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Review on Potentials of *Rhizobia*-Leguminous Plants Symbiotic Nitrogen Fixation for Sustainable Agriculture

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Abstract

Agricultural sector throughout the World is facing problem of supplying sufficient amount of food for the increasing human population without affecting the sustainability of the environment. To supply sufficient food, increasing agricultural productivity is required, which in turn need sufficient application of nitrogen fertilizer which has negative effect on human health and on the environment. To overcome, the negative health and environmental effect of chemical nitrogen fertilizer in put in agriculture replacing or supplementing nitrogen inputs from biological nitrogen fixation (BNF) has been in use for more than a century. Form all Biological Nitrogen Fixation (BNF) systems; the symbiotic nitrogen fixation between leguminous plants and rhizobia is the most efficient in supplying fixed nitrogen for the host plant. The use of Biological Nitrogen Fixation (BNF) system to replace and or supplement the chemical nitrogen fertilizer is limited in the developing nations like Ethiopia. In addition, BNF is affected by various environmental factors like soil acidity, salinity, temperature, moisture, and the rhizobial strain(s) used. As a result, rhizobial strain (s) that has been adapted to the prevailing environmental conditions should be sought. In this review paper, the types of rhizobia and their contribution in biological nitrogen fixation, interaction of rhizobia with host plant and factors affecting symbiotic nitrogen fixation were summarized

Keywords: Legumes, Nitrogen, Biological Nitrogen Fixation, Rhizobia

Introduction

Agricultural sector throughout the world is facing problem of supplying sufficient amount of food for the increasing human population without affecting the sustainability of the environment. To supply sufficient food, increasing agricultural productivity is required, which in turn need sufficient application of nitrogen fertilizer which has negative effect on human health and on the environment. Nitrogen (N) is extremely abundant, comprising about 80 per cent of the atmosphere, but it is in limited supply in soils, since plants cannot convert atmospheric dinitrogen to useful organic forms (Marschner, 1995). Plants acquire N from two principal sources: the soil, through commercial fertilizer, manure, and/or mineralization of organic matter; and the atmosphere through N_2 fixation (Vitousek and Howarth, 1991). An excessive and unwise use of nitrogen fertilizer has negative effect on health and atmosphere (Wolfe and Patz 2002). As a result, replacing or supplementing chemical nitrogen fertilizer by biological nitrogen fixation has been recommended (Peoples *et al.*, 1995). From all biological nitrogen fixing systems, the legume- rhizobia symbiotic nitrogen fixation is the most efficient in supplying fixed nitrogen to host plant. Symbiotic nitrogen fixing bacteria with legumes has been grouped in to nine monophytic group, seven families, 13 genera and 46 species (Sawada *et al.*, 2003). Rhizobia bacteria infect the roots of legumes and induce tumor-like growth called nodules. Initiation of nodule formation on compatible host plants results from a molecular dialogue between the host and the bacteria (Dénarié *et al.*, 1993; Schultze and Kondorosi, 1998; Perret *et al.*, 2000; Spaink, 2000). This reduction of molecular nitrogen to ammonium is catalyzed in all nitrogen-fixing organisms *via.,* the nitrogenase enzyme complex in an ATP-dependent, highly energy consuming reaction (Dighe *et al*., 2010; Vitousek and Howarth, 1991). Nodulation and nitrogen fixation in legume-*Rhizobium* associations are adversely affected by various nutritional and environmental factors (Mohammad *et al*., 1991) including soil acidity, soil temperature, soil water status and salinity (Graham, 1992; Serraj *et al*., 1999; Bordeleau and Prevost, 1994).

