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SYMBIOTIC N_2 FIXATION AS AN ALTERNATIVE SOURCE OF NITROGEN - A REVIEW

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ABSTRACT

The role of N_2 fixation through symbiotic relationship between legumes and root nodule bacteria or rhizobia could not be over emphasized, as an alternative nitrogen source for legumes and other crops. The N_2 fixed by legume-rhizobia symbiosis ranges between 16 - 145 kg N ha^{-1} $year^{-1}$ in fertile soils and 15 -123 kg N ha^{-1} $year^{-1}$ in poorly fertilized soils, while annual chemical fertilizer use has been reported as 89.7 kg per ha $year^{-1}$. This paper reviews legumes, their interaction with rhizobia in N_2 fixation and importance to agricultural systems. More than 732 genera of legumes have been discovered, with more than 19, 321 species. Legumes, through N_2 fixation,directly influence their own growth and development, simultaneously benefit companion crops in mixed cropping and subsequent crops in rotation. Likewise, there are diverse nature of rhizobia, and more genera and species are being discovered. This therefore, emphasizes the need to intensify legume production, through identifying and utilizing relevant rhizobia as inoculants for particular legumes to optimize the benefits of N_2 fixation. The review addresses legumes and rhizobia in relation to their role in N_2 fixation and benefits derived from incorporating legumes in farming systems. It also discusses the current taxonomy of rhizobia, the concepts of specificity and promiscuity among both symbionts. Likewise, it gives highlight on the genera and species of rhizobia currently described and the dynamic nature of their discovery. There now exist more than 15 genera containing over 120 species of rhizobia, with discovery rate of 10 species per annum.

INTRODUCTION

Biological dinitrogen (N_2) fixation is the second most important biological process on earth, after photosynthesis (David, 2005). It taps from the vast atmospheric N_2, which is almost 80% of the atmospheric gases (Unkovich *et al.*, 2008). Consequently, everyhectare of land at sea level has about 78,000 tonnes of the inert gas above it (Wani *et al.*, 1995). Ordinarily, N_2 gas in the atmosphere, which is much higher in concentration than every other gas,is not directly available to plants (Giller, 2001; Unkovich *et al.*, 2008). Inorganic nitrogen fertilizer obtained through Herber Bosch process has become a serious threat to the environment and human health (Dabessa *et al.*, 2018). Thus, finding alternate source of nitrogen is a viable option in ensuring sustainable agricultural system. Atmospheric nitrogen, only made available to legumes naturally through biological N_2 fixation (BNF) is the option. It is defined as the ability of living organisms to convert the inert N_2 gas into nitrogen containing organic compounds such as ammonia, nitrate and nitrogen dioxide, through asymbiotic or symbiotic processes (Karanja *et al*., 2011; Dabessa *et al*., 2018). It has also been defined as a process whereby, a number of species of bacteria use the enzyme nitrogenase to convert N_2 into ammonia (NH_3), a form of nitrogen that can then be incorporated into organic compounds such as proteins and nucleic acids of the bacteria and associated plants (Unkovich *et al*., 2008). Rhizobia uses the enzyme nitrogenase in the presence of leghaemoglobin to convert N_2 intoforms such as ammonium (NH_4^+) and nitrate (NO_3^-) which are readily usable by the legumes. The legumes in turn provide the rhizobia with shelter (root nodule) and photosynthetic products as food (Moróti and Kondorosi, 2014). The fixed N is assimilated *in situ* by the legume as organic compounds, such as amino acids and nucelotides for direct development (Unkovich *et al.*, 2008; Karanja *et al*., 2011; Sprent *et al*., 2013). The N_2 fixed however,has been reported as larger in fertile fields (16 -145 kg N ha^{-1} $year^{-1}$) than in poorly fertile fields (15 -123 kg N ha^{-1} $year^{-1}$) (Kermah *et al*., 2018), while the current rate of annual chemical fertilizer use is 89.7 per ha $year^{-1}$ (Shah and Wu, 2019). The N_2 fixed, subsequently, becomes available to all forms of life through the nitrogen cycle (Rivas *et al*., 2009). The contribution of legume BNF to N-economy of any ecosystem is mediated by the efficiency of the system, contribution to the soil N pool and the total amount of N fixed that is actually recycled (Karanja *et al*., 2011). Effective strains of rhizobia are indispensable for any adequate N_2 fixation by legumes. This paper, therefore, reviews the role ofthe symbiosis between rhizobia and leguminous plants as an alternative source of nitrogen for immediate benefit of the legumes, other crops and the whole agricultural system.

