total fertilizer use (kg ha^{-1})
Tanzania	-38	4.5
Malawi	-48	14.6
Mozambique	-23	3.5
Zambia	-13	5.7
Zimbabwe	-20	49.3

Source: Henao & Baanante, 1999a as cited in Mafongoya, Bationo, Kihara, & Waswa, 2006.

Figure 2. Average annual nutrient depletion (NPK) in Africa, 1993-95



Source: Henao & Baanante, 1999b

Low levels of N and other soil fertility problems have severe poverty, malnutrition and environmental degradation consequences for SSA. Sanchez (2002) reported that soil fertility depletion is "the fundamental biophysical cause of stagnant per capita food production in Africa". Expansion of farmland contributes to decreased biodiversity and increasing deforestation. Addressing nitrogen depletion is essential for increasing yields, food security, nutrition, and income in SSA. The purpose of this literature review is to examine the prospects for expanding biological nitrogen fixation (BNF) technology in SSA in addressing these challenges.

Biological Nitrogen Fixation

The process by which atmospheric N is converted into N compounds that can be used by living things is called nitrogen fixation. Nitrogen fixation can occur through non-biological processes such as lightning, combustion, and most commonly, the Haber–Bosch process, a method developed in the early 1900s which produces ammonia from N and hydrogen gas using an iron oxide catalyst under extremely high temperatures and pressure. Ammonia produced through the Haber–Bosch process can then be used to manufacture N fertilizer.

Although N fertilizer use increases plant productivity in areas where N is deficient, environmental concerns and constraints to use in SSA make exploring other N replenishment options advisable. Excess or inappropriate N fertilizer use has been shown to increase atmospheric nitrous oxide (N_2O, a greenhouse gas), leach nitrates (poisonous to humans) into groundwater, and pollute run-off and surface waters, leading to extensive damage of freshwater sources (Armstrong-Gustafson & Krey, n.d.). In addition, the Haber-Bosch process is energy intensive, using a substantial proportion of worldwide natural gas production. Lastly, N fertilizers in SSA cost two to six times more than the worldwide average, making application of a sufficient amount to replenish soils greatly out of reach for most subsistence farmers in SSA (Sanchez, 2002).

Biological nitrogen fixation (BNF or biofixation) offers an alternative or additional means to increase plant-available nitrogen. Through a symbiotic relationship, an N-fixing bacterium (usually from the genus *Rhizobium*) infects a plant (usually a legume) and forms nodules on the roots of the plant in which N fixation occurs. The plant uses the fixed N for its own needs and unused N remains in the soil after the plant dies, increasing soil N levels.

The ability of soybeans and other legumes to increase soil fertility has been known for centuries but the process of BNF was not discovered until Martinus Willem Beijerinck first described the process in the late 1870s (Armstrong-Gustafson & Krey, n.d.). Since then, knowledge of the N fixation process and BNF technologies has vastly expanded and agronomists and farmers worldwide have recognized BNF's potential as a sustainable way to increase yields while also providing an inexpensive protein source through soybeans and other legumes. In the mid-1990s, it was estimated that about 20% of all N available to the world's crops is due to rhizobial fixation (Smil, 1999).

A nitrogen-fixing bacterium is known as a symbiotic diazatroph. The most common symbiotic diazatrophs are rhizobia which associate with legumes, but there are also other lesser-known diazatrophs as well. Not all legumes are able to partner with diazatrophs to fix N, and those that can vary considerably in the amount of N they are able to fix. See Table 2 for a summary of BNF legumes commonly used in BNF.

Table 2. Estimated Amounts of N Fixed by Various Legume Crops under Field Conditions[1]

Plant		Nitrogen fixed (kg N/ha/yr)
Food legumes		
Calapo	Calopogonium mucunoides	370-450
Horse bean	Vicia faba	45-552
Pigeon pea	Cajanus cajan	168-280
Cowpea	Vigna unguiculata	73-354
Mung bean	Vigna mungo	63-342
Guar	Cyanopsis tetragonoloba	41-220
Soybean	Glycine max	60-168
Chick-pea	Cicerarietinum	103
Lentil	Lens esculenta	88-114
Groundnut	Arachis hypogaea	72-124
Pea	Pisum sativum	52-77
Bean	Phaseolus vulgaris	40-70
Forage legumes		
Tick clover	Desmodium intortum	897
Sesbania	Sesbaniacannabina	542
Leucaena	Leucaenaleucocephala	74-584
Centro	Centrosema pubescens	126-398
Alfalfa	Medicago sativa	229-290
Subclover	Trifolium subterraneum	207
Ladino clover	Trifolium repens var. gigantea	165-189
White clover	Trifolium repens	128
Stylo	Stylosanthes spp.	34-220
Vetch	Vicia villosa	110
Puero	Pueraria phaseoloides	99