Nowadays chemical nitrogen fertilizer is widely used to boost the crop production in order to supply sufficient amount of food to the highly growing population of a nation. The use of chemical nitrogen fertilizer has some draw backs, in a season very small portion of the applied nitrogen fertilizer is used by the crop and the remainder is lost through leaching, volatilization and denitrification. The released mineral nitrogen from soils-plant system negatively affects the environment as well as has negative effect on human health. This is due to high mobility of nitrogen. The applied N fertilizers pollute rivers and lakes and cause eutrophication of terrestrial and aquatic systems and increase global acidification and stratospheric ozone losses (Galloway *et al*., 2003). In addition to the greenhouse effects of the nitrous oxide released by the activity of denitrifying bacteria, the quantity of nitrogen needed for agriculture is projected to increase in the next decades, which could lead to greater environmental pollution. Lesser dependence on fertilizer N and more attention to practices that favor BNF in farming systems will benefit both agriculture and the environment. To balance the need to increase crop productivity and in order to minimize the negative impact of chemical nitrogen fertilizer on environment and human health, to supplement or replace the soil nitrogen requirement form biological nitrogen fixation in the agricultural production system is necessary. Although there are different research activities thorough out the country on biological nitrogen fixation especially with that of leguminous plants-*Rhizobium* symbiotic association the basic biological i nteraction with the host plant and symbiont and different environmental factors affecting the biological nitrogen fixation is limited. So, this review article is intended to fill the available information gap by discussing different types of rhizobia and their contribution in supplying fixed nitrogen to agriculture, interaction of rhizobia with host plant and factors affecting symbiotic nitrogen fixation.

Sources of nitrogen for plants and their effects

Mineral nitrogen is subjected to various forms of losses and as a result it is in limited supply in soils. It is amongst the most limiting nutrient for plant growth, as it is a constituent of proteins, nucleic acids and many other bio-molecules (McCammon and Harvey 1987; Marschner, 1995). As a result, production of high-quality, protein-rich food is extremely dependent upon availability of sufficient N (Socolow, 1999). The entire nutritional N required by humans is obtained either directly or indirectly from plants. Since 1970s, management of N inputs into agricultural systems has become a controversial issue (Oenema and Pietrzak, 2002; Smile, 2000). In one hand, to keep the food supply with the highly increasing world population, increasing the food production is needed which in turn requires optimum supply of nitrogen fertilizers.

The supply of optimum amount of nitrogen fertilizer to the crop fields has some disadvantages (Leach *et al*., 2012). Usually only 30 to 50% of the inorganic nitrogen fertilizer applied is used by the crop and the rest will be lost from the soil-plant system. The processes through which this loss occurs are: through leaching, volatilization, denitrification and other factors. The nitrogen lost from soil plant system can cause pollution to the environment (Agrawal *et al.*, 1999; Leach *et al.*, 2012). Some of the adverse environmental effects of excessive and unwise use of nitrogenous fertilizers are: (i) metheamoglobinemia in infants due to NO_3 and NO_2 in waters and food, (ii) cancer due to secondary amines, (iii) respiratory illness due to NO_3, aerosols, NO_2 and HNO_3, (iv) eutrophication due to high concentration of N in surface water, (v) material and ecosystem damage due to HNO_3 in rainwater, (vi) plant toxicity due to high levels of NO_2 and NH_4 in soils, and (vii) depletion of stratospheric ozone due to NO and N_2O (Erisman *et al.*, 2011; Leach *et al.*, 2012; Wolfe and Patz, 2002).

On the other hand, use of inorganic fertilizers by small holder farmers in sub-Saharan Africa is inadequate due to high costs of chemical nitrogen fertilizers, unavailability and sometimes lack of knowledge on usage. Last but not least, the industrial process by which inorganic nitrogen fertilizers are manufactured requires high temperatures and pressures, obtained by burning natural gas or other fossil fuels. The manufacture of inorganic nitrogen fertilizers therefore represents the largest single energy input in all crop production systems, since 2 per cent of the entire world's fossil fuel consumption is used for this purpose (Moomaw, 2002). Developing countries which lack resources of fossil fuels must import either the manufactured nitrogen fertilizer or the fossil fuels themselves, creating a serious drain on limited supplies of foreign exchange (Moomaw, 2002). Over all, rising concerns over possible environmental effects of chemical fertilizers, as well as their cost for small-scale farmers in developing countries, it is essential to expand the use of the biological nitrogen fixation (BNF) technologies that offer the greatest environmental and economic benefits for each specific agro-ecosystem. For many poor farmers, BNF is a viable, cost-effective alternative or complementary solution to industrially manufactured N fertilizer (Peoples *et al.*, 1995; Postgate, 1998; Tamm, 1999; Vistousek and Howorth, 1991,

