Chapter 9
Prospects for Developing Effective and Competitive Native Strains of Rhizobium Inoculants in Nigeria

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9.1 Introduction

Most legumes possess a unique ability to fix N_2 through a mutualistic relationship with root nodule rhizobia, which are unique soil- and nodule-living bacteria (Nyoki and Ndakidemi 2013). This interaction could be advantaged to enhance crop yield especially in sub-Saharan Africa (SSA) (Osei et al. 2018), where yields are below their expectation (Abaidoo et al. 2013). Farmers in this region have traditionally used chemical fertilizers in the past centuries for improved crop yields. However, they realized that such fertilizers affect soil fertility negatively by hampering many beneficial microorganisms that positively enhance the growth and yield of crops. These chemical fertilizers detrimentally affect humans as well. Biofertilizers thus became an alternative, as they were eco-friendlier to both the environment and farmers (Devi and Sumathy 2018). Biofertilizers do not contain environmentally toxic substances and readily enrich the soil, and therefore their use safeguards soil health. Microbial inoculants can play an increasingly significant role in the agricultural advancement of developing countries (Alori and Babalola 2018). Using an effective and persistent *Rhizobium* strain would reduce or eliminate the need for synthetic nitrogen-based and other chemical fertilizers (Baez-Rogelio et al. 2017).

9.2 Historical Perspective

The inoculation of plants with beneficial bacteria can be, however, traced back to antiquities (Bashan 1998). Farmers knew, from experience, that when they took soil from a previously legume-cropped area and mixed with soil in which nonlegumes

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were to be cultivated, yields often improved (Bashan 1998). The act of blending "inherently inoculated" soil with seeds turned into a prescribed method for legume inoculation in the USA by the end of the nineteenth century (Smith 1992). Since the commercialization of this soil enrichment approach, the practice of legume inoculation with rhizobia eventually has become common.

Inoculation with such non-symbiotic, associative rhizosphere bacteria, as *Azotobacter*, was utilized on a vast scale in Russia during the 1930s and 1940s, the outcomes of which were inconclusive leading to the approach being dumped later (Rubenchik 1963). An endeavor to utilize *Bacillus megaterium* for phosphate solubilization during the 1930s on a large scale also failed in Eastern Europe as reported by Macdonald (1989). Before embarking on a lengthy program of selecting inoculant strains of root nodule bacteria, it is vital to understand whether there is a need to inoculate and this can be achieved through three fundamental treatment experiments (Brockwell and Bottomley 1995; Brockwell et al. 1995; Date 2000). The lack of these experiments could have led to the failure of the large-scale historical trials, as many producers lack the appropriate background skills of adequately interpreting the results obtained (Date 2000).

In the late 1970s, two breakthroughs were experienced in plant inoculation technology. Firstly, *Azospirillum* was found to enhance nonlegume plant growth (Döbereiner and Day 1976), by a direct effect on plant metabolism (Bashan and Holguin 1997a, b). Secondly, biocontrol agents, mostly *Pseudomonas fluorescens* and *Pseudomonas putida*, were introduced and began to be intensively investigated by many researchers (Bashan 1998). Many works, including Kloepper (1994), Tang (1994), and Tang and Yang (1997), reported that different such other bacterial genera as *Bacillus*, *Flavobacterium*, *Acetobacter,* and a few *Azospirillum*-related microbes were thus additionally examined and evaluated.

Most soils used for leguminous crops production in Nigeria are nutrient-deficient, especially of total N, hence, their relatively poor productivity (Machido et al. 2011; Laditi et al. 2012). The soils are also usually low in available P and organic C, thereby making them even inherently worse in their fertility status (Yakubu et al. 2010; Machido et al. 2011). Several other biotic and abiotic factors like crops uptake and removal, denitrification, volatilization, and leaching further make the soils vulnerable to nutrient loss. In order to maintain or maximize agricultural productivity, amelioration of the depleted nutrients, primarily N and P, is paramount and is usually achieved by the application of environmentally less friendly mineral fertilizers (Udvardi et al. 2015; Song et al. 2017). Their staggering costs and a relatively lesser availability in the region (Sanginga 2003; Rurangwaa et al. 2018) pose another hitch to their judicious use by the resource-deficient farmers. These and related factors fuelled the drive towards biological nitrogen fixation (BNF), which has potentials for mitigating negative impacts associated with using mineral fertilizers (Yakubu et al. 2010). However, many soils lack adequate amounts of native rhizobia and some naturally occurring strains are lacking in terms of effectiveness or competitiveness, and fail to effectively achieve an enhanced BNF process (Westhoek et al. 2017) and hence the dire need for providing external sources of rhizobia to enable effective nodulation and consequent N_2 fixation, known as inoculation (Date 2000).

Legumes, on another hand, will generally only respond to inoculation where: (1) compatible rhizobia are absent, (2) the population of compatible rhizobia is small, and (3) the indigenous rhizobia are less effective in N_2 fixation with the intended legume than selected inoculant strains (Vanlauwe and Giller 2006). Although a commercial legume inoculants' production and use in the USA and the UK dated back to as early as 1895 (Nelson 2004), local production of this type of biofertilizer only started in the 1980s and 1990s in Africa (N2Africa 2013). There was, therefore, no regular use of rhizobial inoculants by West African mainstream farmers, including those of Nigeria (Bala 2011a). Since the introduction of these BNF inoculants, soybean production, for example, has continuously and dramatically increased in South Africa from 84,000 t, in 1987, to 1,320,000 t, in 2016. In Nigeria, an increase was also recorded from 40,000 t to 680,000 t in the same 1987 and 2016, respectively (Khojely et al. 2018).

9.3 Important Terms and Definitions

9.3.1 Bacterial Inoculant

Bacterial inoculant is a formulation that usually contains one or more beneficial bacterial strains (or species) in an easy-to-use and economical carrier material (Alori and Babalola 2018). The material can either be natural, inorganic, or derived from specific molecules. The inoculant is the means by which bacteria are transported from the industry to the living plant via the soil. The needed impacts of the inoculant on plant growth and development can be in the form of leguminous BNF, enhancement of mineral uptake, biocontrol of soil-borne diseases, weathering of soil minerals, and nutritional and hormonal impacts. Bacterial inoculants may, however, require bureaucratic and, hence, costly registration processes in several countries (O'Callaghan 2016).

9.3.2 Biofertilizer

This is a widely used term which also refers to a "bacterial inoculant." It also refers to preparations of microorganism(s) for a complete or partial substitution for chemical fertilization, for example, rhizobial inoculants. Many other effects of the bacteria on plant growth are, however, ignored. The word "fertilizer" is used in some countries to allow easier registration of the commodity for commercial use, as observed by Bashan (1998). The term biofertilizer (microbial inoculants) can generally be defined as any preparation that contains live or latent cells of efficient strains of nitrogen (N_2) fixing, phosphate solubilizing, or cellulolytic microorganisms used for application on seeds, soils, or composting materials/areas to increase the

populations of such microorganisms and hence accelerate a given microbial process and compliment the level of plant-available nutrients (Mohammadi and Sohrabi 2012). The term biofertilizer may, therefore, broadly be used to mean all organic resources (manure) used for plant growth and rendered into plant-available forms, which may be through microorganisms and plant associations (Akhtar and Siddiqui 2009). Biofertilizers are essential components of integrated nutrient management. These potential biological fertilizers play a crucial role in the productivity and sustainability of soil and also protect the environment (Mohammadi and Sohrabi 2012). They are cost-effective, eco-friendly, and a renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural systems (Malusá et al. 2012). Beneficial microorganisms in biofertilizers speed up and ameliorate plant growth and protect plants from pests and diseases (El-yazeid et al. 2007). The most common organisms used as biofertilizer component are nitrogen-fixers (N_2-fixers), potassium-solubilizers (K-solubilizer) and phosphorus-solubilizers (P-solubilizers), or in combination with molds or fungi. The bacteria used in biofertilizers mostly have a close relationship with plant roots. *Rhizobium*, for example, has a symbiotic relationship with legume roots, and rhizobacteria inhabit root surfaces or rhizosphere soil (Mohammadi and Sohrabi 2012).