[1] The amount of N_2 fixed by legumes varies with host genotype, *Rhizobium* efficiency, soil and climatic conditions and methodology used in assessing fixation.

Source: La Rue & Patterson, 1981 and Nutman, 1981 as cited in FAO, 1984

Expansion of BNF

Although species of rhizobia are present to some degree in all soils, simply planting legumes does not necessarily result in increased soil fertility and crop yields. Each species of rhizobia can only nodulate with certain legumes and vice versa, which presents the primary practical challenge in developing BNF technologies. Therefore, development and expansion of BNF technologies generally pursues one of three options:

1. **Develop legumes compatible with indigenous rhizobia.** If sufficient numbers of native rhizobia are present, introducing legumes which are compatible with native microbes is an effective way to increase soil fertility and yields without the need of any outside rhizobial inoculation. Finding and developing more “promiscuous” legumes (able to nodulate with a

wide diversity of rhizobial strains) is a key focus of this research (Mpepereki, Javaheri, Davis, & Giller, 2000).

2. **Develop rhizobial inocula compatible with indigenous legume species.** Sufficient numbers of compatible rhizobia are often not naturally occurring in the soils of interest (Marufu, Karanja, & Ryder, 1995). In these cases, rhizobia can be developed and introduced in the form of an inoculant for application to seeds or as a "slurry" to be applied to soil during planting. Legume inoculation began shortly after Beijerinck's discovery of BNF and today, an estimated 12 –20 x 10^6 hectares of soybeans are inoculated yearly (Catroux, Hartmann, & Revellin, 2001).

3. **Develop compatible legume and rhizobial inoculum.** If rhizobia are not naturally occurring and/or native legumes are not available or are low-yielding, development of compatible legumes and rhizobia can be done simultaneously.

<u>Benefits of BNF</u>

BNF has many advantages, both in general and when compared with nitrogen fertilizers.

- **Environmental.** Because biologically fixed N is bound in soil organic matter, it is much less susceptible to leaching and volatization into nitrous oxide than fertilizer. This results in fewer nitrous oxide emissions and less water contamination (Armstrong-Gustafson & Krey, n.d.). This also means that more N remains within the soil for plant use, making it a more efficient form of N than fertilizer (Smil, 2002).

- **Nutrition.** Food legumes offer a high-quality, low-cost source of protein that is extremely valuable to populations with diets normally heavy in carbohydrates. Few studies exist on the direct nutritional impact of BNF technologies, but initial improvements in child weight-for-height have been found in both Nigeria and Malawi (see detailed results in "BNF in Africa" section).

- **Better yields.** Dozens of studies have shown that using BNF technologies increase crop yields of both the legumes and successively planted crops, as demonstrated by dozens of studies. In Burma, rhizobial inoculation is credited with 20-60% increases in yields with chickpeas, groundnuts, and green gram. In the Philippines, inoculation has been credited with 200-700% yield increases in soybeans (FAO, 1984). In the Regional Bean Inoculation Program in Kenya, Tanzania, and Uganda, yields of soybean, common bean, and groundnut increased by 7 to 47% (Kannaiyan, 2003). In Malawi, rotations of soybeans and maize resulted in 75% higher yields of maize than compared to maize following maize (Sanginga et al., 2001).

- **Fodder.** Fodder legumes and non-edible portions of food legumes offer a source of animal feed as well, addressing some of the integrated needs of subsistence farmers.