Symbiotic nitrogen fixation for sustainable agriculture

Biological nitrogen fixation

The Biological nitrogen fixation is the process by which free nitrogen (N_2) of the air is converted to plant utilizable form of nitrogen such as ammonia. The ability to reduce inert atmospheric nitrogen to ammonia is restricted to some prokaryotes that are able to break the strong triple bond within the N_2 molecule, with the key participation of the enzyme nitrogenase, to produce ammonium. The process requires high doses of energy and the nitrogenase is rapidly inactivated by oxygen. (Graham *et al.*, 2004). There are different forms of biological nitrogen fixation:1) symbiotic nitrogen fixation with legumes and nodule forming bacteria generally called rhizobia, 2) symbiotic nitrogen fixation with Actinorhizal bacteria and non-legumes, 3) associative nitrogen fixation with different cereals and 4) free living nitrogen fixation by different Prokaryotic microorganisms. In addition to their great economic interest and indirect impact on human health, most Biological Nitrogen Fixation (BNF) technologies have the potential to generate global environmental benefits by reducing greenhouse gas emissions and water pollution and promoting more sustainable use of agricultural land (Peoples *et al.*, 1995).

The biological reduction of atmospheric N_2 to ammonium (nitrogen fixation) provides about 65 per cent of the biosphere's available nitrogen (Peoples *et al.*, 1995). Most of this ammonium is contributed by legume–rhizobia symbioses (Pedrosa, 1999), which are initiated by the infection of legume hosts by rhizobia bacteria resulting in formation of root nodules (Seefeldt *et al.*, 2004). Within the nodules, rhizobia are found as bacteroids, which perform the nitrogen fixation: to do this, they obtain sources of carbon and energy from the plant, in the form of dicarboxylic acids (Vance, 2000; Poole and

Allway, 2000). There are two group of symbiotic nitrogen fixation with legumes: rhizobia; the Alpha and Beta protobacteria and all are Gram-negative. The alpha-proteobacterial genera are: *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Sinorhizobium*, and all harbored nodule-forming bacteria, and the beta-proteobacterial *Burkholderia* and *Cupriavidus* (Lindström and Martínez-Romero, 2007; Chen *et al*., 2005; Estrada-delos *et al*., 2001). The taxonomic classification of rhizobia followed standard procedures and was based on the phylogeny of housekeeping genes and whole-genome similarities (Lindström *et al*., 2006). Since nodulation functions did not evolve until long after bacterial housekeeping properties, it is thus not always possible to distinguish nodule formers by their names. Only genera in which nodulating bacteria were first discovered have "rhizobium" in their names, whereas *e.g.* *Burkholderia* (former *Pseudomonas*) species were first recognized through other properties (Lindström *et al*., 2006; Young, 1992).

Symbiotic nitrogen fixing bacteria with legumes has been grouped in to nine monophytic group, seven family, 13 genera and 46 species (Sawada *et al*., 2003). The classification and naming of rhizobia to class/order/family and genus level is shown in Table 1. Not all rhizobial species/strains have the same ability to nodulate and fix nitrogen. There is a host-micosymbiont reference which is influenced by the chemical dialogues between the partners.

The ability of different legumes to form nodules with symbiotic relationship with different rhizobia is significantly different and also the amount of nitrogen fixed in this symbiotic relationship based on partners compatibility (Frankow-Lindberg and Dahlin, 2013; Unkovich *et al*., 2010). Table 2 shows the local name of some selected leguminous plants, their scientific name, the modulating rhizobial species, and the amount of nitrogen fixed per hectare per year.

Table 1: The monophyletic group, class/order/ family and genus of rhizobia, and number of species in each genus (Sawada *et al*., 2003)

Monophytic group	Class/Order/ Family	Genus	Number of species
1	Alphaprotobacteria Rhizobiales Rhizobiaceae	*Rhizobium*	13
		Allorhizobium	1
2	Rhizobiaceae	*Sinorhizobium*	10
		Ensifer	1
3	Phyllobacteriaceae	*Mesorhizobium*	8
4	Bradyrhizobiaceae	*Bradyrhizobium*	5
		Blastobacter	1
		Photorhizobium	1
5	Methylobacteriaceae	*Methylobacterium*	1
6	Hypomicrobiaceae	*Azorhizobium*	1
7	Hypomicrobiaceae	*Devosia*	1
8	Beta Protobacteria Burkholderiales Burkholderiaceae	*Burkholderia*	2
9	Burkholderiaceae	*Ralstonia*	1