9.4 Rhizobial Taxonomy

According to Bergey et al. (1923), bacteria were only included in rhizobia when they had the capacity of nodulating. However, when they had similar morphologies but could not nodulate, they were excluded from rhizobia. Nodulation, host range, and behavior on growth media were also later considered (Baldwin and Fred 1929; Fred et al. 1932) for rhizobial classification. Based on growth behavior on media, Fred et al. (1932) classified rhizobia as either fast or slow growing (Young 1996). Rhizobia, therefore, is a selected bacterial group capable of forming root nodules on legumes, and occasionally on the stems of some legumes, and can as such fix atmospheric nitrogen (N_2) to fully or partially meet the nitrogen (N) requirements of the legume host plant (Gage 2017). Frank (1889) proposed the name "rhizobia" to describe root nodule bacteria. All nodule-forming bacteria have from then been known as rhizobia. Biological nitrogen fixation, which is an N_2-fixation process via different prokaryote members (specifically diazotrophs), approximately contributes about 16% of the total N input in croplands (Ollivier et al. 2011). Rhizobia are, therefore, significant contributors to BNF, and the legume-rhizobium symbiosis can fix as much as up to 450 kg N ha^{-1} $year^{-1}$ (Unkovich and Pate 2000).

Moulin et al. (2002) reported that as a group, rhizobia are not monophyletic and have, therefore, been classified as alpha- and beta- (α- and β-). Rhizobia currently consist of 61 species in 13 different genera, namely *Rhizobium, Mesorhizobium, Sinorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium, Methylobacterium, Burkholderia, Cupriavidus, Devosia, Herbaspirillum, Ochrobactrum,* and *Phyllobacterium*. The taxonomy of rhizobia is in constant flux (Ahmad et al. 2008).

Rhizobium, Mesorhizobium, Sinorhizobium, Bradyrhizobium, Azorhizobium, and *Allorhizobium* belong to the alpha-Proteobacterial subdivision of the purple bacteria, an incredibly diverse group (Pierre and Simon 2010). *Rhizobium* contains 33 species, 24 originating from legume nodules while *Sinorhizobium* includes nine species isolated from legume nodules. Also, *Bradyrhizobium* has seven species from legume nodules, and *Azorhizobium* has two species nodulating legumes.

The complete list of known rhizobia species is continuously updated (Khan et al. 2010). The technological advancements in morphological, biochemical, physiological, serological, and sequence analysis used for taxonomic classification could still make classification unstable (Manvika and Bhavdish 2006). Further studies on the genetic diversity of rhizobia will, however, help in understanding the evolutionary histories of the rhizobium-legume symbioses. This will, consequently, help in devising worthwhile planning strategies aimed at reaping the utmost benefit from the symbioses.

9.5 Rhizobial Ecology and Diversity

Studies have targeted to uncover the nature of rhizobial symbionts in their native environments as it has been discovered that one of the significant problems in the application of BNF technology is the establishment of the introduced inoculant strains. Nodulation and nitrogen fixation in this symbiosis require that host and microorganism are compatible, but also that the soil environment is appropriate for the exchange of signals that precede infection (Hirsch et al. 2003; Zhang et al. 2002). Earlier reviews have reported the influence of biotic and abiotic soil factors on rhizobium ecology (Amarger 2001; Sessitsch et al. 2002). A problem identified by many of the reviews adequately describing changes at the population level. Tools, like intrinsic antibiotic resistance (Beynon and Josey 1980), serology (Bohlool and Schmidt 1973; Purchase et al. 1951), and multilocus enzyme electrophoresis (Pinero et al. 1988; Eardly et al. 1990), have all facilitated the acquisition of insight into rhizobial population structure in the soil, and how this could be influenced by the host and environment. However, it is only with the development of advanced molecular (Hirsch et al. 2003; Thies et al. 2001) and computational tools that the consideration of large populations of rhizobia on a routine basis been possible. The nodule formation on the leguminous host keeps on being viewed as the essential phenotypic characteristic due to the evident agricultural significance of rhizobia. Techniques such as fatty acids methyl esters (FAME) (Leite et al. 2018), whole-cell protein analysis using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Dekak et al. 2018) and multilocus enzyme electrophoresis (MLEE) (Van Berkum et al. 2006), and recently whole-genome sequencing (Seshadri et al. 2015) have effectively been utilized to characterize and classify obscure strains and depict novel rhizobial species.

Traditionally, rhizobial variation has been determined using characteristics such as growth rate and colony morphology (size, shape, color, texture, and general

appearance) and antibiotic resistance methods (Graham et al. 1991). However, these methods cannot sufficiently discriminate between all the variations exhibited in the target species. They cannot delineate sources of observed phenotypic variation that may be due to environmental factors or underlying genetic factors. Molecular means have now been accessible to appreciate the diversity and structure of the bacterial population. The *16S rRNA* gene sequencing is a very crucial parameter in rhizobial classification and techniques that depend on the disparity in ribosomal RNA genes have been regularly connected to the identity of species (Laguerre et al. 1994). The traditionalist idea of *16S rRNA* genes has, however, restricted its utilization due to strain level discrimination. The intergenic spacer (IGS) existing between 16S and 23S rRNA genes was depicted to be much diverse (Massol-Deya et al. 1995) and restriction fragment length polymorphism (RFLP) of the polymerase chain reaction (PCR)-intensified IGS was used in the characterization of rhizobia (Nour et al. 1994; Sessitsch et al. 1997). The advancement in PCR prompted new fingerprinting strategies. For example, techniques like Random Amplification of Polymorphic DNA (RAPD) using subjective oligonucleotide PCR primers of irregular grouping have now been used to produce strain-explicit fingerprints of rhizobia (Koskey et al. 2018).

Studies have shown that tropical rhizobia are diverse with subgroups of varied symbiotic specificity and effectiveness. Studies by Bala and Giller (2007) showed rhizobia of the same phylogenetic grouping nodulating *Calliandra calothyrsus, Gliricidia sepium,* and *Leucaena leucocephala* in some soils, but failing to nodulate at least one of the hosts in other soil, thus suggesting that rhizobial phylogeny and host range (infectiveness) were only weakly linked. Rhizobia are heterotrophic, competent bacteria that can survive as large populations for decades in the absence of host legumes (Giller 2001), but the presence of a compatible host legume confers protection to the microsymbionts against environmental stresses (Andrade et al. 2002). On the other hand, a greater diversity of rhizobia in soil populations broadens the range of legume hosts that can be nodulated in such soils. Therefore, a mutual benefit between aboveground (legume) and belowground (rhizobia) biodiversity exists.

9.6 Determinants of Host Specificity in Rhizobia

Host specificity plays a vital role in rhizobia, especially in establishing an effective symbiosis. There is a difference in the specificity of interaction between leguminous species and rhizobia. A few legume-rhizobia symbioses are more specific, for example, when a legume host specifically forms root nodules only when infected with a particular rhizobium. Some other legumes will, however, form their nodules with a variety of rhizobia (Vance et al. 2000). Broughton et al. (2000) observed that specificity encompasses the recognition of a bacterium by a host and vice versa, via signal compounds exchange, which instigates differential expression of the gene in both.

Albeit many host plants, only some symbionts can lead to the development of nitrogen-fixing nodules. Such tropical leguminous trees as Acacia, Prosopis, or Calliandra can, exceptionally, form the nodulation symbioses with diverse rhizobia from various genera. The specificity existing between symbiotic accomplices, however, limits the development of non-fixing ineffective nodules by the host legume as observed by Perret et al. (2000). The formation and development of root nodules require additional energy and nutrients from the host. Rhizobia are different in their reaction to various signal molecules that are produced by legumes. A few rhizobia have a restricted host range and therefore form nodules with a few legumes. *Azorhizobium caulinodans*, *Sinorhizobium saheli,* and the sesbaniae biovar of *Sinorhizobium terangae,* for example, only nodulate *Sesbania rostrata* (Boivin et al. 1997) and *Rhizobium galegae* is the main symbiont of *Galega officinalis* and *Galega orientalis* (Lindström 1989). Conversely, some rhizobia have an expansive host range and, hence, are fit to nodulate a wide range of legumes with different degrees of promiscuity. For it has recently been reported that two rhizobial strains, *Mesorhizobium japonicum* (strain Opo-235) and *M. kowhai* (strain Ach-343) could nodulate a wide range of host including species of diverse legume genera from two tribes (Galegeae and Trifolieae) (Safronova et al. 2019).

