The Bioscientist Vol. 10 (2): 167-180, March 2022
Available online at *http://www.bioscientistjournal.com*



Full Length Research Paper

SUSTAINABLE WAY OF IMPROVING GRAIN LEGUMES PRODUCTIVITYAGAINST FAILURE OF INTRODUCED RHIZOBIA INOCULANT

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Received: 4 April; Accepted 10 May, 2022.

ABSTRACT

Rhizobia inoculation has been established worldwide as a cheaper, more effective way of ensuring adequate nitrogen supply to legumes than even the application of nitrogen fertilizer. The development of rhizobia inoculant industries in many countries has largely been motivated by the desire to introduce legume species to new areas, where appropriate ones do not exist. Competition from naturally occurring populations of rhizobia is usually a significant factor determining the establishment of introduced rhizobia inoculant strains in the field. Application of commercial inoculants introduced to new environments is usually associated with tendencies of failure, mostly due to the nature of the indigenous strains such as their population large size, positional advantage, higher competitive ability in nodule occupancy, saprophytic survival, ability to stand bacteriophages or epiphytic bacteria and superior adaptation to local and environmental factors, such astemperature, pH and nitrate content. Hence, selective enrichment of legume rhizosphere with environmentally adapted and highly effective indigenous strains of rhizobia may be the only way to improve legume nodulation, N_2 fixation and productivity under field conditions. There is need to follow standard procedures for obtaining the appropriate rhizobia for indigenous inoculant development, considering the legume genotype, environmental conditions and management practices to achieve the desired results. This will improve on the low yield of legumes, relative to their potential yields obtained by farmers due to low soil fertility, particularly in savanna zones through their influence on the legume growth, nutrition, and

productivity. The review also highlighted how indigenous rhizobia provides an alternative to costly inorganic nitrogen fertilizers and commercial inoculants developed from rhizobia originating from over environments.Indigenous rhizobiacould ensurelegumes productivity and sustainabilitythrough their biological N_2 fixation that reduces in the adverse environmental effects of inorganic nitrogen fertilizers such as soil acidification, release of greenhouse gases and contamination of nearby and underground water bodies.Thus, checking climate change, ensuring environmental safety, and supporting the ever-increasing human population through higher productivity of the legumes.

Keywords: Grain legumes, inoculants, nitrogen fixation, productivity, rhizobia, sustainability.

INTRODUCTION

The success of a legume grain crop in N_2 fixation is dependent on its capacity to form effective symbioses with root-nodule bacteria (rhizobia) to convert inert atmospheric nitrogen (N_2) into forms the plants can utilize (Khalid *et al*., 2020). Rhizobia inoculation is a cheaper, and usually more effective, way of ensuring adequate nitrogen supply to legumes than even the application of nitrogen fertilizer (Nelson, 2004). Legume-rhizobia technology is usually attractive to smallholder farmers due to the more affordable cost in comparison to the inorganic nitrogen fertilizer (Chidua, 2021). Hence, commercial production and use of rhizobia inoculants commenced as early as 1895 in the U.S.A. and the U.K., by 1993 the production has commenced in many countries across all continents (Nelson, 2004). The development of rhizobia inoculant industry in many countries has largely been motivated by the desire to introduce legume species to new areas, which is associated with difficulties in establishment, due to limitation in compatible rhizobia symbionts (Crisóstomo *et al*., 2013).

Several authors outlined circumstances in which availability of suitable inoculants has been and currently is a limiting factor to successful cropping of legumes in many regions of the world (Date, 2000; Mendoza-Suárez *et al*., 2021). Inoculation is thus, essentially required in such areas, when (i) a legume has not been grown previously on a particular land, (ii) the legume is not symbiotically related to any other legume grown in the immediate past, (iii) the legume grown previously on the land was poorly nodulated, (iv) a legume follows a non-legume in a rotation or (v) when land reclamation has been undertaken (Date, 2000). Albareda *et al*. (2008), on the other hand, suggested quantifying the specific natural populations of rhizobia for the legume to be sown by the most probable number (MPN) or any other suitable method to determine the need for inoculation.