Table 2: Estimates of amount of nitrogen fixed by various legumes: local name of legumes, Scientific name, nodualting rhizobia and amount of nitrogen fixed /ha/year (Frankow-Lindberg and Dahlin, 2013; Unkovich *et al*., 2010; Sawada *et al*., 2003)

Legume Plant	**Scientific name**	**Nodulating symbiont**	**Nitrogen fixed (kgN/ha/yr)**
Horse bean	*Vicia faba L.*	*Rhizobium leguminosarum* *Bv. viceae*	45-552
Pigeon Pea	*Cajanus cajan* L. Millspaugh	*Sinorhizobium arboris, S.fredii*	168-280
Cow pea	*Vigna unguiculata* L. Walp	*S.arboris, S.fredii*	73-354
Mung bean bean	*Vigna mungo* L. Hepper	*R.gallicum Bv. gallicum*, R. *tropici, S.mrelense*	63-372
Soya bean	*Glycine max* L. Merr	*S.arboris, S.fredii, S. xinjiamgense, MesMesorhizobium tianshanense,* *B. japonicum, Bradyrhizobium liaoningense,*	60-168
Chick pea	*Cicer arietinum* L.	*M. conceri, M.mediterraneum*	103
Lentil	Lens esculenta **Moench**	*R.leguminosarum, Bv. viceae*	88-114
Pea nut	Arachis hypogeae L.	*R.hainanense*	72-124
Pea	*Pisum sativum* L.	*R.leguminosarum Bv. Viceae*	55-77
Bean	*Phaseolusvulgaris* L.	*R.etli, Bv: phaseoli* *R.leguminosarum Bv.phaseoli* *R.mongalense, R. tropici* *Type A, Type B*	40-70
Leucaena/umpy-bean/faux Mimosa	*Leucaena leucocephala* (Lam.) de Wit	*R.gallicum, Bv. Gallicum, R. tropici, Type A, Type B, S.mrelense,S.sahei, S.terangae, Bv acacia, Ensifer adhaerens, M.plurifarium*	74-584
Alfafa	*Medicago sativa* L.	*Allorhizobium undicola, S.arboris,* *S.fredii, S. meliloti* *Sub group A, Ensifer adhaerens*	229-290
Clover	*Trifolium spp.*L.	*R.leguminosarum Bv. trifoli*	128-207

Interaction of *Rhizobia* and legume plants

In symbiotic relationship between legume and rhizobia, signal exchange occurs through all stages of nodule development. *Rhizobia* bacteria infect the roots of legumes and induce tumor-like growths called nodules (Seefeldt *et al*., 2004). The centre of each mature nodule is packed with billions of di-nitrogen fixing bacteria. The host legume plant supplies the energy needed to drive di-nitrogen fixation by capturing energy from sunlight by the process of photosynthesis (Vance, 2000; Poole and Allway, 2000). The overall efficiency of this complicated symbiosis is thus dependent on the individual efficiencies of both the legume plant and the bacteria, since it involves several 'handshaking" or "lock and key" steps, each of which provides opportunity for interaction. Initiation of nodule formation on compatible host plants results from a molecular dialogue between the host and the bacteria (Dénarié *et al*., 1993; Perret *et al*., 2000; Schultze and Kondorosi 1998; Spaink, 2000).

Legume roots secrete a variety of iso-flavonoids which induce symbiotic genes in homologous bacteria (Cohn *et al*., 1998; Long, 1996; Perret *et al*., 2000; Spaink, 1995). Bacterial recognition of the particular flavonoids produced by a host species provides the first opportunity for plant choice, as only the correct flavonoid/s induce symbiotic gene expression in a particular *Rhizobium* strain. A common genetic determinant for rhizobia is the presence of genes encoding nodulation and nitrogen fixation functions (nod, nol, noe, nif and fix genes). These genes are often carried on plasmids or other accessory elements, such as symbiotic islands, and properties encoded by them can be easily lost or gained (MacLean *et al*., 2007). The nod, nol and noe gene products are involved in production of a nodulation signal, the Nod factor, which is a lipo- chitooligosaccharide. These factors are composed of four to five β 1–4 linked N-acetyl glucosamine units (a chitin backbone) and a fatty acid. Nod factors can vary in their fatty acids, the lengths of their sugar backbones, and the saturation of the acyl unit and decorations (glycosylation, sulfation, methylation) of the reducing and non-reducing ends of the backbone (Perret *et al*., 2000).

