HISTORY OF RHIZOBIA INOCULANTS USE FOR GRAIN LEGUMES IMPROVEMENT IN NIGERIA - THE JOURNEY SO FAR

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ABSTRACT

Even though, the use of rhizobium inoculants for improvement in N-fixation and productivity of grain legumes has been well established in developed countries; it is still in the developing stage in most parts of sub-Saharan Africa, Nigeria inclusive. Grain legumes are generally grown in low fertility soils in the country. The most frequently deficient nutrient is nitrogen, while nitrogen fertilizers are costly, inadequate, and not timely in supply, as well associated with side effects on use. This makes rhizobia inoculants a cheaper, easier and safer option to improve the N_2-fixation and productivity of grain legumes. However, the use of inoculants in Nigeria, though initiated as far back as 1970s, still has a long way to go. This was through the use of imported inoculants. Initially there was poor response to the inoculants, due to incompatibility in the new environment. A lot of studies on the use of these inoculants were conducted on Soybean (*Glycine max* (L.) Merrill), mostly the "US type" which requires specific inoculation with *Bradyrhizobim japonicum*for optimum productivity, leading up to over 100% increases in yield. Studies on inoculation of cowpea (*Vigna unguiculata* (L.) Walp), were also conducted, but rarely Bambara groundnut (*Vigna subterranea* (L.) Verdc.) and groundnut or peanut (*Arachis hypogaea* L.), being naturally more adapted and promiscuous. Unfortunately, the results ended with the researchers, mostly not applied by farmers. Since 1980s, the International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria, introduced the promiscuous soybean cultivars; Tropical Glycine Cross (TGX), to relieve the farmers from difficulties in obtaining inoculants. These cultivars nodulate without inoculation, fix a large amount of nitrogen and still have high grain yields. However, some experiments indicated the requirement of some of the cultivars for inoculation with up to 40 - 45% increases in yield. Hence, the ultimate option remains the development of inoculants that are adapted to the environment. Highly effective or elite strains need to be selected for the production of bespoke inoculants for particular crops from the diversity of indigenous strains. Therefore, attention needs to be paid to the establishment of industries that could meet the farmers' needs. Thanks to the recent efforts of the project; Putting Nitrogen fixation to work for smallholder farmers in Africa (N2Africa) in that direction.

Key words: grain legumes, N-fixation, nitrogen, nodulation, rhizobia inoculants.

Introduction

Legumes have been a source of food since mankind first tilled the soil many thousands of years ago. They have been recognized from very early times as 'soil improvers' (GRDC, 2013). The unique ability of legumes to fix atmospheric N through symbiotic association with root nodule bacteria could be used to improve the yields of legumes in sub-Saharan Africa, since current yields are only a small fraction of their potential (Abaidoo et al., 2013). Moreover, most of the soils used for legumes production in the region, particularly, in Nigeria are poor in terms of their nutrient status, especially total-N, therefore, relatively unproductive (Machido et al., 2011; Laditi et al., 2012). The soils are also low in organic carbon and available phosphorus; consequently, these soils are inherently low in fertility. The situation is further worsened by nutrient depletion by crops and other related processes, such as leaching, denitrification, volatilization and removal of crop residues for alternative uses (Yakubu et al., 2010; Machido et al., 2011). Hence, replenishment of depleted nutrients, especially N depends largely on addition of inorganic fertilizers, which rank first among the external inputs to maximize output in agriculture, but in turn contributes substantially to environmental pollution (Bohlool et al., 1992). On the other hand, most farmers cannot afford the inorganic fertilizers due to their high cost and non-availability on time in the region (Sanginga, 2003; Yakubu et al., 2010). This has renewed the interest of farmers on biological nitrogen fixation (BNF), which provides a continuous supply of N for plant growth in situ, adds organic matter to the soil and is economically viable (Nelson, 2004; Yakubu et al., 2010).

The success of a legume grain crop is dependent on its capacity to form effective nitrogen-fixing symbioses with root-nodule bacteria. Many soils however, do not have adequate amount of native rhizobia in terms of number, quality or effectiveness to enhance biological nitrogen fixation (FAO, 1984). These situations call for provision of external source of rhizobia to enable effective nodulation and N_2 fixation, known as inoculation. Three such situations were identified, that legumes generally need inoculation; (1) where compatible rhizobia are absent (2) where the population of compatible rhizobia is small and (3) where the indigenous rhizobia are ineffective or less effective in N_2-fixation with the intended legume than selected inoculant strains (Date, 2000; Vanlauwe and Giller, 2006).

The benefits, production and utilization of rhizobia (soil bacteria that fix nitrogen) inoculants for legumes improvement has been recognized for a very long time in developed countries, but still developing in sub-Saharan African countries, including Nigeria. Australian agriculture, for instance, has this recognition for more than 100 years, which includes substantial increases in legume nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels (GRDC, 2013). Similarly, commercial production and use of legume inoculants commenced as early as 1895 in the U.S.A. and the U.K., by 1993, inoculants were produced and used in many countries in all continents (Nelson, 2004). However, initiatives to establish local inoculant production in Africa started only in the 1980s and 1990s led by organizations such as; Nitrogen Fixation in Tropical Agricultural Legumes (NifTAL), Food and Agriculture Organization (FAO) and the Regional Microbiological Resources Centres (MIRCENs), which established small scale inoculant production industries in many countries throughout Africa, Nigeria exclusive, but only a few developed to a large scale in production (N2Africa, 2012). Hence, there is hardly any country in the whole of West Africa, including Nigeria where rhizobial inoculants are regularly used by farmers, except in experiments that are limited to research farms (Bala, 2011).

The introduction of soybean to Nigeria initiated studies on the use of inoculants on soybean in the country and the introduction of promiscuous soybean cultivars by IITA, Ibadan, Nigeria further facilitated it. Soybean is known to have been cultivated in Africa since the early

1900s, although it is likely that the crop was introduced much earlier through extensive trade around the Indian Ocean (Giller, 2008). Precisely it was introduced to Nigeria in 1904 (Ezedimma, 1964), hence it is a relatively new crop to the country (Bala et al., 2011). Mostly the "US-type" soybean that necessarily needs to be inoculated with specific strains of *Bradyrhizobium japonicum* was predominantly cultivated in most soybean growing areas of the country (Osunde et al., 2003). Positive responses of these soybean cultivars to inoculation has been reported in Africa with yield increases of up to179% in situations where indigenous *Bradyrhizobia* cells were fewer than 10 cells per gram of soil or where the rhizobial populations were not effective, RENEASA (cited in Ojo et al., 2007).