- **Reduced cost.** While cost comparisons for BNF can vary widely based on species of legume, the type of inoculation treatment, and country (FAO, 1984), in general it seems that use of BNF is much less expensive than N fertilizers. One 2000 estimate reports that for one hectare of use, \$3.00 in rhizobial inoculants was equal to \$87.00 in fertilizer for the amount of N used by crops but no details are provided on how the figures were calculated (NifTAL, 2000). An older estimate for soybean BNF in the United States found a 17:1 cost ratio when comparing an estimated \$2.93/ha for inoculant application for soybeans (labor and inoculant cost) to a fertilizer cost of \$50/ha for the equivalent amount of N (100 kg/ha at a cost of \$0.50/kg) (FAO, 1984). These cost comparisons generally do not include the costs of research, but if effectiveness is only considered from the farmer's point of view, it appears that BNF is much more cost effective than fertilizer. Additionally, while no cost comparisons were found for BNF without inoculation, it might be assumed that the cost savings could be even more significant if legumes that were compatible with native rhizobium and didn't require inoculation were used. For future efforts, cost ratios can be prepared using the following formula:

$$\frac{\text{N fixed by legume in kg/ha * cost of fertilizer per kg * fixed N per kg fertilizer}}{\text{Labor cost to apply inoculant per ha + Cost of inoculant per ha}}$$

Constraints to Adoption

Although the potential benefits of BNF are well established, numerous challenges in the production, distribution, application, and marketing of technologies can result in low adoption rates both in SSA and other parts of the developing world. Addressing these constraints would be a vital part of any strategy to increase BNF adoption in the future.

Physical and Environmental

Efforts in Kenya to expand use of BNF inoculants have been hindered by problems with transport and storage (Adame, 1997). Small producers of inoculants also have difficulty meeting seasonal peaks in demand (Kannaiyan, 2003). Inoculants need to stay cool, which could obviously be a limitation for subsistence farmers without access to refrigeration but is also a difficulty for transportation (Kannaiyan, 2003). Some success in keeping inoculants cool in traditional clay pots in Zimbabwe, however, has been reported (Mpepereki et al., 2000).

Chemical

The degree to which rhizobia survive, successfully infect the target crop, and fix N is dependent on many soil factors including temperature, moisture, acidity, and adequate nutrition for the rhizobia being present in the soil (Montañez, 2000). Although it seems ironic that a degree of soil quality needs to exist in order to improve the soil with BNF, low soil fertility was reported to be the limiting factor to BNF adoption in Burundi (Marufu et al., 2001).

- **Acidity**. Rhizobium bacteria are sensitive to both low and high pH. Using acid-tolerant rhizobia, applying lime, and pelleting of inoculated seeds can improve N fixation in acidic soils (Roy et al., 1997) and use of gypsum can help rhizobial survival in alkaline soils (Kannaiyan, 2003).

- **Temperature and moisture.** The soil climate affects survival and ability of rhizobia to nodulate and fix N (Montañez, 2000). Placement of inoculum in deeper soil layers, usage of cover crops, and no-tillage management practices can help ameliorate this problem (Montañez, 2000).

- **Molybdenum.** Molybdenum (Mo) is an element required for BNF, which is often at low levels in acidic soils and therefore, may need to be supplied as an external input (Roy et al., 1997). In an experiment in Brazil, additional molybdenum application increased soybean yield from 3100 to 3400 kg ha^{-1} (Andrade & Hungria, 2001).

- **Phosphorus.** Phosphorus is another element required for BNF. A study in northern Guinea (Nigeria) found that far less phosphorus was available to legume crops than the amount required (about 30 kg/ha for optimal growth), due to low usage of phosphorus fertilizer (Sanginga, 2003). This suggests that selecting legume species or rhizobia with low phosphorus requirements would be beneficial.

- **Other nutrients.** Many other elements are involved in the BNF process and adequate levels of these elements must be present in order for BNF to take place at optimal rates. Calcium (Ca) and Boron (B) have been shown to be involved in infection and nodule development (Roy et al., 1997). Mo, Iron (Fe), and Sulfer (S) are all components of the enzyme nitrogenase which transforms atmospheric N into ammonia during N fixation. A holistic look at the chemistry of targeted soils is needed to understand limiting factors and mitigate adverse effects.

- **Fungicide and pesticide.** Use of fungicide or pesticide can be harmful to inoculant and therefore needs to be avoided within 24 hours of seed treatment (Roy, Finck, Blair, & Tandon, 2006).