The core of the Nod factor molecule is encoded by canonical nodA, nodB and nodC whereas, for example, nodFE are involved in polyunsaturation of the fatty acyl group attached to the core molecule (Long, 2001). Other nodulation genes encode enzymes which add a variety of substituents to the core, as in the case of Nod factors produced by *Azorhizobium caulinodans (*Mergaert et *al*., 1993). The Nod factor acts as an elicitor of root nodule formation by the plant by triggering a developmental program leading to construction of the root nodule and entry of rhizobia into the nodule (Long 2001; Geurts and Bisseling, 2002; Gage, 2004). Only the correct nod factor/s induce root hair curling and infection thread formation (the first steps in nodule formation) in a particular legume species.

Hence, the diversity of nod factors produced by rhizobia, and discrimination of these factors by plants, contribute the second level of specificity to the interaction and create an opportunity for partner choice by the plant (Perret *et al*., 2000). Host plant cells then provide energy in the form of dicarboxylic acids (malate and succinate) and ensure a low but steady oxygen flux *via*., leghaemoglobin regulation (Waters and Emerich, 2000). In an effective symbiosis, the bacteroids carry out nitrogen fixation and ship ammonia (possibly *via*., an alanine shuttle) to the plant across the peribacteroid membrane (Waters and Emerich, 2000). The Nod factor paradigm was challenged by Giraud *et al*., (2007), who discovered that certain photosynthetic, stem- and root-nodulating Bradyrhizobia do not possess canonical nodABC genes but use other mechanisms for signalling to the plant. Their experiments led them to hypothesize that a purine derivative might play a role in triggering nodule formation instead of the Nod factor (Giraud *et al.*, 2007). This points to the complexity of the symbiotic system and shows that bacteria have employed diverse strategies to gain. The interaction of the *Rhizobium* and the host root is shown by Figure 1. The effective nodules formed on the faba bean root is shown by Figure 2.

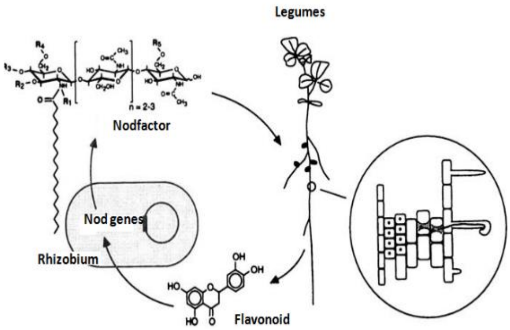
Fig 1: Signal exchange in Rhizobium-plant symbiosis.

Flavonoids produced by the host plant induce rhizobial nod genes. This leads to production of Nod factors.The insert shows an infection thread passing the root cortex toward a cluster of dividing cells that will become a root promordium. (Tamimi, 2014))

Molecular nitrogen fixation and nitrogenase

The fixation of molecular nitrogen into bioavailable compounds for cellular anabolism is a process restricted to some bacteria. Such bacteria are termed diazotrophs, as they obtain all their nitrogen by fixing molecular nitrogen.

Fig 2: Nodules formed on faba bean roots



(Wakjira Bekele, 2017)

During biological nitrogen fixation (BNF) molecular nitrogen is reduced in multiple electron transfer reactions, resulting in the synthesis of ammonia and the release of hydrogen (Kim and Rees, 1997). Ammonium is then used for the subsequent synthesis of biomolecules. This reduction of molecular nitrogen to ammonium is catalyzed in all nitrogen-fixing organisms *via.*, the nitrogenase enzyme complex in an ATP-dependent, highly energy consuming reaction (Fig 3). The nitrogenase complex is comprised of two main functional subunits, dinitrogenase reductase (azoferredoxin) and dinitrogenase (molybdoferredoxin) (Dighe *et al.*, 2010)). The structural components of these subunits are the Nif (nitrogen fixation) proteins NifH (α_2 homodimeric azoferredoxin) and NifD/K (α2β2 heterotetrameric molybdoferredoxin). Basically, three types of nitrogenases are known based on the composition of their metal centers: iron and molybdenum (Fe/Mo), iron and vanadium (Fe/V) or iron only (Fe). The most common form is the Fe/Mo-type found in cyanobacteria and rhizobia (Dighe *et al.*, 2010) (Fig 3).