Legumes and their role in N_2 fixation

The legume family *Leguminoseae* or *Fabaceae* is the third largest family of dicotyledonous plants, traditionally divided into three sub-families associated with distinct flower types; *Caesalpinoideae*, *Mimosoideae* and *Papilionoideae* (Giller *et al*., 2016), among which the *Caesalpinoideae* is the oldest and ancestral sub-family from which the other sub-families diverged (Vanlauwe and Giller, 2006). The total number of legume genera so far discovered has reached 732, with more than 19, 321 species,and more are still continuously being discovered (Giller *et al*., 2016). Leguminous plants are very diverse in morphology, habitat, and ecology; ranging from arctic annuals to tropical trees and are of great importance for ethnobotanical purposes, such as medicines, poisons and fibres (Giller *et al*., 2016). They are grown worldwide as sources of food, feed and edible oil (Rivas *et al*., 2009). They help to improve, particularly, the nutritive status of poor human population who cannot afford animal products, provide means of income at the household level and foreign currency earnings in many countries (Keneni and Imtiaz, 2010).

Legumes have been used in agriculture since ancient times and their seeds or pulses are among the first source of human food since their domestication (Singh *et al*., 2011). Rivas *et al*. (2009) reported that some 25% of the world’s major crop production and more than one-third of humanity’s nutritional nitrogen requirement come from legumes. Legumes are also very important ecologically and agriculturally because they are responsible for a substantial part of the global flux of nitrogen from atmospheric N_2 to fixed forms, such as ammonia, nitrate, and organic nitrogen (Rivas *et al*., 2009). The capability of legumes to fix atmospheric N_2, relative to non-leguminous plants allows them to grow even in N-impoverished soils (Manyong *et al.*, 2001). Hence, N_2 fixation is a critical and key process in sustainable agricultural systems in tropical soils, which are frequently deficient in N and susceptible to leaching of plant nutrients (Kermeh *et al*., 2018). It is an alternative to the popular Herber Bosch process of producing N fertilizers, which leads to many environmental disadvantages over time (Rao, 2014).

Leguminous plants associate with soil bacteria, collectively known as rhizobia, which colonize the legumes' roots (rarely stems), forming specialized organs known as nodules (Denison and Kiers, 2004;Elboutahiri*et al*., 2009;Singh *et al*., 2011;Sprent *et al*., 2013). Within the nodule, the bacteria (bacteroids) reduce ("fix") atmospheric nitrogen (N_2) to ammonia (NH_3) that is passed over to the host plant for assimilation into organic compounds such as amino acids and nucleotides (Sprent *et al*., 2013). Hence, cultivating legumes has been shown as one way of improving N content of the plants and eventually the soils (Mungai and Karubiu, 2010).

Continuous cereal-based cropping leads to rapid decline in soil fertility (Kermeh *et al*., 2018). Legumes under crop rotation and/or mix cropping with cereals rejuvenates depleted soil N through the decomposition of the whole or part of the plant, liberating the biologically available N fixed by the legume into the soil (Manyong *et al*., 2001; Cummings, 2005; Machido *et al*., 2011). They may also spare companion crops in mixed cropping with the mineral N applied, while utilizing the N fixed *in situ* into their tissues for growth and development. Even so, it is a fact that nodulation and nitrogen fixation by legumes is adversely effected by high doses of fertilizer N, because it is an energy intensive process (Dogra and Dudeja, 1993). Shen and Chu (2004) reported that at low rate of applied N, rice (*Oryza sativa*) could utilize the N applied and even obtainmore N from groundnut (*Arachis hypogaea* L.) during the period of their core growth. The legumes may also utilize the fixed N themselves or excrete it through their root nodules into the rhizosphere soil for use by the companion crops (Shen and Chu, 2004). Other benefits of legumes beside this include improvement in nitrogen cycling, and serving as ‘‘break crops’’ to a number of pests and pathogens, hence a reduction in the requirement for pesticide application, thereby, providing sustainability to small-holder farming systems (Keneni and Imtiaz, 2010, Cummings, 2005). A summary of major benefits derived from incorporation of legumes into agricultural systems is shown in Table 1.