Biological

Indigenous flora and fauna can prevent survival of rhizobial inoculants (Kannaiyan, 2003). In India, failure to obtain the desired inoculation response was mostly attributed to the introduced strains' inability to displace native strains and other indigenous antagonists (Kannaiyan, 2003).

Technical

- **Low quality inoculants.** In a test of inoculants from developing countries, NifTAL found nearly 50% were of such poor quality that they would not have benefited farmers at all (CTAHR, 2007, see Box 1). This uncertainty of quality is one of the most important reasons farmers do not believe inoculants will work (Kannaiyan, 2003). Quality control issues are discussed in more depth in the "Regulations" section of this paper.

- **Shelf life.** Most inoculants have a shelf life of three to six months and inoculants stored longer than this have fewer viable rhizobia.
- **Raw material.** High-quality peat, a common carrier for inoculants, is not abundant in tropical regions. Local suitable substitutes are therefore necessary (Kannaiyan, 2003).
- **Infrastructure.** Lack of infrastructure is a severe limitation for large-scale production of high quality inoculants (Kannaiyan, 2003).

Marketing

Marufu et al. (1995) reported that lack of awareness of the technology by peasant farmers is the most important barrier to adoption of BNF in Zambia and Zimbabwe and Odame (1997) reports that the vast majority of farmers in Kenya are not aware of the existence of BNF. Greater awareness campaigns and participatory research are required for farmers to realize the gains to be made from BNF. In addition, poorly developed market networks and retail outlets are constraints for inoculants (Kannaiyan, 2003).

Limited and uncertain market access is also a constraint for legume adoption. Along with unstable legume prices, uncertainty limits legume adoption as a cash crop (Kerr et al., 2007).

Education

The application of BNF technologies can be a complicated process and most farmers lack the knowledge to use them effectively without the help of technical assistance (Kannaiyan, 2003). A shortage of both qualified extension workers and illustrative and explanatory materials exacerbates this constraint (Bohlool, Ladha, Garrity, & George, 1992).

Cost

Although the cost of inoculants is usually not more than 1% of seed cost, the cost may be a disincentive to use for subsistence farmers who normally do not purchase seed off the farm (Bohlool et al., 1992).

Farmer Preferences

Moderate yield of legumes compared to cereals and tubers and the high labor requirement of legumes (for a crop with initially slow growth) are additional farmer constraints to adoption (Kerr et al., 2007). On-farm research in Malawi showed that resource-poor farmers are only willing to adopt improved legume varieties if they anticipate short-term nutritional outcomes and stable markets (Kerr et al., 2007).

Success Stories

Despite the practical challenges associated with BNF, this strategy has long been used successfully worldwide. Among the most well-known examples are the transformation of the infertile Cerrado soils of Brazil and Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL)'s success worldwide in developing and distributing BNF technologies.

Soybeans in the Cerrado Region of Brazil

The Cerrado, or Brazilian savannah region, is named from Portuguese words meaning "closed, inaccessible land". Today, however, the region provides 50% of Brazilian grain, due largely to BNF technology with soybeans, which were not widely grown in Brazil until the 1960s (Melby, n.d.). At that time, a government campaign to increase wheat production in the country involved introducing a crop rotation with soybeans to increase wheat yields.

The Brazilian Enterprise for Agriculture and Livestock Research (EMBRAPA) first introduced BNF into the Cerrado region in 1981. This strategy, along with other technologies (e.g. lime to correct for soil acidity, crop and livestock integration, direct seeding, and no-tillage systems) has resulted in enormous gains in soybean yields. Estimates of BNF's economic impact in Brazil include approximately $350 million per year directly from the BNF industry (Melby, n.d.) and between $2.5 and 6 billion per year in N fertilizer savings (Alves, Boddey, & Urquiaga, 2002; Hungria et al., 2006).

Today, BNF through *Bradyrhizobium* inoculation has completely replaced the use of N fertilizer on soybean crops in the Cerrado region and in fact, attempts to apply N fertilizer on inoculated crops often result in lower yields (Mendes, Hungria, & Vargas, 2003). Success of BNF technology in Brazil is attributed to three factors (Alves et al., 2002):

1. Soybean breeding efforts to increase adaption to short days, pest- and disease- tolerance, and acidic soil conditions so soybean could be grown in almost any region.