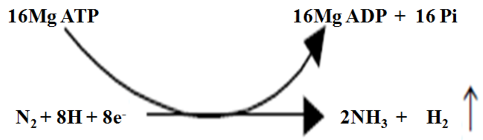
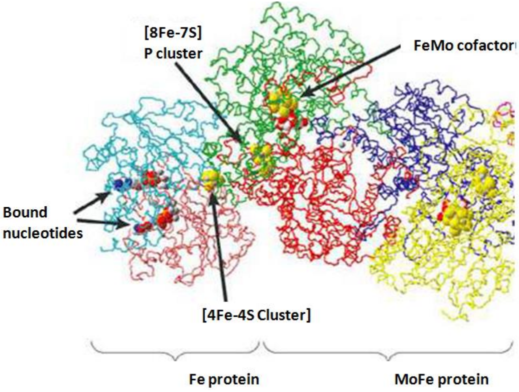


Fig 3: Reaction and its energy requirement of nitrogen fixation by nitrogenase enzymes

An important feature of the nitrogenase enzyme complex is its extreme sensitivity to even minor concentrations of oxygen. In aerobic environments and in photoautotrophic cyanobacteria, where oxygen is produced in the light reactions of photosynthesis (Fay, 1992), nitrogenase activity must be protected. This protection is realised by different mechanisms in nitrogen fixing bacteria, depending on their cellular and physiologic constitutions. Aerobic bacteria like *Azotobacter* limit high intracellular oxygen concentrations by high rates of respiratory metabolism in combination with extracellular polysaccharides to reduce oxygen influx (Dighe *et al.,* 2010)). In some filamentous cyanobacteria, BNF is restricted to specialized cells, the heterocysts, which are separated from other cells, and show reduced photosynthetic activity without oxygen production (Burdon *et al.,* 2003). Unicellular cyanobacteria combine photosynthesis and nitrogen fixation within the same cell and show a temporary separation of these two pathways where BNF is restricted to the dark period, when the oxygen-levels are low. In addition to these protections, the concentration of oxygen can be decreased by biochemical pathways like the Mehler-reaction or by special oxygen scavenging molecules such as cyanoglobin and leghemoglobin, the latter playing a major role in rhizobia-plant interactions (Appleby 1984; Hill *et al*., 1996). Fig 4 shows the organization and complexity of nitroginase enzymes.

Fig 4: Complex of the nitrogenase proteins



(Fe protein and MoFe protein).

The subunits of the protein dimers are coloured in cyan, brown, magenta and grey. The subunits of the MoFe protein are coloured in green and yellow, with b subunits in red and blue. The oxygen-sensitive metalloclusters and bound nucleotides are shown in space-fill (Dixon and Kahn, 2004).

Factors affecting BNF

Several environmental factors such as soil pH, soil salinity conditions, temperature extremes, and soil moisture content, imposed limitations on the symbiotic association between the host plant and *Rhizobium* (Hungaria *et al*., 2000; Zaharan, 1999). Furthermore, the amount of nitrogen fixed by symbionts are variable; depending on the host legume, cultivar, soil texture and composition, bacterial species and growing conditions-especially the presence of soil nitrogen (Hungaria *et al*., 2000)

Effects of salinity of the soils

Survival and growth in saline environments were the result of adaptive processes, such as ion transport and compartmentation, osmotic solute synthesis and accumulation, which lead to osmotic adjustment and protein turnover for cellular repair (Nabizadeh *et al*., 2011); Paul and Cockburn, 1989). Legumes have been suggested as appropriate crops for the enhancement of bio productivity and the reclamation of marginal lands, because these plants not only yield nutritious fodder, protein-rich seeds and fruits, but also enrich soil nitrogen in symbiotic association with rhizobia (Alexander, 1984). Nodulation and nitrogen fixation in legume-rhizobia associations are adversely affected by salinity, which can preclude legume establishment and growth, or reduce crop yields (Mohammad *et al*., 1991).