A breeding programme at IITA, Ibadan in the country later developed "promiscuous" or soybean cultivars that naturally nodulate freely with indigenous rhizobia, thus obviating the need to inoculate with *B. japonicum* (Kueneman et al., 1984; Pulver et al., 1985). This has led to soybean becoming an increasingly important crop in Nigeria, spreading especially to large parts of the Guinea savanna where it is well adapted (Smith et al. 1993). However, some studies have shown responses of the promiscuous soybeans to inoculation (Sanginga et al., 2000; Osunde et al., 2003), since it may not be possible to have a cultivar that nodulates effectively with indigenous rhizobia in all locations (Sanginga et al., 2000). Hence, recent research mostly dwell on the evaluation of the responses of the promiscuous soybean cultivars to inoculation with selected indigenous and imported inoculant stains along with other commercial products, with records of quite good responses (Yusuf et al., 2012; N'cho et al., 2013). Few other studies focused on groundnuts, cowpea and bambara groundnuts; which show sporadic responses (Bala, 2011) due to their long adaptation and compatibility with indigenous rhizobia that naturally nodulate with them, being promiscuous.

This paper attempted to review the history of the use of inoculants in Nigeria as a sub-Saharan African country relative to developed countries, with a view to highlighting problems and ways of future improvement of the situation. Particularly, how soybean as an introduced crop, took over the scene relative to other grain legumes, and how the efforts by Nigerian researchers on inoculants ended mostly without reaching the farmers. Finally, the glad tidings brought by N2Afica project on inoculants development and use that will hopefully lead the smallholder farmer a long way from poor income, food insecurity and N degraded soils, not only in Nigeria, but Africa in general.

Rhizobia inoculants and their use in the world, sub-Saharan Africa and Nigeria up to 1970s

Since the discovery of BNF in the late 19th century, research works have been reported on its potentiality as an alternative to inorganic N-fertilizer in agriculture (Bala, 2011a; N'cho et al., 2013). This knowledge soon led to the practice of inoculation, with early adoption achieved by transferring soil from field to field, or soil to seed before planting. However, this was quickly replaced by the use of pure cultures on agar slants, and later as broths (Bala, 2011a). Hence, rhizobia inoculants - used to deliver nitrogen fixing bacteria (collectively termed rhizobia), have been on the commercial market for over 100 years in many developed countries (Nelson, 2004; Giller, 2008; GRDC, 2013). There are nearly 70 million tonne of soybean inoculated annually with *Bradyrhizobium japonicum* in the USA, in addition to 34 and 53 million tonne in Argentina and Brazil, respectively (Bala, 2011a). The benefits of inoculation coupled with the application of deficient nutrients, mostly phosphorus vary with location and soils (Figure 1). Inoculating legumes with rhizobia has been used to achieve substantial increases in legume nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels over this period. These gains are usually highest when the inoculated legumes are grown in nil-rhizobia or low-rhizobia soils, but marginal in soils already containing high number of compatible rhizobia (GRDC, 2013).

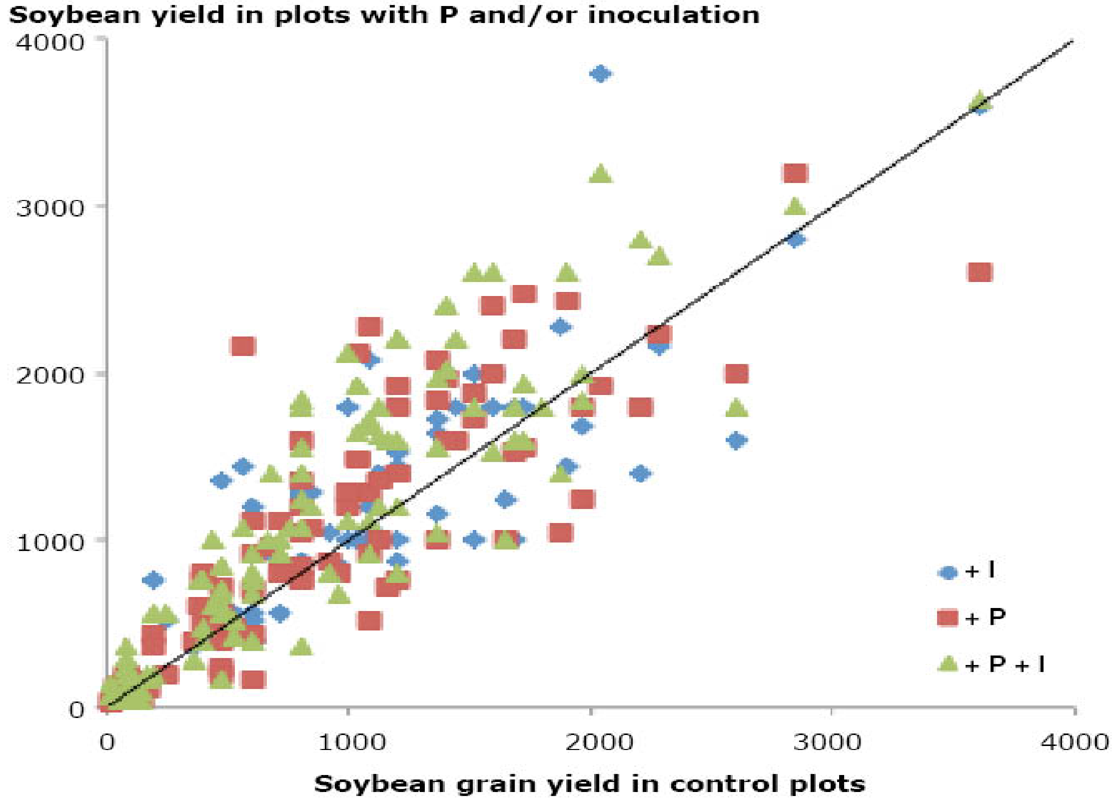

Figure 1. Range of responses to *Bradyrhizobium* inoculation (I) and phosphorus (P) application. Adapted from Abaidoo *et al.* (2013)