TABLE 1. BENEFITS FOR INCORPORATION OF LEGUMES INTO FARMING SYSTEMS

S/No.	Specific value	Forage	Pulses
1.	N_2-fixation benefiting companion or rotational non-legume	†††	† †
2.	High protein human food in green parts, seeds or tubers	†	†††
3.	High protein animal feed	†††	†
4.	Increased soil fertility and structure, decreased erosion risk	†††	†
5.	In permanent settings, provide biodiversity, shelter (tree legumes) and natural beauty	†††	†
6.	High protein, high value cash crop	†	†††
7.	Reduced growth of weed species	††	†††
8.	Break disease and pests' life cycle	††	†††
9.	Capacity for high water use^{+}	†	††
10.	Capacity for nutrient cycling^{+}	†	††
11.	Deliver operational flexibility in farming systems	††	††

†, ††, ††† Increasing applicable value

$^{+}$Greater for trees and for perennial, rather than for annual forage species

Source: O'Hara *et al*. (2002b)

Rhizobia and their role in N_2 fixation

The beneficial association between rhizobia and legumes has been known for more than a century when Beijerink obtained the first pure bacterial culture from a legume's nodule, responsible for N_2 fixation, in 1888. A year later, Frank named this bacteria *Rhizobium leguminosarum* and from that date the root-nodule forming bacteria were named "rhizobia" (Peix *et al*., 2015). Rhizobia is a common name for a group of gram negative, soil inhabiting, motile soil bacteria, belonging to the family *Rhizobiaceae*, which are part of α and β-proteobacteria (O'Hara *et al.*, 2002a; Rajasundari*et al*., 2009; Vinay and Kiran, 2011; O'Hara *et al*., 2016).

The Rhizobia are host plant specific in their interaction with legumes. Specific strains of rhizobia enter into N_2 fixing symbioses with specific host plants, almost exclusively legumes. Successful recognition of each other between compatible bacterial and plant partners elicits the development of the novel plant organ called the root nodule (Sprent *et al*., 2013). The bacteria invade the legume roots and lead to the initiation of the development of the root nodule, inside which they differentiate into the N_2 fixing "bacteroid" (Boogerd and van Rossum, 1997). They are capable of establishing effective N_2 fixing symbiosis system with the legume plants inside the specialized structure, using the enzyme nitrogenase. This is the most important biological mechanism for providing nitrogen to host plants, as an alternative to the energy expensive nitrogen fertilizers production process (Rajasundari *et al*., 2009; Vinay and Kiran, 2011; Sharma *et al*., 2011). Rhizobia are, therefore, popular for this function of making atmospheric N_2 gas available to plants through symbiosis with the legumes and hence, of particular interest in agriculture (Denison and Kiers, 2004;Elboutahiri *et al*., 2009).

Ecologically, rhizobia can exist in two fundamentally different modes. Firstly, they could live in soils either as free-living saprophytesor heterotrophs in the absence of their host and secondly, as legume-host-specific N_2 fixing symbionts in the presence of their host (Sadowsky, 2005). This dual mode of existence gives rhizobia several distinct advantages with respect to survival and persistence over most other soil bacteria (Sadowsky, 2005). There is a close relationship between the growth of legumes in a soil and the occurrence and proliferation of rhizobia populations in the soil (Woomer *et al*., 1988). This is because rhizobia are facultative symbionts that are independent of their host legumes in the saprophytic state, while under certain nitrogen conditions they are not dependent on symbiosis with legumes. This developed as an ecologically convenient mechanism in which the occurrence of one symbiont frequently accounts for the presence of the other (Woomer *et al*., 1988). Rhizobia require access to adequate concentrations of mineral nutrients, such as phosphorus, calcium, boron, copper, molybdenum, iron, nickel and cobalt for their metabolic processes, to enable their survival and growth, both as free-living soil saprophytes, and/or in their symbiotic relationship with legumes (O'Hara, 2001; Weisany *et al*., 2013).

Current taxonomy of rhizobia

Initially only bacteria belonging to the genera *Rhizobium* and *Bradyrhizobium* were descovered. Later four more genera were recognized (*Azorhizobium*, *Mesorhizobium*, *Allorhizobium* and *Sinorhizobium*)to have the ability to nodulate legume hosts,collectively referred to as "the rhizobia" (Denison and Kiers, 2004; Vinay and Kiran, 2011; Singh *et al*., 2011). Improved knowledge led to discovery of more genera in the rhizobia group that later increased the number of genera of rhizobia to seven (*Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Sinorhizobium* and *Methylobacterium*). These were reported to contain about 40 species as alpha-proteobacteria (Berrada *et al*., 2012). Subsequently, more genera were discovered, which include *Ochrobactrum*, *Devosia*, *Blastobacter*and *Methylobacterium* in the alpha-Proteobacteria, *Burkholderia*, *Cupriavidus*in the beta-Proteobacteria and some unclassified strains in the gamma-proteobacteria (Berrada *et al*., 2012). Angelini *et al*. (2011) earlier used a wider extension of the prokaryotic partners which cover other members of the family *Rhizobiaceae*, among the "rhizobia", making it eleven genera (*Bradyrhizobium*, *Rhizobium*, *Mesorhizobium*, *Ensifer*, or *Sinorhizobium*, *Azorhizobium*, *Allorhizobium*, as well as other taxa; *Burkholderia*, *Ralstonia*, *Methylobacterium*, and *Devosia*). Currently, more genera and species are continuously identified to be in symbiotic relationship with legumes for N_2 fixation.According to O'Hara *et al*. (2016), there are 15 genera containing over 120 species of rhizobia, with discovery rate of 10 species per annum (Table 2).