2. Inoculation with best rhizobium strains and no application of N fertilizer.

3. Continuous communication between breeders, agronomists, and rhizobiologists, which led to rhizobium strain development in parallel with soybean breeding.

Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL) Project

In 1975, the United States Agency for International Development (USAID) established an interdisciplinary unit at the University of Hawaii's College of Tropical Agriculture and Human Resources dedicated to the development of BNF technology for international development goals. The ultimate purpose of the project was to help farmers maximize BNF inputs to their cropping systems, thereby increasing the production and quality of high protein foods while reducing their dependence on N fertilizers, which were especially expensive at the time due to the oil crisis.

NifTAL's early work included supporting country legume programs through research support and training in BNF, developing and testing effective symbionts for target legumes, and field testing legume response to inoculation throughout the tropics. Later work focused on development of appropriate BNF technologies for tropical agriculture.

Results of NifTAL's national assessments of the macro-economic impact of BNF technology are described below (CTAHR, 2007). Further details on how inoculant values were calculated were not found but likely exist in internal reports named in "Additional Resources" section.

- **Thailand**. A $3.5 million USAID loan funded the initiation of the NifTAL/DOA Resource Center for South and Southeast Asia (BNFRC). Activities at BNFRC focused on strain development, inoculation trials, extension training and demonstration, and human resource development. Activities resulted in $121.40 million of net income from soybean, groundnut, and mungbean and replacement of an estimated $25.92 million worth of N fertilizer from 1980 to 1993. The BNFRC helped the Bangkok Seed Company to enter the inoculant market. The company marketed 400,000 units of inoculant yearly in Thailand as of 2007.

- **Zambia**. For four years, NifTAL and the University of Illinois teamed up to provide technical assistance, research, facilities design, and market development. From 1984 to 1992, 92,981 hectares were inoculated, resulting in an additional $17.88 million worth of soybeans and $5.37 million worth of fertilizer replaced by BNF. Results were achieved with approximately $550,000 of investment.

- **Indonesia**. Assistance from BNFRC/NifTAL in the form of technical assistance, facilities design technical training, and germplasm resulted in a private company, Rhizogin PTY, distributing 1.0 million packets of inoculant per year. In 1986, the future value of inoculant use through 2000 was estimated to be $35.5 million, but results were not subsequently measured.

Current projects by NifTAL were not found.

BNF in Africa

Currently, BNF is being used in Burkina Faso, Democratic Republic of the Congo, Guinea Bissau, Madagascar, Rwanda, South Africa, Sudan, Tanzania, Uganda, Zambia, and Zimbabwe (Roy et al., 2006). In West Africa, legumes are traditionally a part of farming systems (Sanginga, 2003). Inoculants are produced in Kenya, Uganda, Tanzania, South Africa, Zambia, and Zimbabwe (Kannaiyan, 2003). BNF expansion through legume introduction and inoculation technology has been attempted for nearly fifty years in Africa and while these efforts have been met with varying degrees of success, it is clear Africa has yet to fully benefit from the technology (Danso, 1992).

Soybean in Zimbabwe

Legume inoculant production began in 1962 at a government owned factory in Zimbabwe (Marufu et al., 1995). The factory served mostly commercial soybean farmers until a promotion program in the Hurungwe District began promoting soybean BNF to smallholders in 1986 (Mpepereki et al., 2000). Introduction of improved varieties resulted in yield improvements of 30-40% and inoculants were somewhat effectively stored under cool conditions in traditional clay pots for up to four months before use. Unfortunately, when the promotion program ended in 1989, soybean production declined significantly as the supply of commercial rhizobial inoculant was cut off (Mpepereki et al., 2000). However, production continued for farmers who were planting local promiscuous varieties (Mpepereki et al., 2000).

In 1996, another task force was created to increase production of promiscuous soybean among smallholders in 10 districts. Adoption of soybeans increased from 55 farmers in 1996 to 5000 in 1998 (Mpepereki et al., 2000). The pilot phase of the project included farmers planting two promiscuous and two specifically-nodulating varieties with small amounts of basal phosphorus fertilizer and lime. In the second planting season, farmers chose their preferred varieties. Farmers mostly chose to grow both promiscuous and specifically-nodulating varieties because they used the large biomass from promiscuous varieties as fodder and soil amendment and used the specifically-nodulating varieties more for food and cash income (Mpepereki et al., 2000).