Competition from naturally occurring populations of rhizobia is a significant factor determining the establishment of introduced rhizobia inoculant strains in the field. Hence, rhizobia inoculants sometimes face competition from indigenous rhizobia that are well adapted to the native conditions (Chidua, 2021). This is because root-nodule

bacteria are very widespread as a result of natural distribution of the legumes. Even though, there are many soils still devoid of strains of rhizobia effective for particular crops. Thus, where naturally occurring rhizobia are few or absent, introduction of new strains by inoculation of seeds or soil is normally successful, provided effective and compatible rhizobia is used as the inoculant and optimum factors for its efficiency are maintained (Date, 2000; Vanlauwe and Giller, 2006; O'Hara *et al*., 2002; Crisóstomo *et al*., 2013). On the other hand, where large populations of effective rhizobia occur, inoculation becomes invariably futile (Vanlauwe and Giller, 2006; O'Hara *et al*., 2002; Schumpp and Deakin, 2010). Therefore, it is germane to consider how rhizobial populations in the soil can be manipulated to influence legume nodulation, N_2 fixation and productivity. Natural rhizobia populations were not only able to infect groundnut plants but proved highly adapted, competitive and even capable of out-competing introduced rhizobia strains as observed by several authors, such as Yakubu *et al.* (2010), Bala (2011), Abdullahi and Yusuf (2017) and Osei *et al.* (2018). These native rhizobia potentially make improved rhizobia inoculants in the event that they fix higher amounts of nitrogen than the inoculant strain (Chidua, 2021).

The aim of the review is therefore, to clarify the circumstances and identify reasons behind the failure of failure of introduced exotic rhizobia inoculant to yield desirable results and offer possible ways to enhance rhizobia inoculation response by grain legumes in the tropics, by considering harnessing of indigenous rhizobia as a key solution. It also indicates the role of indigenous rhizobia facilitating sustainability legumes productivity and environmental safety.

Challenges facing introduced commercial rhizobia inoculants and relevance of developing local ones

Introduction of commercial inoculants is usually associated with tendencies of failure, mostly due to competition from more adapted indigenous strains and other environmental factors. It is a lamentable fact that after 100 years of seed inoculation with many varied forms of inoculants, they mostly fail to contribute to agricultural production as expected (Nelson, 2004).

Brockwell and Bottomly (1995) asserts that ≥ 90 % of rhizobial inoculants applied on seeds were observed to have minimal effects on nodulation of the developing plants and subsequent N_2 fixation. Many reasons could be advanced for the inefficiency of the introduced commercial inoculants, a key among which is their failure to compete favourably with indigenous rhizobial strains (Deaker *et al*., 2004). The occurrence of indigenous rhizobia populations in soils often leads to inoculation failure due to the large size of the population, their positional advantage, and/or superior adaptation to local environmental and soil conditions, as well as high effectiveness (Sajjad *et al*., 2008; Nievas *et al*., 2012; Wielbo, 2012, Wilson *et al*., 2020). Other reasons responsible for the failure of introduced inoculants beside high population and better adaption of the indigenous populations is their higher competitive ability in nodule occupancy and saprophytic survival in the

soils (Woomer *et al.*, 1988; Babalola *et al*., 2012; Wilson *et al*., 2020). Likewise, high nitrate content of the soil and/or low quality of the introduced inoculant could also cause such failure. Other factors making indigenous rhizobia out-perform exotic commercial inoculants in the occupation of legume nodules include biological, such as the ability to stand bacteriophages or epiphytic bacteria, as well as environmental, such as temperature, pH and nitrate content, characteristic to the area of their existence (Castro *et al*., 1999).