Specificity and promiscuity in legumes and rhizobia

Every legume requires symbiotic nodule-forming bacterial partner, rhizobia for N_2 fixation. However, not all rhizobia are capable of forming nodules on all legumes. Classification of rhizobia into species was initially made purely on phenotypic grounds, and largely on the ability of the rhizobia to form nodules with particular legumes (Karanja *et al*., 2011). This gave rise to the concept of "cross - inoculation" groups, defined as a "group of legume host species nodulated specifically by one set of rhizobia species, and not by rhizobia species that could induce nodules on legumes not belonging to that cross-inoculation group" (Karanja *et al*., 2011). This has been used as an important concept in rhizobia taxonomy when considering the nodulation range of both rhizobia and their legume hosts, in terms of host specificity or promiscuity (Singleton *et al*., 1992; O'Hara *et al*., 2002b; Karanja *et al*., 2011).

TABLE 2. FAMILIES AND GENERA OF ROOT NODULE BACTERIA AND THE APPROXIMATE NUMBER OF DESCRIBED SPECIES (ALSO KNOWN AS GENERA CONTAINING SPECIES USED AS COMMERCIAL LEGUME INOCULANTS)

Family	Genus	Number of described species
α- Proteobacteria		
Bradyrhizobiaceae	*Bradrhizobium*†	15
Brucellaceae	*Ochrobactrum*	2
Hyphomicrobuaceae	*Azorhizobium*	3
	Devosia	1
Methalobacteriaceae	*Methalobacterium*†	1
	Microvirga	3
Phyllobacteriaceae	*Phyllobacterium*	1
	Aminobacter	1
	Mesorhizobium†	29
Rhizobiaceae	*Rhizobium*†	43
	Neorhizobium†	3
	Sinorhizobium/Ensifer	13
	Shinella	1
β - proteobacteria		
Bulkhoderiaceae	*Burkholderia*†	6

† Indicates genera that contain species with strains used as commercial inoculants in agriculture
Source: O'Hara *et al*. (2016).

Legume hosts and rhizobia differ in the range of partners with which they can form symbiosis with (Mpepereki *et al*., 200 0). A legume which nodulates with a restricted number of rhizobia strains (or species) or a rhizobia strain that nodulates with particular legume species is termed 'specific'. An example of specific legume crop is soybean (exception are the later introduced promiscuous soybean genotypes, bred in the

International Institute for Tropical Agriculture - IITA, Nigeria), hence it often requires inoculation (Osunde *et al*., 2003). This requirement becomes necessary when the crop is introduced to new regions, where *Bradyrhizobium japonicum* populations required for its effective nodulation are not endemic, like African soils (Osunde *et al*., 2003). Conversely, promiscuity is the ability of a legume host to nodulate with a wide diversity of rhizobial strains, or the ability of a rhizobia strain to nodulate with a wide diversity of legume host plants (Mpepereki *et al*., 2000). Cowpea (*Vigna unguiculata*), for example, appears to be the most promiscuous legume which has been intensively studied. It nodulates with a wide range of fast and slow-growing rhizobia (Mpepereki *et al*., 2000; Osunde*et al.*, 2003). Similarly, siratro (*Microptilium atropurpureum*) is also very promiscuous, usually employed to trap unknown legume nodulating bacteria from soils (Lima *et al*., 2008; Peix *et al*., 2015).
However, other traits are now given greater weight, partly because of blurring cross inoculation boundaries and partly because of development in computer assisted phylogenetic approaches to rhizobia taxonomy (O'Hara *et al.*, 2002b). Moreover, interactions between rhizobia and leguminous plants, have varying degrees of host specificity. This is usually based on molecular communication, i.e. signal exchange, between the two symbiotic partners (Chen *et al*., 2003; Oldroyd and Downie, 2008; O'Hara *et al*., 2016), a currently important area of study in molecular biology.

CONCLUSION

The paper reviews legumes as very important components of agricultural systems, providing many benefits, the most important among which is symbiotic association with rhizobia to provide an alternative to N fertilizers for legumes and subsequently, other crops. The role of rhizobia has been discussed as important associating micro symbionts of the legumes, showing their diversity, and prospects for further discovery. It is obvious that harnessing the benefits of both organisms would improvesoil fertility and the productivity of both the legumes and thecompanion or rotation crops.

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