Inoculation activities have been on-going in sub-Saharan Africa since the 1950s, mostly on soybean and forage legumes; however, adoption of inoculation on a commercial scale has not been appreciable, except in a few countries, such as Zimbabwe and South Africa, where commercial farms dominate the agricultural sector (Bala, 2011a). Kang (1975) and Rhoades and Nanju (1979) confirmed the importance of soybean inoculation with *B. japonicum* strains in Nigeria. Response of soybean to inoculation in tropical areas like Nigeria as influenced by several adverse factors including high rainfall, high temperature, drought, salinity, acidity and low level of soil mineral nutrients was also established by Freire (1977). Indigenous *Bradyrhizobium japonicum* strain IRj 2180A has been isolated from soybean since 1979 and used for inoculation of soybean (Okogun and Sanginga, 2003; Muhammad, 2010; Yusuf et al., 2012b). The breeding programme to introduce promiscuous soybean was initiated in IITA, Ibadan Nigeria, within this period in 1977 (Sanginga et al. 2000), which later manifested in the 1980s.

Within the period, cowpea rhizobia were confirmed to be fairly well distributed in most Nigerian soils and native rhizobia seemed as effective as rhizobium strains tested at the IITA, Ibadan, Nigeria. Hence, inoculation gave good response with cowpea only in extremely sandy and acidic soils, poor in organic matter and plant nutrients as well as when the native rhizobia population is abundant but ineffective. These observations were made in a study by IITA in 1975 on the competition between indigenous and introduced bacteria in some soils (e.g. Apomu soil), where the soil was inoculated with CIAT 79 *Rhizobia* strain, but no nodule was formed by the inoculant, which was attributed to severe competition from native rhizobium strains existing in the soils (Balasubramanian, 1978).

Research on the use of rhizobia inoculants in Nigeria in the 1980s

Research on use of inoculants in Nigeria in this period was predominantly taken over by the introduction of promiscuous soybean cultivars by IITA Ibadan, Nigeria. Active field studies on soil microbiological aspects of soybean inoculation stopped at IITA, Ibadan Nigeria in 1983. Apparently, none of the *Bradyrhizobium* strains that were used before 1983 has been tested for compatibility with recent selections from breeding programmes (Sanginga et al., 1996). The breeding programme at IITA led to the introduction of “promiscuous” soybean cultivars; Tropical Glycine Cross (TGX), that nodulate freely with indigenous rhizobia (Kueneman et al., 1984; Pulver et al. 1985). The cultivars were developed with the aim of providing high yielding adapted germplasm able to nodulate with indigenous rhizobia in African soils to avoid dependence of soybean production on imported *B. japonicum* inoculants, hence, relieve the farmers from difficulties in obtaining the inoculants (Mpepereki et al., 2000; Osunde et al., 2003). The objective of combining the ability to nodulate with strains of indigenous rhizobia to

African soils, derived from soybean genotypes of Asian origin, with high yielding potential of cultivars bred in North America were thus achieved (Giller, 2001). Despite the problems with the initial nodulation in some soils, the promiscuous soybean cultivars have been widely adopted by farmers in different parts of Nigeria (Manyong et al., 1998; Sanginga et al., 1999). The cultivars nodulate with indigenous rhizobia, presumably strains of *Bradyrhizobium* spp. which nodulate legumes in the "cowpea cross-inoculation" or "cowpea miscellany" group, thus circumventing the need to inoculate with *B. japonicum* (Pulver et al., 1982; 1985).

Thereafter, studies on inoculation in the country turned to evaluation of the response of the promiscuous soybean cultivars to additional inoculation. This is because of the impossibility of having a cultivar that nodulates effectively with indigenous rhizobia in all locations (Sanginga et al., 2000; Osunde et al., 2003). Several of such studies have shown positive responses to inoculation by the cultivars (Sanginga et al. 1996, 2000; Yusuf et al, 2012b). For example; three different experiments were conducted in wet seasons within the period 1982-88 by the Institute for Agricultural Research (IAR), Samaru in the northern Guinea savanna zone of Nigeria to test the effect of inoculation, nitrogen and phosphorus fertilization on some promiscuous and specific soybean cultivars. The first experiment tested the effect of nitrogen (N) fertilization between 1982-84 using two soybean cultivars of Malaysian origin (SAMSOY-1 and SAMSOY-2) which were sown with or without *Bradyrhizobium japonicum* inoculation. The inoculant was a peat culture of *B. japonicum* IRj 2123 (USDA 311b138) with inoculation carried out every year. The second experiment involved four strains of *B. japonicum* (IRj 2114, IRj 2123, IRj 2133 and IRj 2144) tested on five soybean cultivars, three of which are promiscuous (TGx 536-02D, TGx 814-26D and TGx 888-49C), with the two cultivars (SAMSOY-1, SAMSOY-2) of Malaysian in origin. The cultivars were grown under seven inoculation or nitrogen (N) treatments. The final experiment evaluated the effect of nitrogen and phosphorus fertilization on the response of the cultivar SAMSOY-2 to *B. japonicum* inoculation during the 1986-88 using a mixture of *B.*

japonicum strains IRj 2133 and IRj 2144**.** The results indicated *B. japonicum* strain IRj 2123 to significantly increase nodule number and weight. Four of the strains including IRj 2123 when further evaluated either singly or in a mixture improved growth and seed yield of the cultivars relative to the uninoculated control (Olufajo and Adu, 1992).

A different example featuring other legumes was a study on cowpea inoculation by Oloke and Odeyemi, (1988) involving three locally made carriers; lignite, sub bituminous coal and cow manure inoculated with *Bradyrhizobium* compared with peat based imported *Bradyrhizobium* inoculants in two- field studies. The local inoculants were prepared by incorporating three rhizobia strains (Ife CR9, Ife CR15 and *Bradyrhizobium japonicum*) each into one of the carrier materials and were used to inoculate three cowpea varieties; TVU 1190, IT82E-60 and Ife brown. The local inoculants; with lignite, sub bituminous coal and cow manure as carriers increased cowpea yield 72, 54 and 10% respectively, compared to uninoculated control plants.