Legumes may, on the other hand, also be host to only one kind of symbiont (*Galega* spp.) or establish symbioses with a wide range of rhizobia (*Leucaena leucocephala*, *Calliandra calothyrsus*, *Phaseolus vulgaris*). Distantly related rhizobia can nodulate the same host; for example, *Sinorhizobium fredii*, *Bradyrhizobium japonicum,* and *Bradyrhizobium elkanii* all nodulate *Glycine max*. Members of *Rhizobium*, *Sinorhizobium,* and *Bradyrhizobium* are less related to each other than to other non-rhizobial genera. Stem and root-nodulating *Azorhizobium caulidonans* and root-nodulating *Sinorhizobium fredii* and *Sinorhizobium terangae* bv. sesbaniae, both symbionts of *Sesbania rostrata*, also represent two taxonomically distant genera.

9.7 Mechanisms of Biological Nitrogen Fixation

Biological nitrogen fixation (BNF), a system used only by specific prokaryotes, is catalyzed by a two-part nitrogenase complex (Yan et al. 2010). Nitrogenase catalyzes the simultaneous reduction of one N_2 and $2H^+$ into ammonia (NH_3) and a molecule of hydrogen gas, as thus:

$$N_2 + 8H_2 + 16ATP + 2NH_3 + 2NH_3 + 2H_2 + 16ADP + 16Pi$$

The immediate electron donor is ferredoxin, a potent reducing agent. The reaction is driven by the hydrolysis of 2 ATP molecules for each electron transferred (Wheelis 2008). Carvalho et al. (2011) observed that the best-known BNF system occurs between legume hosts and bacteria (rhizobia). The mutual interaction between the legume roots and given soil rhizobia accounts for the development of a specified

organ, the mutual root nodule, that primarily functions in BNF. Root nodules in legumes make a vital contribution to the soil N content, which plays a significant role in agriculture (Alla et al. 2010). Legume root exudates enhance the production of Nod factor signals of rhizobia, which are readily distinguished by compatible plant receptors leading to the formation of nodules, in which are bacteroids, differentiated bacteria and N_2 (Oldroyd and Downie 2008). Maintenance of nitrogenase activity in the root nodule is subject to a fragile equilibrium. At first, a high rate of oxygen respiration is indispensable in order to supply the energy needs of the N reduction activity (Sanchez et al. 2011), but oxygen also inactivates the nitrogenase complex irreversibly. These opposing needs are reconciled by oxygen flux control via a diffusable barrier present in the nodule cortex and by leghemoglobin, an oxygen carrier of the plant which is exclusively present in the root nodules (Minchin et al. 2008).

Besides N_2 fixation, some rhizobial species are capable of growing under conditions of low oxygen using nitrate (NO_3) as an electron acceptor to support respiration in the process of denitrification in which bacteria sequentially reduce NO_3 to nitrite (NO_2) and finally to N_2 (Van Spanning et al. 2005, 2007). In this process, NO_3 is reduced to NO_2 by either a membrane-bound or a periplasmic NO_3 reductase, and NO_2 reductases catalyze the reduction of NO_2 to nitric oxide (NO). Nitric oxide is then further reduced to nitrous oxide (N_2O) by NO reductases and, finally, N_2O is converted to N_2 by the N_2O reductase enzyme. The importance of denitrification in legume-rhizobia symbiosis can best be appreciated when the oxygen concentration in soils decreases (soil hypoxia) due to environmental stresses as flooding of the roots. Following such conditions, the denitrifying process could be a mechanism of generating ATP for the survival of soil rhizobia and also to preserve the functioning of nodules (Sanchez et al. 2011).

9.8 Significance of Biological Nitrogen Fixation

The atmospheric environment is an almost homogeneous blend of gases, the amplest of which is N (78.1%) (Garrison 2006). Around 96% of the N taken by crop plants has been estimated as N derived from the atmosphere (López-Bellido et al. 2006). Biological N fixation includes the transformation of N_2 to ammonium (NH_4), which is a plant-available N form (Vessey et al. 2005). The idea of BNF is that the dinitrogenase catalyzes the response and part triple-bond idle atmospheric N (N_2) into natural ammonia molecule (Cheng 2008). The BNF is viewed as an inexhaustible asset for sustainable agriculture, as it decreases fertilizer use, and hence augments financial farmers gains (Walley et al. 2007). Also, it assumes a vital role in appraisal of rhizobial diversity, adds to global knowledge of soil microbial biodiversity and the handiness of rhizobial accumulations, and to the foundation of long-term methodologies that are aimed at expanding the contributions of biologically fixed N to agriculture. The N_2 fixed by legumes can incredibly contribute to economically

buoyant and environmentally suitable agriculture, as suggested by Odair et al. (2006). It has been assessed that 80–90% of the plant-available N found in the environments is sourced from BNF (Rascio and Rocca 2008). It (BNF) also adds to the renewal of soil N, and hence circumvents the dire need for chemical N fertilizers (Larnier et al. 2005). Biological N fixation offers an economically alluring and ecologically encouraging methods for lessening external N fertilizer demand and input (Yadvinder-Singh et al. 2004). Agricultural systems sought to have gradually metamorphosed towards enhancing environmental quality and exclude its (environmental) deterioration. The use of inoculants that are composed of diazotrophic bacteria and used as N fertilizer alternatives is, therefore, one of the most vibrant methods of bypassing environmental deterioration (Roesch et al. 2007).

9.9 Rhizobial Bio-Prospecting Studies

The Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA) has maintained a collection of nitrogen-fixing legume symbionts for most of the twentieth century (Van Berkum 2002). Although many rhizobial isolation studies appear in scientific literature, there has been little attempt to evaluate global trends across diverse strain collections. The most comprehensive studies focus on a particular rhizobial species recovered from several host legumes at multiple locations or on populations or communities of rhizobia recovered from a particular host legume over a wide geographic range (Han et al. 2008). The absence of a global synthesis can be attributed to the difficulty in comparing studies that use diverse methods for rhizobial sampling and strain typing. The use of diverse sampling strategies means that collections of isolates are rarely equivalent, except in related studies arising from individual research groups. Comparing published studies is also difficult because strain typing methods vary in their discriminatory power and are usually species-specific (Li et al. 2009) and therefore influence the number of strain types identified.

9.10 Rhizobia Identification

A typical rhizobial cell is a small- to medium-sized (0.5–0.9 × 1.2–3.0 μm) Gram-negative, motile rod, exhibiting the characteristic presence of copious β-hydroxybutyrate granules forming 40–50% of the cell dry weight, easily observed using metachromatic granules stains. Most strains produce sticky gum-like substances of varying composition. According to a study by Gupta et al. (2007), rhizobia are typically observed, on Yeast Mannitol Agar (YMA) media, as translucent, viscid, slimy, and individual dome-shaped colonies, having a lifted component with whole edges.

9.11 Inoculant Formulations

9.11.1 Optimal Characteristics of a Carrier for Inoculants

The carrier, a delivery vehicle of live microorganisms, helps in transporting the microorganisms from the production factory to the field where they are utilized and is the significant portion (by volume or weight) of the inoculant. There is presently no universally accepted carrier or formulation available for microbial release into the soil (El-Ramady et al. 2018). Carrier materials and formulation type, therefore, vary, and it can be a slurry or powder (Bashan et al. 2014). A suitable carrier must have the capacity to deliver the right number of viable cells in good physiological condition at the right time (Malusá et al. 2012). Other essential characteristics of a suitable inoculant carrier have been reported (Bashan 1998):

1. A carrier should be nearly sterile or easily sterilized, as chemically and physically uniform as possible with consistent quality and be suitable for as many bacterial species and strains as possible. Wet carriers should also have a high water-holding capacity.
2. It should also be easily manufactured and mixed by existing factories, allow additional nutrients, have an easily adjustable pH, be made from relatively cheap raw materials, and be in adequate supply.
3. A good carrier should also be easy to handle, provide rapid and controlled release of bacteria into the soil, and can be applied using appropriate standard machines.
4. It should be environmentally nontoxic, biodegradable, and non-polluting, thereby minimizing such environmental risks as the dispersal of cells into the groundwater or atmosphere.
5. It should have enough storage shelf life of a year or two when kept at room temperature.