Utilization of indigenous rhizobia as a solution to the problem

Most indigenous rhizobia are usually reported to be in-effective in N_2 fixation or of varying effectiveness. Hence, there is the need to identify and select effective local rhizobia strains before using them for inoculant production. Selective enrichment of legume rhizosphere with highly effective indigenous strains of rhizobia may be the only way to influence legume nodulation under field situations, where ineffective or poorly effective populations are so large that conventional inoculation procedures are ineffective (Brockwell and Bottomly, 1995). Moreover, symbiotic efficiency of rhizobia populations may vary greatly within a local environment, with majority considered to have low efficiency, according to Wielbo (2012). Hence, only selected local inoculants usually form more effective symbioses than commercial inoculants that have been isolated from distant and unrelated soil environments (Sajjad *et al*. 2008). Hence, the identification and selection of local rhizobia strains for adaptation as local inoculants usually leads to significant economic and ecological benefits (Elboutahiri *et al*., 2009; Nievas *et al*., 2012; Khalid *et al*., 2020).

Thus, the selection of indigenous strains with high N_2 fixing capacity, adapted to a wide range of environmental conditions at a specific site, is an important strategy to maximize legume production (Sajjad *et al*. 2008; Wielbo, 2012). Similarly, Saleena *et al*. (2001) reported that selecting effective stress tolerant rhizobia from naturally occurring ones in any particular environment has proved more practical in solving inoculation problems. Bashan *et al.* (2014), likewise suggested for the use of local strains for improved performance of inoculants in any given environment, because no plant growth promoting bacteria (PGPB) strain will perform best in all farming conditions. This is because effectiveness of inoculation depends on multiple parameters, including the target plant species, soil and weather conditions as well as appropriate differentiation and matching of the strains with appropriate crop varieties (Bashan *et al.*, 2014). Hence, the success or otherwise of rhizobia inoculation is influenced by specificity between rhizobia, legume genotype, environmental conditions and management practices involved (Woomer *et al.*, 2014), which allows researchers to make informed decisions about subsequent inoculation practices (Wilson *et al*., 2020).

Procedures for selection of effective rhizobia strain from an indigenous pool

The oldest and still the most commonly employed method of selecting effective rhizobia strains for inoculant production

involves the use of legumes as trap for their specific rhizobia. Effective nodules formed on the trap plant are then cultured and the strains of rhizobia isolated, authenticated and subjected to rigorous selection procedures (Machido *et al*., 2011). The first step in the selection is to screen the whole population and identify potential strains in terms of stress tolerance, nodulation and N_2 fixing efficiency, which would ensure obtaining highly competitive and resistant strains, even under adverse conditions (Saleena *et al*., 2001). O'Hara *et al.* (2002) outlined the strain selection program developed at the Centre for *Rhizobium* Studies, Murdoch University, Australia as having four principal phases: germ-plasm acquisition, isolation of strains, their storage and maintenance in a stable secure environment. Isolates of root nodule bacteria are often obtained from the centres of origin of the host legume and/or stressful soils, as applicable. The strains are then stored as lyophilised cultures, and in deep frozen suspensions in 20% v/v glycerol. The second phase involves glasshouse experiments to authenticate isolates as root nodule bacteria and screen them for effective N_2 fixation. A pool of effective strains is then taken to the field for assessment of their adaptation to the edaphic environment, where they are intended to be used. Field trials are conducted over two or three years to determine the capacity for the strains to survive in the soil. The final phase involves validation of strain performance using a smaller number of strains in larger scale rotation trials in farming systems. This step is often carried out independently by researchers in different locations. The outcome from these processes has been the provision of robust, effective strains of root nodule bacteria for the inoculants industry (O'Hara *et al.* 2002).