Studies on rhizobia inoculants in Nigeria from 1990s to date

Studies in this period were similarly dominated by evaluation of the response of promiscuous cultivars of soybean to inoculation, usually along with other relevant deficient nutrients (Figures 2 and 3), which sometimes turn positive, depending on cultivars and nutrient requirements of the soils, along with few studies on other legumes. Some studies featured both the specific and promiscuous soybean cultivars. For example; response of two soybean cultivars a Malaysian (SAMSOY 2) and TGX (TGX 1448-2E) to *Bradyrhizobium* inoculation with a mixture of R25B and IRj 2180A rhizobial strains tested in the northern Guinea savanna of Nigeria, in a researcher - managed on-farm trial. SAMSOY-2 was not significantly affected by the treatment in terms of vegetative parameters such as shoot and root weight, nodule number, biological nitrogen fixation and grain yield. This was attributed to possible high indigenous rhizobia population, adequate for soybean nodulation. However, significant effect of the treatment was observed in root biomass of TGX 1448-2E. The BNF and grain yield of the promiscuous cultivar was significantly higher than that of SAMSOY-2, showing that varietal differences masked the effect of inoculation (Okogun et al., 2004).

Other studies featured only the promiscuous soybean cultivars; for instance, varying responses of some promiscuous soybean cultivars to inoculation, phosphorus and nitrogen were observed in Kano state, Nigeria (Figures 2 - 4) as reported in trials conducted by Anne et al. (2011). A similar study was conducted on an early maturing promiscuous cultivar (TGX 1485) inoculated with one of four rhizobial strains (R25B, IRj 2180A, IRc 461 and IRc 291) at Minna Nigeria, in the southern Guinea savanna of Nigeria. The four rhizobial inoculants increased all parameters including grain yields over those of the control.

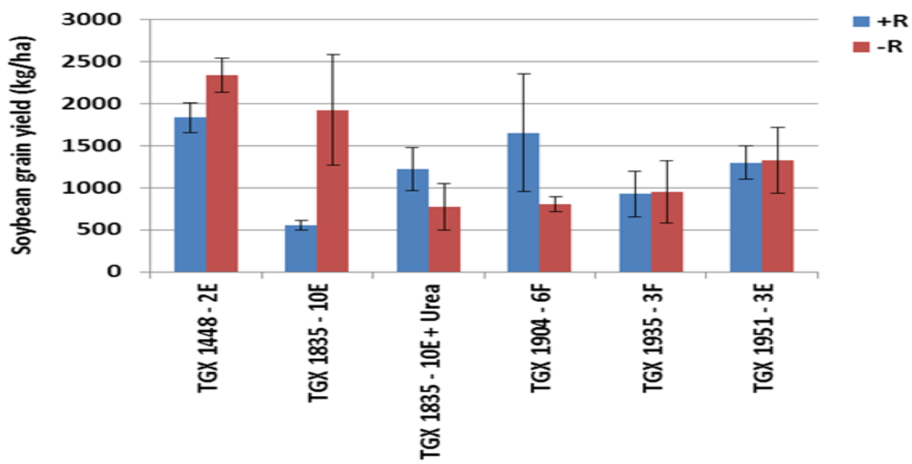

Figure 2. Soybean grain yield as affected by variety and the use of inoculants (R) at Bichi, Kano State, Nigeria, in 2010. Error bars represent standard errors of means. Adapted from Ann et. al. (2011).

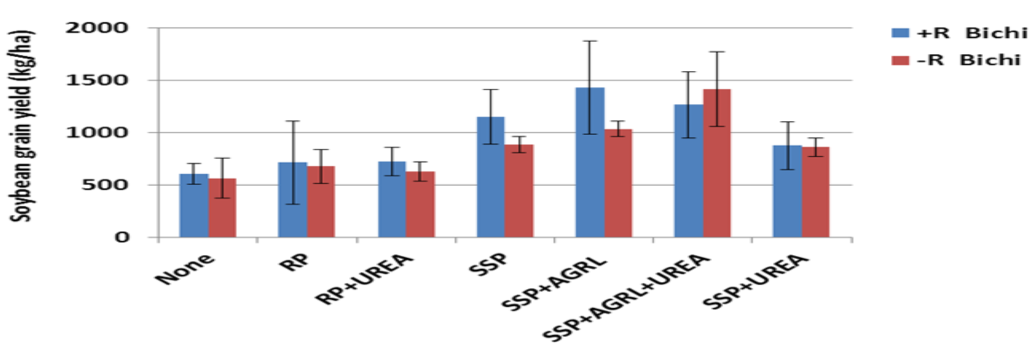

Figure 3. Response of soybean grain yield to nutrient inputs and inoculation (R) at Bichi in Kano State, Nigeria, in 2010. Error bars represent standard errors of means. Adapted from Anne et al. (2011)

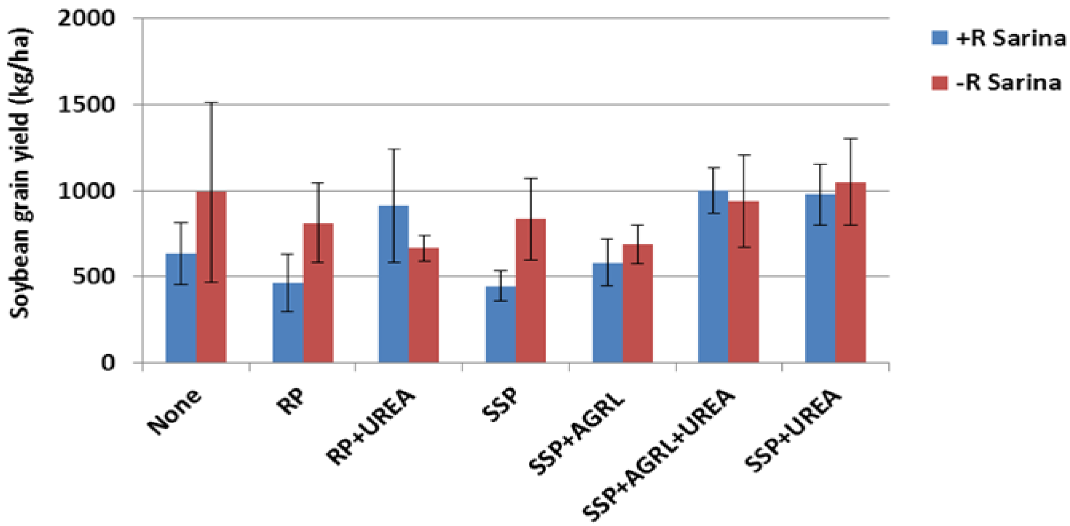

Figure 4. Response of soybean grain yield to nutrient inputs and inoculation (R) Sarina in Kano State, Nigeria, in 2010. Error bars represent standard errors of means. Adapted from Ann et al. (2011)