No single carrier can possess all these qualities; it should, however, have as many as possible to be a good one.

9.11.2 Types of Existing Carriers for Inoculants

Peat is almost the most widely used carrier for rhizobia, the only inoculant being sold in large volume today (Ruíz-Valdiviezo et al. 2015). However, this carrier has records of several disadvantages, some of which include wide and source-dependent variability in its quality, thereby possibly presenting difficulties in inoculant dosage and clear storage conditions (Reddy and Saravanan 2013). Availability of peat is rare, and hence its exorbitant cost in most countries in Asia and Africa (Bashan et al. 2016). Some peats may release compounds toxic to the bacteria when sterilized by heat resulting in low bacterial counts (Kaljeet et al. 2011). The mining of peat has also been regarded as being unfriendly to wetland ecosystems (Margenot et al.

2018). In terms of delivery, peat powder can easily be blown away from seeds by an air-delivery system of the planter. Peat may also interfere with the seed-monitoring mechanism of the planters (Nehra and Choudhary 2015). A possible remedy is an addition of adhesives or slurries to the inoculant during its application to the seeds to improve its adhesion, but this requires additional time, labor, and cost for a process that is already labor-intensive (Nehra and Choudhary 2015). Today, inoculant carriers can generally be divided into five basic categories including soils, waste plant materials, inert materials, plain lyophilized microbial cultures, and oil-dried and liquid inoculants, as reviewed by Bashan et al. (2014)). Due to the drawbacks observed with peat, many alternatives consisting of different formulations of the basic materials have, therefore, been evaluated (Trivedi et al. 2005; Albareda et al. 2008; Nehra and Choudhary 2015).

9.11.3 Inoculant Production

There are several essential issues to be considered in inoculant production, among which include the microbial growth profile, types and optimum condition of the organism and formulation of the inoculum. The methods of inocula formulation and application and inocula product storage are all very critical for a successful biological product.

In the process of inoculant production, the target microorganism can either be introduced into a sterile or non-sterile carrier (Bashan et al. 2014). The former carrier has microbiologically significant advantages over the latter but has not been cost-effective from commercial perspectives in most cases (Bashan et al. 2016). For an inoculant to contain an effective bacterial strain and for its success or failure as a biological agent to be determined, formulation is the most critical consideration. The formulation stage is the industrial process and practice of successfully converting a promising laboratory-proven bacterium into a commercial field product. The biofertilizer formulations are, therefore, expected to conform with all the numerous characteristics mentioned earlier and also surmount two significant constraints against living organisms, that is, (1) viability loss during short storage in growers' warehouse (as most developing African countries lack appropriate refrigeration facilities), and (2) long shelf life and stability over a broad temperature range in the marketing distribution systems (O'Callaghan 2016).

The six main steps paramount in inocula production are the choice of active organisms, isolation and selection of the target microbes, selection of method/carrier material, selection of propagation method, prototype testing, and large-scale testing (Bashan et al. 2016). Active organisms must be decided based on activity objective; isolation is vital in separating target microbes from their habitation. Usually, microbes are isolates from plants' root. Best candidate isolates are selected following various stages of routine selection processes. Also, paramount is deciding the form of inoculant carrier. Selecting a befitting propagation method is mainly

through understanding the optimum growth requirements of organisms, and this can be achieved by obtaining the microbial growth profile under different conditions. The prototype (usually in different forms) inoculant is made and tested at diverse environments with a view to evaluating the effectiveness and efficacy of the product.

9.12 Forms of Inoculants Dispersal

Inoculants are mostly known to come in four primary dispersal forms, as previously reviewed (Bashan et al. 2014; Alori and Babalola 2018).

1. Powders: these are often used as a pre-planting seed coating with particle size typically ranging between 0.075 and 0.25 mm to ensure a better chance for the inoculant to properly adhere to the seeds (Malusá et al. 2012).
2. Slurries are powder-based inoculants formed by suspending the base inoculant material in liquid, usually water, and applying the mixture directly to furrows. The seed can, alternatively, be dipped into the suspension just before planting (O'Callaghan 2016).
3. Granular inoculants are directly applied to furrows together with the seeds. Granular size ranges are from 0.35 to 1.18 mm (Hungria et al. 2005) and are usually used for broadacre applications (O'Callaghan 2016). There are also some bead-like forms that are synthetic variations of these granular forms, and which can be in macro (1 to 3 mm) sizes in diameter and used as a granular form. They can also be in micro size (100–200 μm) used as a powder for seed coating. These types of inoculant are, however, not suitable for developing countries as their application usually requires heavy and sophisticated machinery which in most cases is not available in such countries (Bashan et al. 2014).
4. With liquid inoculants, seeds are either evenly sprayed or dipped into the inoculant before sowing and later be sown after drying (Bashan et al. 2014). This ensures even coverage of seeds with relatively no planter-related problems or inoculum.

Despite the diverse forms of inoculant and the different ways in which they can be applied, the use of any inoculant will depend on its availability, cost, and crop/environment-specific needs. For example, although inoculants applied as seed coating may be cost-effective, their use is severely challenged by the need for proper pre- and post-application, and they are sometimes less effective than granular inoculants that are directly applied to the soil (Jones and Olson-Rutz 2018).

9.13 Potentials for Production and Use of Inoculants in Africa

Despite the high recorded rates of economic growth of more than 5 years, SSA is still by far the poorest region globally (The Economist Intelligence Unit 2014). This is not unconnected with the region's level of food insecurity, which is undermined by sporadic poverty and limited utilization of modern agricultural technologies. Poor soil fertility, pests and diseases and such low-skilled and unsustainable farming methods like continuous cropping are some of the region's direct causes of the food insecurity (Oruru and Njeru 2016). About 60–70% of mineral fertilizers applied to farms is lost, particularly, via leaching, volatilization, and erosion, for example (Hardarson et al. 2003). Only an estimated 30–40% of the mineral fertilizers applied is, therefore, utilized by plants, worldwide (Chianu et al. 2011). In such instances, biotechnology has great potential to increase the productivity in SSA (Chianu et al. 2011; Oruru and Njeru 2016), especially through the conservation and sustainable use of soil microbes (Macdonald and Singh 2014).

Many opportunities could be readily available if countries in SSA could develop a long-term approach to policies on, specifically, BNF and generally on biotechnology. Policies like these should be able to: (1) advance national biotechnology need appraisal and implementation, (2) target research on biotechnology and executing same to needs, (3) give motivating forces and conditions to commercialization of biotechnology research and endeavors, (4) advance partnerships between immediate public research for development (R4D) and multinational biotechnological industries, (5) enhance scientific limits and technological framework for the execution of an ideal biotechnology, and (6) incorporate biotechnology hazard management into existing agricultural, health, and environmental routines. The potential advantages of biotechnology, like *Rhizobium* inoculation, may, otherwise, not be tapped for the enhancement of human welfare in the SSA.