Desired characteristics required for selecting indigenous inoculant strains

It is essential that the selected strains are thoroughly tested to ensure that they are effective in plant growth promotion. This is because a good number of attempts to displace established populations of N_2 fixing bacteria with introduced inoculants have typically been unsuccessful (Date, 2000). Similarly, it must be carefully matched to appropriate crop varieties to maximize the boost in yield and soil nitrogen (Date, 2000; Cummings, 2005). It must also be able to form highly effective nodules on the host (s) for which it is recommended and have the capacity to do so promptly under a wide range of field conditions (Date, 2000). It must also be able to compete for nodule occupancy alongside the indigenous strains. Usually, the competitiveness of the isolate is determined by estimating the proportion of nodules formed by the inoculant strains relative to the indigenous strains (O'Hara *et al.*, 2002; Machido *et al*., 2011). Isolates which exhibit high level of competitiveness are considered promising, provided that they also possess the other desirable attributes. This is because, an inoculant strain needs to dominate in nodule formation, if the anticipated benefits are to be derived from its application (Machido *et al*., 2011). Another essentially desired characteristic for inoculant strains is to be highly effective in N_2 fixation with the intended host species, and in some instances, there is a requirement for the strain to effectively nodulate a wide

range of host legume species (O'Hara *et al.*, 2002; Machido *et al*., 2011). The most acceptable indices used in determining the effectiveness of inoculant strains under field condition are (a) amount of N_2 fixed, usually expressed in terms of kg N ha^{-1} yr^{-1} and (b) grain yield, usually expressed in terms of kg or ton ha^{-1} yr^{-1} (Machido *et al*., 2011). Brockwell and Bottomly (1995), similarly identified other major criteria for evaluating success of inoculation as extent of nodulation, proportion of nodules occupied by the inoculant strain, and indices of plant response which may include the total amount of N fixed, the proportion of plant N due to N_2fixation, and dry matter production. A selected inoculant strain must also have the capacity to persist in the soil in the complete absence of the host legume(s). This portends that, the isolate be able to live saprophytically in the soil as well as withstand periods of harsh conditions typifying the soil. An isolate which possesses this attribute usually has a greater chance of establishing itself as a member of the soil community to which it is introduced. Therefore, serving as an assurance against the need for the inoculant to be repeatedly and continuously applied (Machido *et al*., 2011). The pragmatic approach presumes that the strain which performs best in the field is the most desirable strain for field inoculation (Brockwell and Bottomly, 1995; Howieson *et al*., 2016). This emphasizes the importance of strain testing under field situations. The ability of an inoculant strain to grow and survive in chosen inoculant carrier for a long period of time under ideal storage conditions is also an essential requirement (Date, 2000; Machido *et al*., 2011). This is because the viability of the inoculant strain from the point of production to the point of application determines its usefulness (O'Hara *et al.*, 2002; Machido *et al.*, 2011). A good inoculant carrier must support growth of rhizobia cells, maintain the desired population over time, display high water holding capacity, non-toxic to the inoculant strain, neutral pH, provide rapid release of rhizobia cells upon use, be in abundant supply and environmentally safe (Tiwari *et al.*, 2012). Peat is the most popular and widely used carrier due to its protective properties and ability to support and maintain high levels of rhizobia cells over time, when no other contaminating organisms exist (Tiwari *et al*., 2012). However, materials such as soil, charcoal, oils and similar materials are also used as inoculant careers due to their abundant supply (Machido *et al*., 2011). Nowadays, preliminary polyphasic taxonomic approaches comprising phenotypic and genotypic characterization are recommended as a rhizobia identification and classification tools for identification of many novel rhizobial taxa that could serve as effective inoculant prior to field testing (Khalid *et al.*, 2020).