Inoculant strain IRj 2180A produced yields comparable to plots supplied with 60 kg N ha^{-1} (Muhammad, 2010). Another study was on two cultivars, TGX1456-2E and TGX1660-19F tested by Osunde et al. (2003) in a 2-year trial for their response to rhizobia inoculation in five farmers' fields within a 60-km radius of Minna town, in the Southern Guinea savanna zone of Nigeria. Three treatments were imposed only in the first cropping season; uninoculated control, inoculation with a mixture of *Bradyrhizobium* strains R25B and IRj 2180A, and 60 kg N ha^{-1} control. The inoculant strains appeared to be less competitive, but more effective, than indigenous populations. The proportions of nodules occupied by the inoculant strains were 17% in the first cropping season, and 24% in the second. However, inoculation increased grain yield by 40% in the first cropping season, while no such yield differences occurred in the second season. As well the proportion of nitrogen derived from N_2 fixation ranged from 27% to 50% in both cropping seasons (Osunde et al., 2003).The effect of inoculation on N_2-fixation and biomass in the study is shown in Table 1. A different study examined the symbiotic effectiveness of two promiscuous soybean breeding lines (TGX 1660-19F and TGX 1456-2E) and a cowpea cultivar (IT 849-92) using twenty *Bradyrhizobia* strains isolated from soybean and cowpea in Ibadan and Zaria soils in Nigeria, in pot experiments. Two *Bradyrhizobia* isolates (R25B and IRj 2180A) had an average symbiotic effectiveness (SE) of 2.36-fold and 1.62-fold of the uninoculated control when inoculated on TGX 1456-2E and TGX 1660-19F, respectively. These strains, however, were less effective on cowpea having an SE of 1.20-fold. The best isolates (R25B, IRj 2180A and their mixture) and one cowpea *Bradyrhizobia* (IRc 461) were further tested on these lines under field conditions at three sites in different agro ecological zones in the moist savanna (Fashola, Mokwa and Zaria) of Nigeria. The mixture of *Bradyrhizobia* isolates R25B and IRj 2180A increased grain yield of 1456-2E by 30 and 25% at Zaria and Mokwa relative to the control but failed to do so at Fashola (Sanginga *et al.*, 2000).

On the other hand a study tested the effect of microbial inoculants and foliar fertilizers application on promiscuous soybean (TGx 1448-2E) under smallholder farmers' conditions in the northern Guinea savanna, of Nigeria. Three microbial inoculants; *Bradyrhizobium* spp. (RACA 6), arbuscular mycorrhizal fungi (Rhizatech) and *Trichoderma harzianum* (Eco-T) and two foliar fertilizers; Agroleaf high P and Agolyser were used. Grain yield was relatively increased by the application of RACA 6 + TSP, RACA 6 + Agrolyzer and RACA 6 + Rhizatech + Agrolyser compared to the control. The authors suggested that soybean co-inoculation with rhizobial or fungal inoculants and/or foliar fertilizers could lead to improved grain yields (Ncho et al., 2013). A similar study was conducted for screening of 15 commercial and laboratory rhizobium inoculants in Kadawa (Sudan savanna) and Samaru (northern Guinea savanna) to identify effective and promising products on a promiscuous soybean genotype (TGx 1448-2E) and a Malaysian genotype (SAMSOY-2). Application of three commercial products and seven strains resulted in increased nodulation relative to the control in Kadawa, while two commercial products and six strains increased nodulation, but only two strains resulted in significant increases in biomass and/or grain yield. The authors recommended 1495 MAR, USDA 4675, USDA 110, TSBF 531 and TSBF 560 as effective inoculants to consider for improved grain yield in Samaru (Yusuf et al., 2012b).

Table 1. Total N fixed and shoot biomass of promiscuous soybeans as affected by farmers' fields, inoculation and cultivars in two cropping seasons. Adapted from Osunde et al. (2003).

	N_2 fixed (%)		Shoot biomass (g/plant)	
	1999	2000	1999	2000
Farmers' field				
Bosso	46.27a	32.47b	54.29c	59.90d
Shakodna	30.28a	36.04b	124.66ab	90.79bc
Vemu	43.89a	47.91b	139.74a	73.51cd
Numui	45.55a	59.85ab	132.26a	123.06a
Gidan Kwanu	50.38a	76.25a	119.78b	107.80ab
Inoculation				
Uninoculated	46.00a	49.22a	68.31c	90.13a
Inoculated	49.70a	48.66a	99.97a	90.56a
60 kg N ha^{-1}	25.90b	45.96a	81.72b	92.73a
Cultivars				
TGX 1456-2E	42.51a	51.97a	98.45a	103.48a
TGX 1660-19F	38.56a	43.92a	70.26b	80.05b
Interactions	NS	NS	NS	NS

For each treatment, values within the same column followed by the same superscript are not significantly different (P>0.05; NS Not significant).