Soybean in Nigeria

In Nigeria, the International Institute for Tropical Agriculture (IITA) has worked to increase soybean productivity in Nigeria since the late 1970s. A 1999 review of progress found that new varieties (requiring no inoculants) were being grown by 75% of male farmers and 62% of female farmers eight years after their introduction in 1989 and over 47,000 farmers (including 30,000 women) had been trained in production and potential utilization of soybean in their families' diet (Sanginga et al., 1999). After adoption, 42% of men and 47% of women ranked soybean as their most important source of cash income and the majority of farmers reported substantial increases in material and human capital acquisition, including school, health, and goats. As previously reported, rotations of soybeans and maize resulted in 75% higher yields of maize than maize planted continuously (Sanginga et al., 2001). Most importantly perhaps, soybean consumption and soybean income were significant determinants of children's nutrition status (weight-for-height) when controlling for other relevant variables (Sanginga et al., 1999). Determinants of adoption of improved soybean varieties included gender (women less likely to adopt), village location (villages with better market access for soybeans more likely to adopt), age of farmer (younger less likely to adopt), and number of contacts with extension workers (more led to higher adoption). Success of the project was greatly influenced by household trainings and extension efforts including awareness campaigns, on-farm adaptive research, and household trainings on how to integrate soy into their diets. IITA's Cereals and Legumes Systems research continues today with projects all across the tropics.

Soybean in Malawi

The Soils, Food, and Healthy Communities (SFHC) project is another example of success in soybean introduction without inoculant. The project was started in 2000 by a hospital in Malawi, collaborating with researchers from Canada and Malawi (Soil, Foods and Healthy Communities, 2008). The program aims to improve the health, food security, and soil fertility of resource poor households in 80 communities in northern Malawi through increasing adoption of legumes. Since its initiation, 4000 farmers have tested and gained knowledge of legumes (SFHC, 2008), with farmers (especially women) mostly choosing to adopt maize and edible legumes crop rotations as opposed to maize intercropped with a forage legume that may increase soil fertility more (Kerr et al., 2007). Focus groups revealed that food use was the primary reason for legume adoption with soil fertility a secondary concern. Soil fertility was accessed using focus groups as well, with more than half of farmers reporting a dark green leaf color for a maize crop grown after a legume (Kerr et al., 2007). More recently, a paper in review reports that children in villages most intensely involved the project had improvements 0.7 in weight-for-age Z-scores as compared to uninvolved villages (Kerr, Berti, & Shumba, in review).

Bean Inoculation in Kenya

A BNF technology dissemination project in Kenya was the only true failure in SSA found. The project, which cost $43,500, ran from 1983 to 1989, aimed to disseminate rhizobial inoculants to smallholders but only 0.2% of targeted farmers ended up using the inoculants (International Development Research Centre (IDRC), n.d.). Two thirds of farmers did not attend farmer training courses, farm demonstrations, or field days; and did not read farm newspapers or listen to agricultural radio shows. Researchers from the project recommended that future efforts keep in mind farm size, cultural practices, and ecological conditions (IDRC, n.d.)

Regulation

Regulation of the quality and safety of microbial inoculants is at various stages around the world. International trade agreements usually have regulations regarding the transport of living organisms across borders but within borders, African countries generally do not have explicit regulations regarding safety of inoculants unless the inoculant includes genetically modified organisms. Details of inoculation quality regulations are outlined below.

Quality Control

High quality legume inoculant products require many factors; the most important include high numbers of live rhizobia capable of nodulation and N_2 fixation with the target host, and minimal or no contamination (Lupwayi et al., 2000). Because internal manufacturing controls are expensive, and because farmers cannot judge inoculant quality at the time of purchase, there is often little incentive for inoculant producers to institute quality control measures in the absence of government regulation. More than half of the inoculant products sampled from 12 developing country markets had less than 10^8 rhizobia per gram whereas 2×10^9 rhizobia per gram is preferred for best N fixation (Singleton et al., 1997), and there have been examples of inoculant products in both developed and developing countries that did not contain any rhizobia at all (Lupwayi et al., 2000; Bashan, 1998). Poor quality products cause farmers to lose confidence in the technology.