Improvement in legumes productivity by indigenous rhizobia in low fertility soils

Low soil fertility is key constraint to agricultural production (Loks *et al*., 2014; Mohammed *et al*., 2016). This is prominent particularly in savanna zones with high potential for crop production, however, yield levels obtained under farmers' conditions are usually low relative to potential yields (Hamma and Ibrahim, 2013). The major causes of these low yields are low levels of

external sources of nutrients and the near absence of fallows that are long enough to restore the soil nutrients base (Vanlauwe *et al.,* 2001). The soils share common features of low content of organic carbon, total nitrogen and phosphorus, making them inherently low in fertility (Machido *et al.,* 2011). Moreover, majority of small-holder farmers could not afford the high cost and non-availability of inorganic fertilizers, especially nitrogenous mineral fertilizers (Vanlauwe and Giller, 2006; Mekontchou *et al.,* 2007; Yakubu *et al*., 2010).

The ability of legumes such as cowpea to convert inert nitrogen from the atmosphere through biological nitrogen fixation in to forms the plants could utilize is a cheap and sustainable alternative to inorganic fertilizers (Nyaga *et al*., 2020). The benefits of growing these legumes in rotation or mixtures with cereals, relative to continuous cropping of cereals have renewed the farmers' interest on biological nitrogen fixation (BNF). This is because it provides a continuous supply of N for the legume growth in situ, thus sparing N applied to companion cereal crops in a mixture, adds N rich organic matter to the soil, in form of shoot and root residues for the benefit of subsequent crops in rotation and is economically viable (Yakubu *et al*., 2010). Therefore, legumes feature prominently in the multiple cropping systems, such that improvement in the N_2 fixing capacity of the legumes with a view to enhancing their contributing to nitrogen-fertility of and hence productivity of soils (Machido *et al.,* 2011).

Several studies have reported that inoculation with rhizobia to improve the legume's growth, nutrition and production (Takács *et al*., 2018; Aserse *et al.,* 2020). Unfortunately, inoculation with proven rhizobia inoculants as well as some commercial inoculants sometimes failed to yield positive results in regions with different agro-environmental conditions to the original habitat of the rhizobia in the inoculant (Bala, 2011, Yusuf *et al*., 2011; Abdullahi and Yusuf 2017). Indigenous rhizobia inoculated on different legumes including cowpea, common bean, green grams, soybean, and lentils have enhanced legume productivity (Tena *et al*., 2016; Koskey *et al.,* 2017). This has been linked to the indigenous rhizobia's' growth-enhancing traits as well as being well-adapted to the soil and environmental stresses, hence forming effective interactions with the host crops (Mwangi *et al.,* 2011).

Therefore, collection of a pool germplasm of indigenous rhizobia in an area yields effective isolates for local inoculant to improve N_2 fixation and productivity of legumes on screening through glasshouse and field experiments over time (Tiwari *et al*., 2012; Hungria *et al*., 2016; Howieson *et al.,* 2016). According to Yakubu *et al*. (2010) indigenous rhizobia inoculation improved N_2 fixation and productivity of local genotypes of three legume crops, cowpea (*Vigna unguiculata* (L.) Walp.), groundnut, and bambara groundnut (*Vigna subterranean* (L.) Verdc.).

Role of indigenous rhizobia in sustainable legumes production

The most efficient biological nitrogen fixation (BNF) is conducted in symbiotic systems comprising bacterial microsymbionts collectively known as rhizobia and plant hosts belonging to *Leguminosae* family (Wielbo, 2012). Legume-rhizobia symbiosis is a free source of nitrogen that can be exploited by resource-poor farmers for increased crop yields (Giller and Cadisch, 1995), making it one of the most important microbiological processes on earth; globally (Jaiswal *et al*., 2021). It is an environmentally friendly and inexpensive alternative to the use of chemical nitrogen fertilizers in legume crops production. It is an alternative to the popular Herber Bosch process of N fertilizer production, which is relatively costly and leads to many environmental disadvantages (Rao, 2014). Even if affordable, such mineral fertilizers derived from the process are associated with adverse environmental consequences, such as release of greenhouse gases, soil acidification, contamination of underground and nearby water bodies as a result of volatilization, leaching and similar processes (Yakubu *et al*., 2010). Moreover, the interactions between rhizobia and leguminous plants, has varying degrees of host specificity, which is usually based on molecular communication, i.e., signal exchange, between the two symbiotic partners (Chen *et al*., 2003; Oldroyd and Downie, 2008). This determines the quantity of nitrogen that will fixed, preventing unnecessary losses.