Other legumes were not left out in research at this period. A study was conducted in Awka Anambra state, Nigeria to determine the suitability of decomposed rice husk, charcoal and coal to act as carrier materials for cowpea *Bradyrhizobia* inoculants, using TAL 209, TAL 379 and TAL 173 strains imported from US. The study showed decomposed rice husk as better carrier materials. A follow up pot experiment also confirmed the ability of *Bradyrhizobia* in decomposed rice husk to nodulate, increase shoot dry matter and total N in cowpea in different soils relative to uninoculated controls (Okereke and Okeh, 2007). Inoculation trials were also conducted on Eutric Cambisols (EC) and Rhodic Nitisols (RN) soils in a greenhouse study. Soybean (TGx 1448-2E), cowpea (IT90K-277-2) and groundnut (SAMNUT 21) were used as test crops along with rhizobial inoculants (MAR 1495, TSBF Mixture, Legumefix, HiStick and IRj 2180A) to determine their response to soil type and ability to form symbiotic relationship with the crops. Rhizobia strains MAR 1495 and TSBF mixture showed similar ability to improve the productivity of soybean and groundnut and thus recommended for use as common inoculants for the two crops (Aliyu et al., 2013). The influence of rhizobia inoculation on N-fixation by cowpea (*Vigna unguiculata* (L.) Walp.), groundnut (*Arachis hypogaea* L.) and bambara groundnut (*Vigna subterranea* L.Verdc.) was evaluated under field condition in Maiduguri, Sudano-Sahelian zone of Nigeria. Strains isolated from the same crops the previous year were used to inoculate them the next year. The results showed rhizobia inoculation to increase the amount of N- fixed by 46% over the control. Cowpea fixed 42.68 kg N ha^{-1} while groundnut and bambara groundnut fixed 27.19 and 32.53 kg N ha^{-1}, respectively. This indicates a higher potential of cowpea to alleviate soil nitrogen deficiency over the other legumes, particularly where the indigenous rhizobia nodulating the crops are inadequate (Yakubu et al., 2010). A field trial was also conducted in 2010 to evaluate the effects of three P sources and three rhizobium inoculants on the yield and yield components of groundnut in the northern Guinea savanna of Nigeria using rhizobium inoculants; biofix, vault and mixture of the two.

There was no significant difference in nodulation and shoot dry matter yield between inoculated and uninoculated plants. Similarly, there were no significant differences in pod and haulm yields among the rhizobium inoculants. Instead, the uninoculated plants produced significantly higher pod (20%) and haulm (28%) yields than the average yield of the inoculated plants (Table 2).

Table 2. Effects of rhizobium inoculation and P sources on nodulation, biomass, haulm and pod yield of groundnut at Samaru, Zaria, Nigeria. Adapted from Yusuf et al. (2012a).

Treatment	Nodule number plant^{-1}	Nodule dry weight (mg plant^{-1})	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Haulm yield (kg ha^{-1})	Pod yield (kg ha^{-1})
Rhizobium Inoculant (I)						
Biofix	150	262	18.25	2.47	3436	1029
Vault	132	257	17.74	2.34	3202	1015
Biofix + Vault	150	237	16.18	1.96	2910	934
No inoculation	129	256	18.30	2.30	4071	1188
Mean	140	251	17.62	2.27	3405	1042
SED	19.17	28.00	1.19	0.19	281.51	65.07
P-Source (P)						
Agroleaf	140	269	18.62	2.19	3349	1082
SSP	126	224	16.81	2.11	3583	1068
TSP	139	252	16.39	2.37	3468	1044
No P	155	267	18.65	2.40	3219	973
Mean	140	251	17.62	2.27	3405	1042
SED	19.17	28.00	1.19	0.19	281.51	65.07
I x P						
SED	38.33	55.15	2.38	0.38	563.03	130.14

The authors attributed the results to long history of groundnut cultivation, necessitating the need to introduce more competitive and efficient strains of *Rhizobium* to ensure adequate N_2 fixation for maximum growth and yield of the host plant (Yusuf et al., 2012a).

Reasons why research on inoculants and their use in Nigeria is mostly on soybean

A number of reasons could be responsible for the soybean domination of inoculants research in Nigeria. More than 90 percent of rhizobial inoculants worldwide are used on soybean, which differs from many other tropical legumes such as cowpea, groundnut and common beans for its “specific” requirements in terms of the type of rhizobia able to form nodules on its roots and actively fix nitrogen. Moreover, their positive response to inoculation is more frequent, relative to other grown legumes in the country. Soybean - *Bradyrhizobium* symbiosis could lead to high increases in yield and fix high amounts of nitrogen. It has been reported to fix up to 300 kg N ha $^{-1}$ under good conditions and the proportion of N derived from fixation varies substantially from zero to as high as 97%, with most estimates falling between 25% to 75% (Keyser and Li, 1992). Moreover, soybean is becoming one of the most cultivated grain legumes in sub-Saharan Africa, since it has the greatest potential of producing the cheapest source of food protein and other essential nutrients for farm households (Rao and Reddy, 2010).

Other legumes form nodules with a wide range of rhizobia that are present in most soils, termed “promiscuous” or “naturally-nodulating” grain legumes, and make effective use of the inherent soil biodiversity of rhizobia (Giller, 2008). Inoculation of cowpea and groundnut was met with sporadic reports of positive responses with cowpea, and less evidence of responses with groundnut. This makes the crops urgently needing research and promotion of inoculants to arrive at the highest quality of both strains and formulations (N2Africa, 2012).

Factors responsible for lack of adoption of inoculants research results by Nigerian farmers

Many factors are responsible for lack of adoption of the few existing results of inoculant studies by farmers not only in Nigeria but West Africa in general, though some attempts have been made. Most of the rural poor population, who live in sub-Saharan Africa practice subsistence agriculture on marginal soils, do not have access to or cannot afford high capital investments in agriculture and lack access to knowledge on how to improve their situation (Pretty, 1999). Investment on inoculants is not affordable by most farmers, because countries in sub-Saharan Africa do not have industries to produce viable inoculants at affordable prices to smallholder farmers, with only few exceptions, (Kueneman *et al*., 1984) and up to date the situation has only little improvements. Generally, the production and distribution of inoculant in many parts of Africa is difficult (Keyser and Li, 1992). West Africa lacks large-scale commercial production of soybean and intensive livestock industry, two major elements that appear to drive widespread adoption of inoculants and inoculant technology (Bala et al., 2011). This could be because soybean is a relatively new crop in the region. Although the area of cultivation continues to expand rapidly, virtually all the soybean grown is the promiscuous cultivars, which readily nodulate with indigenous rhizobia. Hence the incentive for inoculant adoption is minimal (Bala *et al*. 2011). The percentage use of inoculants and/or phosphorus on three important legumes among Nigerian farmers, indicating very low adoption inoculants and on soybean alone is shown in Table 3.