Unlike their host legumes, rhizobia can survive in the presence of extremely high levels of salt and show marked variation in salt tolerance. Some strains are inhibited by 100 mM of NaCI (Nabizadeh *et al.,* 2011), whereas strains of *Rhizobium meliloti* and *Rhizobium fredii* grew at salt concentrations above 300 mM (Sauvage *et al*., 1983; Yelton *et al*., 1983). Moreover, some *Acacia* and *Prosopis* strains can tolerate up to 500 mM NaCI (Zeghari *et al*., 2000). Osmotolerant *Rhizobium* strains can support large modifications in the osmolarity without decrease in the number of viable cells (Singleton *et al*., 1982). Consequently, their multiplication in the rhizosphere of the plant host is not affected in saline soils, as it is the case in sensitive strains. The osmoadaptation of most microorganisms involves the accumulation of K ions and one or more of a restricted range of low molecular mass organic solutes, termed collectively as 'compatible solutes' (Welsh, 2000). These solutes are accumulated to high intracellular concentrations, in order to balance the osmotic pressure of the growth medium and maintain cell turgor pressure, which provides the driving force for cell extension.

Water deficiency and drought

Water deficiency is a major limiting factor of plant productivity and symbiotic nitrogen fixation in many arid regions of the World. One of the immediate responses of rhizobia to water deficiency concerns morphological changes (Busse and Bottomley, 1989; Shoushtari and Pepper, 1985). The modification of rhizobial cells by water stress eventually leads to a reduction in infection and nodulation of legumes (Graham, 1992; Hungaria *et al*., 2000). In addition to its depressive effect on nodule initiation, water deficit also results in the restriction of nodule development and function (Serraj *et al*., 1999).

The wide range of moisture levels characteristic of ecosystems where legumes have been shown to fix nitrogen suggests that rhizobial strains with different sensitivity to soil moisture can be selected. In vitro studies have shown that sensitivity to moisture stress varies among rhizobial strains (Busse and Bottomley, 1989), while nodulation and N_2-fixation in alfalfa (*Medicago satina*) was improved by inoculating plants with competitive and drought tolerant rhizobia (Hungaria *et al*., 2000), suggesting that rhizobial strains can be selected with moisture stress tolerance within the range of their legume host which is generally more sensitive to moisture stress than bacteria. Several mechanisms have been suggested to explain the varied physiological responses of several legumes to water stress. Under osmotic stress, a balance between internal and external water potentials can be reached if the cells accumulate compatible solutes or osmoprotectants. These include potassium ions, glutamate, glutamine, proline, quaternary amines (glycine betaine) and sugar trehalose, sucrose and glucosylglycerol. However, compatible solutes help to maintain the stability of proteins during osmotic stress via a 'preferential exclusion mechanism' (Potts, 1994).

High temperature and heat stress

In arid regions, high soil temperature affects both the free-living and symbiotic life of rhizobia (Hungaria *et al.,* 2000). For most rhizobia, the optimum temperature range for growth is 28 to 31°C, and many were unable to grew at 38°C (Graham, 1992). Some strains of the rhizobia surviving under heat stress could lose their infectivity, due to plasmid curing or alterations in cellular polysaccharides necessary for infection (*Hungaria et al., 2000*). High soil temperature (35 to 40°C) usually results in the formation of ineffective nodules; however, some strains of rhizobia (for example, *Rhizobium leguminosarum* Bv. *phaseoli*) were reported to be heat tolerant, thereby forming effective symbioses with their host legumes (Hungria and Franco, 1993; Michiels *et al*., 1994). The heat tolerance ability of this strain of rhizobia could be more relevant for growing inoculated legumes in arid climates.