Furthermore, approaches such as BNF and *Rhizobium* inoculation should be able to circumvent the need to: (1) make agricultural and non-governmental organizations (NGOs) stronger as they diligently serve the interests of subsistent farmers as they embrace biotechnology, (2) upgrade their capability, and (3) enhance their support in adjusting and testing BNF and *Rhizobium* inoculation advancements. The SSA countries must settle on an integrated biotechnology approach instead of their much-adopted ad hoc approaches. The former, however, also needs an intervention of policy as observed by Brenner (1996). The (integrated) approach will guarantee that biotechnology research readily takes care of the grievances of resource-poor farmers. For example, Botha et al. (2004) reported that soybeans CB 1809 strain was up to 60% superior to other isolates tested in efficient BNF from Bergville and Morgenzon, in South Africa, and was almost 73% of isolates from Koedoeskop. Mpepereki et al. (2000) reported that inoculation with *Bradyrhizobium japonicum* in the SSA increased soybean (*Glycine max* (L.) Merrill) yield from 500 to 1500 kg ha^{-1}. Mugabe (1994) had earlier observed that the majority of countries in Africa could reduce much of the expenditures incurred on fertilizer imports via a

full utilization of BNF. *Rhizobium* alone could provide an estimated >50% of the fertilizers required for crop production in most of the marginal environments of Kenya, Tanzania, and Zimbabwe. Unlike most African countries, the agricultural sector is overwhelmingly dominated by a detectable level of commercial farms in South Africa and Zimbabwe. This is translated into an easier adoption of commercial inoculant production and use (Bala 2011b). Chianu et al. (2011), however, observed that socio-economic and policy constraints were the most critical challenges that seriously undermined the much-needed production and use of *Rhizobium* inoculants in SSA. The limited capacity of most national agricultural research systems in SSA causes an absence of expertise in setting preferences and priorities in the use of biotechnology. This condition militates against the development and production of BNF-based technologies. Research and development (R&D) programs in the African continent are presently, more or less, isolated, with low-level monitoring and evaluation, vis-à-vis severely low funding. Inherent variability in legumes' response to inoculation (Ronner et al. 2016) and an over-dependence of BNF on such factors as legume agronomy; and edaphic and other environmental factors (van Heerwaarden et al. 2018) are some of the severe hindrances to effective inoculation programs. Also, such aspects as inoculant source, variety, and management types and level usually differ between countries. There also exist variations in climatic and edaphic conditions across various proximities. This, invariably, makes it even more difficult to draw reliable conclusions on the efficacy of inoculants derived from local trials (van Heerwaarden et al. 2018) and hence suggests that the use of inoculants and diverse varieties may need to be directed towards specific frameworks as observed by Ronner et al. (2016). This, therefore, indicates the dire need for comparative studies focussing on the efficacy of legume technologies that contain various pulses being implemented across SSA.

In early studies conducted on inoculation in various parts of Africa, there was a grain yield advantage in soybean (*Glycine max*) in tropical Africa (Sivestre 1970; Nangju 1980; Bromfield and Ayanaba 1980). Also, Sivestre (1970) observed yields, in inoculated soybean, of 1440 kg ha^{-1} compared to uninoculated ones, which had a yield of only 240 kg ha^{-1}. In a paper presented at an international workshop on *Rhizobium* inoculation, held in Tanzania, in 2008, Bala had cited work as reporting yield increase of 80–300% due to inoculation in the Democratic Republic of (DR)-Congo. Ndakidemi et al. (2006) observed in an on-farm trial conducted with rhizobial inoculants (*Rhizobium tropici* strain CIAT 899, for common bean, and *Bradyrhizobium japonicum* strain USDA 110, for soybean) at Moshi and Rombo, two districts in northern Tanzania, that at harvest, soybean and common bean (*Phaseolus vulgaris*) development was significantly higher with *rhizobial* inoculation when compared with an uninoculated control or N and P supply. Grain yields of *P. vulgaris* were also increased by 60–78% due to inoculation alone and 82–95% due to inoculation and P application at 26 kg P_2O_5 ha^{-1}, relative to the uninoculated and/or unfertilized plots. There was also a 127–139% increase in grain yield via inoculation only and 207–231% via inoculation and 26 kg P_2O_5 ha^{-1} application. Hence, the combined application of inoculants and P fertilizer to *G. max* and

P. vulgaris increased grain yield and biomass production when compared with the use of only N and P or strains of rhizobia (Ndakidemi et al. 2006).

To isolate and test the effectiveness of N_2-fixing bacteria from Africa's large-biodiversity ecosystem, vis-à-vis supervising factors that affect legumes, the rhizobia, and their symbiosis while ensuring for effective rhizobia, is paramount because it may eventually result in identifying superior inoculants that can readily improve legume growth, development, and yield. This will, later, provide for an economic boom for legume farmers. Expanded and emphasized research activities are, therefore, direly needed to promote this eco-friendly and cheap technology for the many resource-poor smallholder farmers in Africa (Simon et al. 2014). Some earlier studies conducted in South Africa, as reported by Van Rensburg and Strijdom (1969), revealed that some local soybean varieties formed a specific symbiosis with *B. japonicum*. It is, however, of paramount importance to appreciate that even promiscuous soybeans that seldom require inoculation, and that are popular in a few parts of Africa, at times respond to inoculation. Osunde et al. (2003), in a study carried out at five locations in Nigeria's moist savanna region, revealed that the promiscuous soybean cultivars (Tropical Glycine cross (TGx) 1456-2E and TGx 1660-19F) favorably responded to inoculation. *Magoye*, a Zambian, exceptionally promiscuous line released in 1981, however, readily nodulated in all tested southern African soils and seldom responded to inoculation in Zambia and Zimbabwe (Mpepereki et al. 2000). Sanginga et al. (2001) reported that the principal criterion for selection in the IITA, for more than a decade, was promiscuity without in-depth microbiological studies. It was based on these results that IITA introduced a program on soybean breeding in 1978 aimed at developing "promiscuity" in soybean cultivars that nodulate with local *Bradyrhizobia* in the soil, thereby excluding the necessity of inoculation (Kueneman et al. 1984). However, studies conducted in the early 2000s on symbiotic effective nature of local rhizobia that nodulate promiscuous soybean in 92 soils of Zimbabwe led to the identification of three isolates that were of utmost N_2-fixing potential in the *Magoye* cultivar than MAR 1491, which is a commercial strain (Musiyiwa et al. 2005). The M3 isolate was, however, later identified to be more superior to the commercial strains MAR 1491 and MAR 1495, as reported by Zengeni and Giller (2007). Okogun and Sanginga (2003) observed no statistically significant difference between the yield of inoculated and uninoculated crops (promiscuous soybean varieties—TGx 1485-1D, TGx 14562E, TGx 1448-2E, and TGx 1660-19F)) at three sites in the savanna of Nigeria, even though the native rhizobial population in soils at these sites were to a certain extent different.

Nitrogen-inoculants and BNF have had an extended history in Africa. It dates to the colonial agricultural research days when attempts were made to develop pasture legume inoculants to boost exotic cattle productivity (Odame 1997). Also, the United Nations Educational, Scientific and Cultural Organization (UNESCO) established a few Microbiological Resource Centres (MIRCENs), spread within the five continents, which were supported by the United Nations Environment Programme (UNEP) and the Food and Agriculture Organisation (FAO) of the United Nations (UN) to elevate BNF status of third world countries (Odame 1997). The main functions of MIRCENs in Africa, located in Cairo, Dakar, and Nairobi,

included, among others, the collection, identification, testing, and maintenance of strains, and preparation and distribution of inoculants or their cultures that were compatible with local crop plants. Other functions were to deploy local *rhizobia* inoculant technologies, promote research, and provide advisory services, training, guidance, and counseling to institutions and individuals engaging in rhizobiology research activities. The Nairobi MIRCEN project, for example, promoted and transferred BNF technologies such as pulses' inoculants, pasture legumes, and trees to research scientists and other stakeholders of agricultural relevance in Kenya and other East African countries. It (Nairobi MIRCEN) also extended its activities into *Rhizobium* strains screening for adaptation to abiotic stresses like soil acidity, extremely high temperatures, and drought, especially while considering the various environmental stresses that hamper successful BNF and as two-thirds of agricultural land in Kenya is vulnerable to these stress types. The whole idea was aimed at gradually intensifying screening trials for rhizobia that could be adaptable to such ecological menaces. This project also used the potential of a symbiotic association with a fungal strain (mycorrhiza), on plant roots that aid the plant to extract water and P from the soil environment. The MIRCEN also developed *Biofix*, a very marketable biofertilizer (Odame 1997). Kenya Institute of Organic Farming (KIOF) and the Organic Matter Management Network (OMMN), which are Kenya-based nongovernmental organizations (NGOs), played a serious role to reckon with in the distribution of *Biofix* to farmers. Researches are also being conducted with *Biofix* in Nigeria. Over time, however, the active involvement of KIOF in promoting *Biofix* had over-stretched and, therefore, its human and financial resources waned (Chianu et al. 2011).