The situation is however, hopeful since farmers are very responsive and keen to get hold of new legume cultivars. They were often surprised to see large growth responses to small applications of phosphorus fertilizer throughout the region. Responses of soybean to inoculation are sometimes massive - more often with at least 20% increase in biomass (Giller, 2010). Although low literacy level in rural areas of both Ghana and Nigeria provide challenges for learning and dissemination, farmers in Ghana called rhizobium inoculum “black medicine” - but were proud to dig up plants and explain that the inoculum led to formation of nodules that could capture nitrogen from the air (Giller, 2010).

Table 3. The percentage of farmer using specific legume inputs in their own fields, based on data from the N2Africa impact survey in Nigeria. Extracted Huising and Franke, 2013.

Crops	No use of inputs	+ inoculant only	+ P fertilizer	+ P fertilizer and inoculant
Soybean	6	11	57	26
Cowpea	18	-	82	-
Groundnut	24	-	76	-

Recent giant strides of N2Africa project towards production and use of inoculants in Nigeria and Africa.

The N2Africa project is a research-and-development partnership program that aims to develop, disseminate, and promote appropriate N_2-fixation technologies for smallholder farmers, focusing on major grain legumes (Abaidoo et al., 2013). Particularly objective 4 phase I of the project focused on delivery of legume inoculant technologies to farmers throughout sub-Saharan Africa, including Nigeria. It is targeted at influencing farming households to participate in inoculant technology dissemination activities providing a nucleus for farmer-to-farmer adoption and creating sufficient demand to reinforce private sector investment in legume and inoculant technologies. The project is particularly supporting an initiative to establish inoculant production facilities in Nigeria where soybean is produced by millions of smallholder farmers, and where there is confidence of a large future growing market (Giller, 2012). Inoculant production is accessed to be almost nil in Nigeria. Hence their use is minimal and usually restricted to imported products. The history of implementation in eight N2Africa project operating countries could be seen in Table 4. The project collaborates with Institute for Agricultural Research (IAR), Ahmadu Bello University, Samaru, Zaria, Nigeria. The intention is to boost value chain promoters of legume crop in the country such as Grand Cereals Limited, Jos; the largest soybean and groundnuts processing company, Sunseed Nigeria PLC, Zaria; processing soybean, Fortune Oil Mills Nigeria Limited, Kano; processing groundnut and soybean, Yakasai Oil Mills Kano; processing groundnut and soybean, among others (Bala and Rusike, 2011). Their existence led to the current trend and increasing demand of the major grain legumes in the country as indicated by future projections as presented by Tropical Lugume II project (TLII) (2011) (Tables 5 and 6). Foreign exports are also in view .

Table 4. Partner laboratories involved in N2Africa Rhizobiology activities. Adapted from Bala, (2011)

S/No	**Country**	**Laboratory**	**Location**	**Year Established**	**Inoculant Production**	**Quantity Produced in 2010**
1	DRC	Soil Microbiology Laboratory	Bukavu		No	None
2	Ghana	Soil Research Institute	Kumasi		No	None
3	Kenya	MIRCEN/University of Nairobi	Nairobi	1977 (1981)	YES	ND
4	Kenya	MEA Fertilizer Ltd.	Nakuru	1977 (2008)	YES	25,000
5	Malawi	Chitedze Research Station	Chitedze, Lilongwe	1964	YES	15,000
6	Nigeria	Institute for Agricultural Research (IAR)	Samaru, Zaria	1922	No	None
7	Rwanda	Institut des Sciences Agronomique du Rwanda (ISAR)	Robena	1984	YES	ND
8	Mozambique	IIAM	Nampula	Under Construction	No	None
9	Zimbabwe	Soil Productivity Research Laboratory (SPRL)	Marondera	1964	YES	80,000

Year in parenthesis is the year inoculant production commenced; ND = Not determined

Table 5: Trends of major grain legumes of Nigeria. Adapted from TLII (2011)

Crop	Area		Grain Yield		Production	
	1000 HA	*ROG (%)	kg/ha	ROG (%)	1000 MT	ROG (%)
Cowpea	2,207	-3.95	700	0.94	1,546	-3.01
Groundnut	2,159	-0.82	1191	-0.04	2,571	-0.85
Soybean	581	0.91	910	5.28	529	6.24
Total/Average	4,947	NA	NA	NA	4,646	NA

*ROG-Annual Rate of Growth

Table 6: Projected production and demand for cowpea, groundnut and soybean in Nigeria. Adapted from TLII (2011)

Comodity	Production (1000MT)			Demand (1000MT)		
	2010	2015	2020	2010	2015	2020
Cowpea	2,761	3,364	4,097	4,092	5,273	6,906
Groundnut	3,275	3,563	3,784	3,335	3,497	3,726
Soybean	633	709	793	643	748	869
Total	6,669	7,536	8,674	8,070	9,518	11,501

N2Africa, however considers a number of reasons towards the establishment of inoculant industries in participating countries such as Nigeria as outlined by Giller (2012); the current market volume is too small to make an inoculant production plant a viable economic enterprise, the skills base in terms of both scientist and technicians trained in rhizobiology for inoculant production and quality control is too weak, hence initial research will be needed to test, select and refine carriers and develop appropriate formulations. Finally, West Africa has only one rainy season a year. This implies that to produce inoculants, with shelf-life of only six months, staff would have to be paid for half of the year without production to maintain their expertise. This will be necessary to keep the factory productive, unless the facilities will be used to make other related products. Thus focus is now on import of high quality inoculants, ensuring an effective supply chain for inoculants in the areas where N2Africa is working until there is sufficient demand to warrant establishment of local production (Giller, 2012). Efforts have also been dedicated to capacity building, in terms of training African MSc and PhD students from various countries in recognized institutions on inoculants and related technologies.