Acidity in Soils

Air pollution stresses, like 'acid rain' and enhanced N deposition, affect soils by accelerating soil acidification. Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they perform symbiotic N_2 fixation (Bordeleau and Prevost, 1994). Acidity is reported to limit both survival and persistence of nodule bacteria in soil, and the process of nodulation itself (Correa and Barneix, 1997). And hence, the failure of legumes to nodulate under acid-soil conditions is common, especially in soils of pH less than 5. The inability of some rhizobia to persist under such conditions is one cause of nodulation failure (Bayoumi *et al.,* 1995), but poor nodulation can occur even where a viable rhizobia population persist (Graham, 1992; Graham *et al*., 1994).

In a study, Evans *et al.,* (1980) found that nodulation of *P. sativum* was 10 times more susceptible to acidity than either rhizobial multiplication or plant growth. Some legumes, for example, *Trifolium subterranean*, *Trifolium balansae*, *Medicago murex* and *Medicago truncatula*, showed tolerance to soil acidity as indicated by dry-matter yield; however, the establishment of nodules was more sensitive to soil acidity in most of these plants than was indicated by the relative yields of dry matter (Evans *et al*., 1990, Howieson *et al.,* 1992). Despite this, elevated inoculation levels have enhanced the nodulation response under acidic conditions in some studies (Pijnenborg *et al*., 1991). For example, the growth, nodulation and yield of *Vicia faba* was improved after inoculation with strains of *R. leguminosarum* bv. *viciae* in acid soils (Carter *et al.,* 1994). It appears that the pH-sensitive stage in nodulation occurs early in the infection process and that *Rhizobium* attachment to root hairs is one of the stages affected by acidic conditions in soils (Caetano-Anolles *et al*., 1989).

Taylor *et al.,* (1991) concluded that acidity had more severe effects on rhizobial multiplication than Al stress and low P conditions. They suggested that colonization of soils and soybean (*Glycine max*) roots by *Bradyrhizobium japonicum* may be adversely affected by acidity which may lead to a decline in nodulation on root systems of tested legumes.

By selection of acid-soil tolerance in both symbiotic partners, annual medics such as *Medicago murex* can be grown symbiotically on soils as acidic as pH 4.3 (Cheng *et al*., 2002). While the genetic control of acid tolerance in *Sinorhizobium* is becoming increasingly understood (Dilworth *et al*., 2001), there is little information on the mechanisms contributing to enhanced nodulation at low pH in host species such as *M. murex* in comparison to *M. sativa* (D'Haeze and Holsters 2002). Cheng *et al*., (2002) reported that the acid-sensitive species of *M. sativa* exhibited delayed nodulation under acid stress relative to the acid-tolerant species of *M. murex*, but that the nodules were eventually formed on both species in the same section of the root.

Conclusion and Future Directions

In attempts of sufficient food production in order to feed the alarmingly increasing human population of the developing countries like Ethiopia is largely based on chemical nitrogen fertilizer input in agricultural sector. The excessive and unwise uses of chemical nitrogen input in the agricultural system have negative health effect and also highly polluting to the environment. To overcome this short-term and long-term effect of chemical nitrogen utilization in the agriculture, looking for ways of supplementing or replacing chemical nitrogen fertilizer from Biological Nitrogen Fixation (BNF) is paramount important.

From all BNF systems, the rhizobia–leguminous plant symbiosis found to be the most efficient in supplying sufficient amount of fixed nitrogen to the host plant and in turn supplied with the photosynthate product required for their growth and reproduction and nitrogen fixation. Although research in BNF has been around for more than three decades in Ethiopia, no single rhizobial inoculants strains which able supply required amount of nitrogen under prevailing agro-climatic conditions was not yet developed and commercialized. There are natural population rhizobia which will persistently survive if their host plant is available or will grow at least in customized crop rotation. But if the agricultural land will be converted in to intensive monoculture and or/ be used for industrializations, contractions and expansion of cities and town, much of the genetic resources of the rhizobium will be lost, which need well organized rhizobial culture collection genetic preservation and aclamatizing the elite rhiobial isolates for mass production of inoculants.

Besides a number of environmental and host factors will affect nodulation and nitrogen fixation by rhizobial–leguminous symbiotic association. This review article can give insights and will motivates microbiologists and agriculturalists to a more detail studies in the rhizobial-leguminous plant symbiotic relationship for supply of biologically fixed nitrogen for agricultural systems, which will contribute for sustainable agricultural production and improving human health and protect environmental pollution.

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