Hence, rhizobia inoculants, applied frequently to legumes as biofertilizers, play an important role in sustainable agriculture (Nyaga *et al*., 2020). Therefore, optimizing microbiome function is essential for sustainable agriculture (Hartmann *et al*., 2015). Ensuring the sustainability of agriculture becomes more important in the light of future challenges such as climate change or rapid growth of the human population (Howieson *et al*., 2008; Schlaeppi and Bulgarelli, 2015). Mendoza-Suárez (2021) observed that the next-generation agriculture will greatly benefit from the development of rhizobia bioinoculants based on elite indigenous rhizobia strains that combine effectiveness and competitiveness under specific field conditions. Also, according to Weilbo (2012) numerous rhizobia strains which comprise rhizobia populations are not self-reliant, but act as part of a community. He added that despite being genetically, and physiologically diverse, rhizobia-legume symbionts may contribute community`s genome and may participate in gene flux and evolution of the indigenous population in a particular environment. Moreover, such strains interact with existing plant hosts as a single differentiated population despite plant-derived mechanism, which seems to be responsible for selection for microsymbionts that are highly efficient in nitrogen fixation. Inoculation of legumes with appropriate rhizobia has been regarded as a sustainable and cost-effective technology to augment the plants' nitrogen needs (Ondieki *et al.*, 2017). The utilization of native rhizobia as inoculants promotes ecologically sustainable management of agricultural ecosystems and enhance legume production due to their growth promoting traits and adaptability to soil and environmental stress (Mwangi *et al*., 2011). This partly due to the ability of

native strains to interact positively with the resident soil microbiota and their adaptability to the local agroecological climatic conditions, which often elucidates their superior performance over the exotic commercial strains (Meghvansi *et al*., 2010). Hence, a better understanding of the factors that guide the existence, specificity and effectiveness in the rhizobia-legume symbiosis in a particular area will be needed if sustainable increases in productivity are required in agricultural systems in order to cope with the increasing population in the world, higher N fertilizer prices and other eminent pressures (Howieson *et al.*, 2008). Hence, it has been widely used as a replacement of nitrogen fertilizers in legume production because of its economic efficiency in the provision of sustainable agroecosystem services (Ouma *et al*., 2016).

CONCLUSION

Rhizobia inoculation is a cheaper, more effective and safer way of ensuring adequate nitrogen supply to legumes than even the application of nitrogen fertilizer. However, competition from naturally occurring populations of rhizobia is usually a significant factor determining the establishment of commercial rhizobia inoculant strains in the field. Hence, they usually fail, mostly due the indigenous rhizobial high population, positional advantage, higher competitive ability in nodule occupancy, saprophytic survival, resistance to bacteriophages or epiphytic bacteria and superior adaptation to their local environments. Environmental factors also contribute to the failure, such as high temperature, extreme pH conditions and high nitrate content in the field. Hence, selective enrichment of legume rhizosphere with highly effective indigenous strains of rhizobia may be the only way to improve legume nodulation, N_2 fixation and productivity such conditions. Moreover, there must be consideration to specificity between rhizobia and legume genotypes, environmental conditions and management practices, to which they will be subjected and following standard procedures to achieve the desired results. The role of indigenous rhizobia in providing an alternative to costly inorganic nitrogen fertilizers and commercial inoculants usually developed from rhizobia originating from environments other than the environment they are applied. They are cheaper means of ensuring legumes productivity and environmental sustainability through their biological N_2 fixation that reduces in the adverse effects of inorganic nitrogen fertilizers such as soil acidification, release of greenhouse gases, which would reduce the effect of climate change and support the ever-increasing human population.

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