The FAO in the 1990s supported a project to select more promising rhizobial strains in Tanzania, as reported by Mugabe (1994). This resulted in the development of Nitrosua, a biofertilizer for profitable soybeans production by Sokoine University of Agriculture (SUA), Morogoro. The SUA, in collaboration with the Ministry of Agriculture and some NGOs, also established extension activities for the dissemination of Nitrosua to local Kenyan farmers. These activities, however, also waned over time, as stated by Bala (2008). At least two firms (Madhavani Ltd. and the BNF of Makerere University, established in 1990 with the help of the USAID) in Uganda have produced inoculants. The two firms, however, functioned until 1997 and produced 14.2 tonnes of soybean inoculants between 1995 and 1997 for the FAO. Inoculant production in Rwanda started at the Institut des Sciences Agronomiques du Rwanda (ISAR) in 1984. Production capacity reached 2.4 tonnes per annum by 1990 (Cassien and Woomer 1998). Unfortunately, activities were, halted by the civil war fought in the 1990s. At the end of the war, however, the laboratory was renovated, and BNF activities were resumed, but pre-civil war records were not yet reached by the early 2000s (Giller 2001).

First commercial quantities of inoculants were seen in the South African markets in 1952, although their quality was highly debatable until the 1970s after an independent quality control system was introduced (Strijdom 1998). All inoculants from 1967 were produced with sterilized peat and contained at least 5×10^8 rhizobial cells g^{-1} of peat (Strijdom 1998). The quality control strategies introduced ensured

for comparison of South African inoculants with the best quality of inoculants produced outside the African continent (Strijdom and van Rensburg 1981). Farmers mainly growing crops like soybean, cowpea (*Vigna unguiculata*), and groundnut (*Arachis hypogaea*) enjoyed the production of the inoculants (Deneyschen et al. 1998). Khonje (1989) reported that the production of commercial quantities of inoculants in Malawi started in the 1970s, where they were made available in 50-g packets for crops like soybean and cowpea. They were produced by Chitedze Agricultural Research Station, Lilongwe. Sales rose dramatically from as little as 450 packets, in 1976, to over 1800, between 1987 and 1988. In Zimbabwe, the presence of a mega and highly established commercial soybean sector readily suggests sporadic spread and use of inoculants in that country (Mpepereki et al. 2000). Soil Productivity Research Laboratory (SPRL) controlled a BNF enhancement technology project in the 1990s in Zimbabwe, which was supported by the International Atomic Energy Agency (IAEA) as reported by Chianu et al. (2011). This project reached an inoculants mass production capacity of 120,000 packets per year, which were distributed via extension services to smallholder farmers. Mugabe (1994) reported that mycorrhizal inoculation research was also undertaken in some regions by The University of Zimbabwe.

A study in Ghanaian cowpea grown in fields have reported a nitrogen fixation of up to 402.3 mg plant^{-1}, resulting in an average of 19.5 kg N-fixed ha^{-1} (Naab et al. 2009). Values between 4 and 29 (i.e., 15% and 56% of plant N) were, in contrast, reported in the semiarid south-western region of Zimbabwe (Ncube et al. 2007). The application of rhizobial inoculants may, therefore, hold potential for increasing plant nutrition and overall soil fertility in areas such as these. Although many strains of effective rhizobia have been identified, and are now readily available, it has still been observed that the rhizobial strains often under-perform in conditions that differ from their original habitat (Zhang et al. 2003; Law et al. 2007). Also, their effective nature relies on environmental factors like soil texture (Law et al. 2007), soil pH (Botha et al. 2004), soil temperature (Zhang et al. 2003; Suzuki et al. 2014), and various types of the host plant (Pule-Meulenberg et al. 2010). This is, especially, relevant for areas like the Botswanan Okavango region given its adverse climate, nature of available local plant varieties, and its soil heterogeneity. Law et al. (2007) already reported a popular strain of inoculant not affecting Botswana-grown cowpea and peanut. Application of soybean inoculant in South Africa, furthermore, boomed seed yields at only one of three sites, as observed by Botha et al. (2004). In a study by Grönemeyer et al. (2014), the authors observed a predominance of distinct genotypes which were only found in SSA, to date, at which point, sometimes the geographic distribution may prove more local. In view of the premise that "the environment chooses," these outcomes indicate that some autochthonous species like *Bradyrhizobia* are highly fitted to particular environmental requirements and, should, as such, be favored for an inoculant formulation (Grönemeyer et al. 2014).

Ever since N2Africa project was launched in Ethiopia, the inoculant production capacity of, for example, Menagesha Bio-tech Industry (MBI), a private company based in the country, experienced a sixfold expansion (Wolde-meskel et al. 2018). This was exemplified by a surge in annual chickpea inoculant production increment

from, not more than, 28,000 to up to 165,000 sachets. Distribution and sales of the inoculants also rose to 7- and 13-fold as reported by Ampadu-Boakye et al. (2017) and Wolde-meskel et al. (2018), respectively. Increased nodulation, biomass production, and N accumulation in soil-grown groundnut were achieved after inoculation with native rhizobium strains of northern Ghana (Osei et al. 2018) and the authors also reported that out of the isolates recently tested in Ghana, KNUST 1002 was observed to be highly effective. Its performance was like that of 32H1, a groundnut reference strain. Apart from only two *Rhizobium* isolates (KNUST 1003 and 1007) studied, all the strains selected in the experiment were intimately related to *B. yuanmingense*, which confirmed the species as a major groundnut microsymbiont (Osei et al. 2018).

9.14 Prospects on Inoculants Production and Use in Nigeria

There is an overwhelming opportunity to increase productivity through the use of *Rhizobium* inoculants in the Nigerian farming systems as it has been established that isolates of indigenous *Rhizobium* can produce fruitful results (Sanginga et al. 1988; Sanginga et al. 1994; Sessitsch et al. 2002). Numerous studies have also demonstrated that inoculants containing indigenous strains outperformed commercially available inoculants (Hungria et al. 2000; Ballard et al. 2003; Aliyu et al. 2014a). The recent hike in the economic recession in Nigeria, due to doom in oil price in the international market (Osalor 2016), has adversely affected many sectors within the country, including agriculture and general food security. This necessitated the inauguration of the Agriculture Promotion Policy (APP) by the Federal Government of Nigeria (FGN) through the Ministry of Agriculture and Rural Development (FMARD). This was an effort of the FGN to shift the nation from oil- to agriculture-based economy (FMARD 2016). This development is a relatively bright future for the agricultural sector and inoculant production and use. Continued significant increase in the price of mineral fertilizers, especially of the N-based, results in ever-increasing food prices (IFDC 2008; Nehring et al. 2008). This, therefore, necessitates the need for developing alternative soil fertility management strategies. The unlimited potential for developing and disseminating BNF and inoculation technologies is, therefore, revealed. Small-holder and resource-deficient farmers who cannot manage the staggering prices of mineral fertilizers may easily access and utilize *Rhizobium* inoculants (Osei et al. 2018). Another reason for the need to explore indigenous strains of *Rhizobium* inoculants is to safeguard the environment against pollution (land, water, and otherwise). Biofertilizers are more environment-friendly than their mineral counterparts (Malusá et al. 2012), even when the availability of the latter to the resource-poor farmers, in Nigeria, is not commendable. Resource-poor small-scale farmers, the primary producers of legumes in Africa, unusually apply fertilizers during legume production. The crop is, therefore, mostly dependent upon biologically fixed N by indigenous N-fixers. Rhizobia isolation for leguminous crops production has always received negligible attention in Africa.