Countries have differing degrees of regulation on the quality of rhizobia inoculants. Brazil, Canada, France, and Uruguay have regulatory authorities supported by legislation to oversee inoculant quality, while voluntary regulations exist in Australia, New Zealand, and South Africa. In many other countries, including the United States and the United Kingdom, product quality standards are left to manufacturers. Some regulations tie the manufacturers' recommended rate of inoculant application to a minimum number of viable rhizobia that must be delivered per seed. Most establish a minimum number of viable rhizobial cells of the appropriate species or strain per unit weight of inoculant, though there is no common international standard (Bashan, 1998), and therefore requirements vary widely. At least 10^9 rhizobia per gram are required in France, Australia, Rwanda, Zimbabwe and Kenya, 10^8 cells per gram in New Zealand and South Africa, and 5×10^7 cells per gram in Thailand (Lupwayi et al., 2000). Even where regulations exist, these regulations may not be enforced, for example, in some countries in Latin America (Bashan, 1998).

The quality of the media in which the rhizobia are delivered is also important. While sterile media ensures that there are no contaminants, these are generally 5-10 times more expensive than non-sterile media, and therefore are generally not used (Bashan, 1998). Non-sterile media can be contaminated with other microbes, which can have detrimental effects on rhizobia (Olsen et al., 1994). A Canadian study showed that contamination levels sometimes exceeded the level of rhizobia, in some cases by several orders of magnitude (Bashan, 1998). France requires that there be no contaminants in rhizobial cultures, while Australia allows contaminants to be no more than 0.1% of the total bacterial populations, and requires high population levels of rhizobia (Bashan, 1998). Rwanda requires less than 0.001% contamination (though Bashan expresses doubt about whether these standards are enforced).

Registration

In some countries (mostly those that are more developed), bacterial inoculants must go through a registration procedure, which can be lengthy and expensive. Within these countries, bacterial inoculants that are labeled as "biofertilizer" may sometimes, though not always, go through different, less cumbersome registration for commercial use (Bashan, 1998). In China, some inoculants are officially registered, while others are not registered and yet are being sold off the shelves of research institutes or with commercial names (Bashan, 1998). Along with registration requirements, specific product labeling requirements (such as identification of inoculant and expiry date) also exist in some countries (Roy et al., 2006).

Safety Regulations

Malawi, South Africa, and Zimbabwe are the only three countries in SSA which have legal regulations for the safe development and application of biotechnology (Mnyulwa & Mugwagwa, 2005). Despite the limited country-specific safety regulations on biotechnology in general, all Southern African Development Community members[1] are signatories on the Cartagena Biosafety

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[1] SADC members include Angola, Bostwana, DRC, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Seychelles, South Africa, Swaziland, Tanzania, Zambia, and Zimbabwe.

Protocol, an addendum to the Convention on Biological Diversity, which regulates the transboundary movement, transit, and handling of living modified organisms. Living modified organisms are defined as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (Mnyulwa & Mugwagwa, 2005), so any regulations pertain only to genetically modified organisms.

Conclusion

Biological nitrogen fixation (BNF) technologies can be an efficient and effective tool for decreasing environmental degradation and increasing soil fertility, yields, income, and food security in Sub-Saharan Africa although many constraints to farmer adoption exist. In order to assure successful adoption of BNF technology (especially by smallholders) soil, infrastructure, and socio-cultural constraints must be overcome. Firstly, a holistic understanding of the characteristics of each targeted farm and area is needed to understand farmer preferences, decide on a technology strategy and to develop rhizome-legume partnerships that are able to thrive within the chemical and biological constraints of the soils. Secondly, if inoculants are used, addressing the production, transportation, and storage barriers is necessary in order to effectively distribute inoculants to rural smallholder farmers. Lastly, and perhaps most importantly is the development of education, training, outreach and technical assistance for farmers. In Africa, common themes for BNF success stories are on-farm participatory research and effective education campaigns. With continued participatory research and field trials, great gains in soil fertility, crop yield, and food security are possible for smallholder farmers in SSA.

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