Future outlook on inoculants production and use in Nigeria

Establishment of effectively nodulated legumes cannot be left to chance but requires the introduction of effective strains of rhizobia into the soil at sowing (Okereke et al., 2001). Knowledge of the indigenous rhizobia nodulating legumes is limited while their symbiotic properties may differ among locations. There is the need for a holistic approach to improve the entire cropping systems, which includes selection of more competitive and efficient indigenous rhizobia that could serve as local inoculants (Machido et al., 2011; Sanginga, 2003). Oloke and Odeyemi, (1988) screened several local cowpea *Bradyrhizobia* strains for effectiveness in search for cowpea inoculants in West Africa, especially Nigeria. Some locally available base carriers were assessed for their availability of support and prolong the shelf life of rhizobia, towards that direction. Machido *et al*., (2011) recommended establishing a database on the occurrence, abundance, distribution and composition of indigenous populations of rhizobia in soils, identifying promising strains from among the native population that could be used as exotic inoculants strains. They also recommended identifying crop combinations, sequences and rotations that would take maximum advantage of the N_2-fixing potentials of different legumes under continuous cropping system as practiced by particular farmers as a solution to the problem of inoculants development and use in Nigeria. A summary of the history of inoculants use in Nigeria is shown in Table 7.

Table 7. Summary of the History of the use/studies on inoculants in Nigeria relative to world.

Period	Landmark	References
Late 19th century to 1970s	Biological Nitrogen Fixation (BNF) discovered as an alternative to inorganic N-fertilizer in agriculture, this led to the practice of rhizobia inoculation technology	Bala, 2011a; N'cho et al., 2013.
	Inoculants exists in commercial market over 100 years ago in developed countries	Nelson, 2004; Giller, 2008; GRDC, 2013
	Inoculation activities in sub-Saharan Africa date back to the 1950s, mostly in in commercial scale only Zimbabwe and South Africa.	Bala, 2011a
	Importance of soybean inoculation with *B. japonicum* strains in Nigeria recognized in 1970s.	Kang, 1975, Rhoades and Nanju, 1979
	Breeding programme for promiscuous soybean initiated in IITA, Ibadan Nigeria in 1977.	Sanginga et al., 2000
	Cowpea rhizobium was observed to be well distributed in Nigerian soils and native rhizobia seemed as effective as rhizobium strains tested such as CIAT 79.	Balasubramanian, 1978
	Indigenous *Bradyrhizobium japonicum* strains IRj 2180A isolated from soybean in 1979 and used for inoculation of soybean.	Okogun and Sanginga, 2003, Yusuf et al., 2012b
1980s	Introduction of "promiscuous" soybean cultivars; Tropical Glycine Cross (TGX) to ease farmers' difficulties of in obtaining inoculants, among other advantages (Refer and add reference).	Kueneman et al., 1984; Pulver et al. 1985, Mpepereki, 2000, Osunde et al., 2003.
	Active field studies on the soil microbiological aspects of soybean inoculation stopped at IITA, Ibadan Nigeria in 1983	Sanginga et al., 1996
	None of the *Bradyrhizobium* strains that were in use before 1983 have been tested for compatibility with recent selections from the breeding programmes then.	Sanginga et al., 1996
	Evaluation of promiscuous soybean (TGX) cultivars' response to inoculation 1982-1984 at IAR, ABU, Zaria using IRj 2114, IRj 2123, IRj 2133 and IRj 2144 strains N and P application.	Olufajo and Adu, 1992
	Inoculation of cowpea with Ife CR9, Ife CR15 and *Bradyrhizobium* strains	Oloke and Odeyemi, 1988
1990 to date	Evaluation of the response of TGX and other cultivars to inoculation, P, N and other deficient nutrients in rhizobia inoculants and other commercial products. Strains used include; R25B, IRj 2180A, IRc 461 and IRc 291, 1495 MAR, USDA 4675, USDA 110, TSBF 531 and TSBF 560, RACA 6.	Sanginga et al., 1996, 2000, Osunde et al., 2003, Okogun et al., 2004, Muhammad, 2010, Anne et al., 2011, Yusuf et al., 2012a , Yusuf et al, 2012b, Ncho et al., 2013
	Inoculation of other legumes; cowpea, groundnut and banbara groundnut at the period.Strains used include; MAR 1495, TSBF Mixture, Legumefix, HiStick , TAL 209, TAL 379 and TAL 173	Okereke and Okeh, 2007, Yakubu et al., 2010, Yusuf et al., 2012a, Aliyu et al., 2013
	Inoculation of cowpea with strains Ife CR9, Ife CR15	Oloke and Odeyemi, 1988
	N2Africa project initiated in 2010, awareness of N_2 fixation, inoculants and phosphorus application among farmers and extension workers. Collaboration with Institute for Agricultural Research, Ahmadu Bello University, Zaria for capacity building and effort to introduce local and imported inoculants.	Giller, 2010, Giller, 2012, Abaidoo et al., 2013
Future out look	Need for a holistic approach to improve legume cropping systems through isolation and selection of effective strains to develop local inoculant for each crop.	Machido et al., 2011; Sanginga, 2003, Okereke et al., 2001, Oloke and Odeyemi, 1988.
	Need to meet demand of increasing capacity of value chain promoters	Bala and Rusike, 2011

Conclusion

The use of rhizobium inoculants for improvement in N-fixation and productivity of grain legumes, though established in developed countries over a long time, is still in the developing stage in most parts of sub-Saharan African countries like Nigeria. The soils used for legumes production in the region are poor, especially in total-N while inorganic fertilizers, which rank first among the external inputs to maximize output in agriculture, are not affordable by most farmers. Biological nitrogen fixation is a better alternative N source, through the use of rhizobia inoculants. Although legume inoculation activities started in sub-Saharan Africa since the 1950s, mostly on soybean and forage legumes, research on inoculation in Nigeria, however started only in the 1970s mostly also on soybean. Promiscuous soybean cultivars (TGX) were then introduced by IITA, Ibadan, Nigeria in the 1980s, which took over research on inoculants to date. Not much studies have been conducted other legumes such as cowpea, groundnut and bambara groundnut and show sporadic responses because of their promiscuity, long adaptation and compatibility with indigenous rhizobia. Results of the Studies end mostly in the hand of researchers, without reaching the farmers. The intervention of N2Africa project towards facilitating the production and use of inoculants by small holder farmers in Nigeria and Africa in general is thus a highly welcomed development that could shortly transform the situation.

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