This is, among other reasons, due to a dearth of much-needed research or lackadaisical attitude of researchers and ignorance of its significance in legume production vis-à-vis lack of proper commitment from skilled personnel to promote the technology. Assessment of the efficacy of isolated rhizobia is vital for the preparation of inoculants, the recommendation of host specificity, and symbiotic effectiveness (Simon et al. 2014).

Rhizobium strains vis-à-vis corresponding inoculation methods developed for certain conditions at a given location under a specific farming system may not perform equally well at another location practicing a different farming system (Sanginga et al. 1994). This, therefore, necessitates exploring the potentials in Nigeria's native strains of *Rhizobium*. Another area of concern is the storage conditions of the inoculants, which presents a severe constraint to the viability of rhizobia within the legume inoculants (Kaljeet et al. 2011; Abd El-Fattah et al. 2013). This will invariably give room for more research opportunities, and hence a bright future for the inoculant industry in Nigeria.

Several organizations are known for funding research activities aimed at adapting inoculant technology to the situations where it will be utilized in tropical countries. These organizations include the United Nations Development Program (UNDP) which supports International Agricultural Research Centre(s) (IARCs) through the Consultative Group on International Agricultural Research (CGIAR) and for a specific research program involving International Institute of Tropical Agriculture (IITA) and Boyce Thompson Institute (BTI)/Cornell University. The UNEP and UNESCO also support inoculant technology under the MIRCEN Project, whereas the FAO is also considering her role in the adaptation of inoculant technology for use in developing country like Nigeria. Also, the USAID, via its contracts with the University of Hawaii (Nitrogen Fixation by Tropical Agricultural Legumes NifTAL Project) and United States Department of Agriculture (USDA), is another effort. Another organization is The Beltsville Agricultural Research Centre (ARC) (World *Rhizobium* Study and Collection Center), which provides grants under Section 211(d) to the U.S. Universities' Consortium on BNF in the Tropics, and through a series of smaller grants that are administered by the USDA Science and Education Administration/Cooperative Research (SEA/CR). The USAID and several other governmental and non-governmental agencies supporting the CGIAR are also sponsoring work at Centro International de Agricultural Tropical (CIAT), IITA, International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), and International Centre for Agricultural Research in Dry Areas (ICARDA) on the adaptation of inoculant technology for use in the tropics. These are among many opportunities that can boost research activities geared towards indigenous inoculant production in Nigeria.

It is evident that the BNF benefit to nonlegumes as the inclusion of legumes in a cropping system is small compared to the level of nitrogenous fertilizer used in the more intensive cereal production systems of the developed world. Thus, the principal contribution of BNF to human nutrition will continue to be via the protein in legume grains. Any suggestion of substantial replacement of nitrogen fertilization of cereals and root crops by biologically fixed nitrogen is unrealistic because these

crops respond to levels of nitrogen fertilizer far more significantly than those currently supplied through BNF by legumes. Thus, there is an urgent need to devise ways to increase the contribution that BNF by legumes can make to cropping systems as a complement to nitrogen fertilizer-based production, rather than as an alternative to it. Yusuf et al. (2009) observed that many rhizobial genotypes had been identified through experiments, and these were the genotypes that significantly improved N balance in the soil. This displays the importance of inoculation, especially in N-poor tropical soils. Numerous studies, past and present, showed a promising trend in the field of inoculation technology in Nigeria. In the early 1980s, Ranga-Rao et al. (1981) reported that a series of field experiments were conducted in 1978, in Nigeria, to screen some N_2-fixation-efficient strains of *B. japonicum* that showed as high grain yield as 100% of two American soybean cultivars (Bossier and TGm 2944), whereas Asian cultivars did not indicate any significant response. Inoculation also led to encouraging grain yield increases of 40–79% in the American soybean cultivars that were grown in the Nigerian southern Guinea savannas (Nangju 1980; Pulver et al. 1982; Ranga-Rao et al. 1984). Bromfield and Ayanaba (1980) also noted that inoculation of soybean in the low pH sands of southeastern Nigeria achieved increases in grain yield of 300–500% after liming and 270–970% without liming.

In an experiment by Aliyu et al. (2014b), four indigenous strains of pasture rhizobia isolates were observed to contribute to nodulation, hence nitrogen fixation of groundnuts (Tables 9.1 and 9.2). The native strains outperformed all others, including the exotic commercial inoculant in terms of both nodule number and dry weight observed (Table 9.1).

In an earlier study, Sanginga et al. (1994) observed that about 96% of the rhizobia found in root nodules consisted of two main serotypes (IRc1045 and IRc1050). Both were confirmed as strains of indigenous rhizobia earlier isolated from Nigerian soils. This further reaffirmed the bright future for indigenous inoculant industries in Nigeria.

9.15 Studies on Rhizobia Inoculants in Nigeria from the 1990s to Date

Studies conducted within this period mostly focused on the assessment of the response of promiscuous cultivated varieties of soybean to inoculation, alongside other vital nutrients that were deficient. A few trials studied specific and promiscuous soybean cultivars. Based on vegetative parameters, the response of two soybean cultivars (SAMSOY 2 and TGX 1448-2E) to *Bradyrhizobium* inoculation (mixed with two other strains: R25B and IRj 2180A) was, for example, not affected significantly, except for root biomass in the TGX 1448-2E. This was under an on-farm researcher-managed trial condition in the northern Guinea savanna (NGS) of Nigeria. The scenario was ascribed to conceivable high populations of indigenous

Table 9.1 Influence of soil type and pasture rhizobia isolates on nodulation

Treatment	Nodule number	Nodule dry weight (mg)
Soil (S)		
CS	224.57	119.81
FS	54.29	29.38
Mean	139.43	74.6
SE	2.94	2.67
Isolates (I)		
CPI01	193.5	114.5
CPI02	141.17	80.5
MUI03	148.33	72.83
MPI04	176	78.83
Biofix	116.5	–
Control	111.33	56.83
Reference	89.17	79
Mean	139.43	39.67
SE±	5.51	5.
Soil (S) × isolates (I)	***	***

Adapted from Aliyu et al. (2014a)
SE standard error
***$p < 0.0001$

Table 9.2 Influence of soil type and pasture rhizobia isolates on nitrogen fixation

Treatment	Nitrogen fixation (mg N)
Soil (S)	
CS	101.87
FS	63.26
Mean	82.56
SE	1.93
Inoculant (I)	
CPI01	95.45
CPI02	90.94
MUI03	78.58
MPI04	71.76
Biofix	90.52
Control	68.13
Mean	82.56
SE	3.35
Soil (S) × inoculant (I)	***

***$p < 0.0001$

rhizobia satisfactory for soybean nodulation. Okogun et al. (2004), however, observed that the promiscuous cultivar outperformed SAMSOY-2 in terms of BNF and consequently the grain yield, demonstrating that varietal variations concealed the inoculation impact.

Other works included only the promiscuous cultivars of soybean; for example, diverse responses of some promiscuous soybean cultivars to inoculation, N, and P were reported from Kano state, Nigeria, as observed in a series of experiments conducted by Anne et al. (2011). Similar trials were carried out with an early maturing TGX 1485, a promiscuous cultivar, which was inoculated with a rhizobial strain at Minna, NGS of Nigeria. All the parameters observed, including grains yield, were significantly increased by the four inoculants when compared to the control.

In another study, groundnuts (*Arachis hypogaea* L.) were inoculated with indigenous strains isolated from cowpea and the rhizobia isolates were proved effective. A higher number of nodules, nodules dry weight, and consequently greater N_2 fixation were observed compared to the control and reference treatments (Aliyu et al. 2014b) (Table 9.3).

Bashan (1998) reported that combinations of microbes, blended as inoculants that synergistically interact, were being conceived. Studies conducted on microorganisms, devoid of plants, demonstrated that a few mixtures enable the bacteria to synergistically associate with one another. This provided nutrients, expelled inhibitory products, and invigorated each other through physical and/or biochemical activities that improved some beneficial aspects of their physiology like BNF. Bashan

Table 9.3 Effect of soil management and cowpea rhizobia isolates on nodulations, shoot dry weight, N uptake, and N_2 fixation of groundnut

Treatment	Nodule number (plant^{-1})	Nodule dry weight (mg plant^{-1})	Shoot dry weight (g plant^{-1})	N uptake (mg N plant^{-1})	N_2 fixation (mg N plant^{-1})
Soil (S)					
Cultivated soil	198.5	94.42	4.92	135.55	103.01
Fallowed soil	31.33	20.67	3.64	98.28	47.47
± SE	4.55	3.58	0.20	4.04	4.68
Isolates (I)					
VUI05	139.00	65.00	3.67	102.14	73.78
VUI06	119.83	68.67	4.26	121.20	77.76
Control	111.50	56.83	4.32	106.79	70.18
Reference	89.33	39.67	4.88	137.52	
Mean	114.92	57.54	4.28	116.91	73.94
SE±	6.430	5.060	0.300	5.700	5.740
$S \times I$	*	NS	NS	**	*

Adapted from Aliyu et al. (2014b)
SE standard error of difference of means
*$p < 0.05$
**$p < 0.01$

and Holguin (1997a) reported that these bacterial synergisms benefited plant growth. Also, some plant experiments indicated co-inoculation of *Azospirillum* with other microbes could metamorphose into more improved mutual impacts on plants than a single inoculation as observed by Bashan and Holguin (1997a, b). Hence, plant growth could be expanded by double inoculation with *Azospirillum* and phosphate-solubilizing bacteria (Belimov et al. 1995). This is because *Azospirillum* is also viewed as a *Rhizobium*-"aide," which stimulates plant metabolism, nodulation, and nodule activity, all of which also invigorate many plants growth factors and plant protection against unfavorable conditions (Fabbri and Del Gallo 1995; Bashan 1998). *Azospirillum* or *Azotobacter* blended with *Streptomyces* (El-Shanshoury 1995), and *Azospirillum* with the fungal biocontrol agent, *Phialophora radicola* (Flouri et al. 1995), include examples of other fruitful mixes (Bashan 1998). Blended inoculation with diazotrophic bacteria and arbuscular-mycorrhizal fungi (AMF) created synergistic interactions that resulted in a noteworthy increment in growth, P content in plants, upgraded mycorrhizal infection, and improved the uptake of mineral nutrients like N, P, copper (Cu), iron (Fe), and zinc (Zn) (Al-Nahidh and Gomah 1991; Barea 1997; Garbaye 1994; Gori and Favilli 1995; Bashan 1998).

Recently, a comparison was made, in a study by Aliyu et al. (2018), between some isolates and commercial inoculants and a control. The control was used as a benchmark for the comparison such that those isolates that statistically surpassed the control were deemed befitting candidates for a commercial inoculant production. A statistically significant difference ($p < 0.001$) was observed between the commercial inoculants and the controls regarding nodule number. Thus, the authors concluded that 70% of the isolates had records of more nodule number when compared to the control. Regarding dry nodule weight, 74% of the isolates recorded higher weights than the controls, although only 26% were statistically significant ($p < 0.01$). Based on the dry matter yield, only 18% of the isolates had a higher record of the studied parameter, and of these, only a single isolate showed a statistically significant difference ($p < 0.05$) in dry matter yield when compared to the control.

Although native *Bradyrhizobium* strains in Africa were employed to nodulate adapted soybean cultivars, thereby eliminating the need for inoculation (Abaidoo et al. 2007), Okereke et al. (2001) warned that a good establishment of effectively nodulating legumes could not be left to chance. The process, therefore, requires the introduction of effective strains of rhizobia into the soil during the planting period. This can be rightly achieved only through inoculation, and hence an opportunity for judicious use of Nigerian native strains of rhizobia. This may be achieved through their isolation from areas of a flamboyant native population and introduction of the same into relatively less populous sites. There is also a need for a holistic approach to be geared towards improving the entire cropping systems. This should include a selection of more competitive and efficient indigenous rhizobia that could serve as local inoculants (Machido et al. 2011; Sanginga 2003), and hence a bright future for indigenous biofertilizer production firms. A rigorous but systematic identification of crops suitable for diverse cropping sequences and combinations vis-à-vis reaping the potentials in N_2-fixing legumes is paramount (Machido et al. 2011). This will

open a new window for more rigorous research activities on inoculant development in Nigeria. Besides, there is a dearth of information on indigenous rhizobia. Where already identified, their symbiotic properties may not fully be understood and may differ depending on their original locations. This may lead to a possible establishment of a location-specific database on the occurrence, abundance, distribution, characteristics, and composition of the indigenous populations of rhizobia strains of Nigerian soils. More promising and versatile strains of the native rhizobial population could in the process, therefore, be identified for subsequent use as registered inoculants containing indigenous strains.

Three main factors limit the effectiveness of an inoculant: (1) its poor quality accompanied with low viability; (2) its inability to compete with indigenous rhizobia; and (3) its inability to tolerate the inherent physical and chemical conditions of the soil to which it is introduced (Cummings 2005). There are, however, many current and potential approaches that may circumvent these and other problems. Chianu et al. (2011) observed some key lessons that would ensure success at farmers' field level, some of which include: (1) an ubiquitous demonstration of the inoculants to the needs of, especially, small farmers; (2) intra-national collaborations, with the involvement of mass media; (3) well-coordinated and collaborative research-for-development programs; (4) involvement of top people of the government; (5) joint efforts of related governmental and non-governmental organizations for a long time; (6) involvement of individuals and the private sector in production and dissemination of the biofertilizers, and (7) effective farmer education on inoculation. The said strategies have been found to work effectively in some pilot areas and should be scaled up to reach more smallholder farmers (Chianu et al. 2011) in order for, especially, grain legumes farmers to maximally reap the diverse dividends of using inoculants in their cropping practices. This scaling could, however, be attained only through a desirable innovation platform involving all stakeholders and appropriate incentives to entice the private sector and industries (Chianu et al. 2011).

9.16 Conclusion

The need for initiating advanced studies on inoculation to address the difficulties confronting the use of inoculants by farmers in Africa, particularly Nigeria, can never be overestimated. Various trials, to be aimed at demonstrating the need for inoculation, should, therefore, include tests for the constraint of BNF by other nutrients like boron (B) and calcium (Ca), for example. Also, there is a dire need for a deeper examination of the economic and social cost-benefit analyses of the *Rhizobium* inoculation. The need for expanding the knowledge base on BNF utilization among farmers in Nigeria should go beyond awareness and use only. It should also include more qualitative aspects of farmers' knowledge, willingness to pay, and the long-term relevance of inoculants in farm objectives. Institutions and policies promoting the development of inoculants and widespread farmer adoption cam-

paigns for increased production of both food and cash legumes must be encouraged. This must especially be accompanied by targeted research to effectively explore the available indigenous strains of *Rhizobium* present in Nigerian soils. This is will invariably counterpoise the problems of N fertility and its consequent cost on smallholder farmers in Nigeria. Problems of poor quality, inadequate and inefficient markets, as well as inadequate extension services on inoculants and their use must also be tackled. Some successful outcomes of many on-station and farmer-condition simulating experiments with *Rhizobium* inoculants have been recorded. These records may be used as an index for the potentials of indigenous rhizobium-based inoculants in Nigeria. Specific measures such as tax motivations and exceptions will be paramount in stimulating the advancement of BNF innovation markets and the formation of nearby inoculant firms.

There is also a need for specific policy incentives to stimulate private sector involvement, at all stages of the innovation process, to install adoption. An array of studies has glaringly made it clear that the enormous diversity of *Bradyrhizobium* species specifically in Nigeria and elsewhere, in SSA, in general, is currently underestimated. Therefore, research in diversity, and characterization of nodule symbionts, in Nigeria, and SSA, should be accentuated. This is basically because numerous strains are bound to be developed into adapted inoculants for green manure and legumes. Of particular importance is tolerance for high temperature of many African *Bradyrhizobial* species, which makes them potential candidate strains to curb the problems of global climate change that foresee increases in temperature. Such future research activities should also focus on, and address, the molecular rationale and/or basis for their tolerance to the usually deleterious temperatures (Grönemeyer and Reinhold-Hurek 2018).

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