Review on the importance of rhizobial inoculation in grain legume production

Dinkinesh Miressa

National soil testing center,
Addis Ababa, Ethiopia
Corresponding Author: Dinkinesh Miressa;

***Abstract*: Grain legumes are an important source of food and feed as they are plant protein sources. They also contribute to soil fertility improvement through biological nitrogen fixation. Biological nitrogen fixation is the conversion of atmospheric nitrogen to ammonia by microorganisms in root nodules of legume plants. Rhizobia are the bacteria associated symbiotically with legumes and fix atmospheric nitrogen to the host plant. This article illustrates the importance of rhizobia inoculation to legumes in many aspects. Inoculating legume seeds with rhizobia improves shoot and root growth, shoot and root biomass accumulation, seeds per pod, pods per plant, grain yield, and straw yield. Rhizobial inoculation of legumes also plays a vital role in the acquisition of plant nutrients such as biological nitrogen fixation, phosphate solubilization, mobilization of macro and micro plant nutrients. Additionally, rhizobial inoculation has functions like disease suppression in grain legumes (root rot; chocolate spot), soil fertility enhancement, economic benefits, and mitigation of climate change.**

***Keywords*: Grain yield, Inoculation, Nitrogen fixation, Rhizobium, Root nodules**

1. Introduction

The soil contains many types of microorganisms such as bacteria, actinomycetes, fungi, and algae. These soil microorganisms are important because they affect the soil's physical, chemical, and biological properties through different processes (Datta *et al*., 2015). Among the soil bacteria, there is a unique group called *rhizobia* that has a beneficial effect on the growth of legumes. *Rhizobia* are soil-inhabiting bacteria that form the root nodules where symbiotic biological nitrogen fixation takes place. In root nodules, the nitrogen-fixing *rhizobia* exist as irregular cells called bacteroids which are often club and Y-shaped whereas regular rod-shaped when they are living freely in the soil (Datta *et al*., 2015; Bahati 2015).

Different groups of *Rhizobia* genera include *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, and *Azorhizobium*, which can form symbiotic associations with different legumes (Sessitsch *et al*., 2002). The formation of root nodules (Nyaguthii, 2017) in symbiotic legumes involves complex molecular signaling between the legume host and the *rhizobial* microsymbiont (Jaiswal *et al*., 2021). *Sinorhizobium* consists of all fast-growing acid-producing *rhizobia* while *Bradyrhizobium* contains the slow-growing alkali-producing *rhizobia* (Nyaguthii, 2017).

Nowadays, global agricultural production increased considerably. But due to the global human population increase, the demand for higher crop production has also increased substantially. Because of this, to achieve higher agriculture yields, farmers have adopted the extensive application of chemical fertilizers and pesticides, which causes soil degradation and a decrease in soil fertility (Díaz-Valle A. *et al*., 2019).

Inoculants are products composed of living microorganisms capable of benefiting the development of different plant species. *Rhizobia* are the first microorganisms used as inoculants. Currently, the use of inoculants is widespread and indicated in agriculture, mainly for legumes such as soybeans, common beans, faba beans, and cowpea, but the production of inoculants for other legumes and non-legumes has to be increased to obtain the greater yield (Emanoel *et al*., 2020). *Rhizobium* inoculants are selected strains of beneficial soil microorganisms cultured in a laboratory and packed in with or without a carrier. They are host-specific, low-cost, and an environmentally friendly source of nitrogen. *Rhizobia* inoculants coated on legume seeds before planting enhances the growth, yield of legume crops, and provide nitrogen and organic carbon for subsequent or associated crops. Seeds coated with *rhizobia* inoculants are not exposed to chemical nitrogen fertilizer. The coated seeds must be planted in moist soil as soon as possible. (Daniel, 2019).

Chemical fertilizers affect the environment negatively in the context of nutrient supply, crop growth, and environmental quality. The advantages need to be integrated to make optimum use of each type of fertilizer and achieve balanced nutrient management for crop growth. One of the major concerns in today's world is the pollution and contamination of soil. The use of chemical fertilizers and pesticides has caused tremendous harm to the environment. Biofertilizer is the solution for all these problems which is environmentally friendly and now used in most countries (Lennox *et al*., 2020)**.**

There are many types of research and some articles which tell us the importance of *rhizobia*. But still, there is a gap in using inoculants widely. Therefore this article is needed to address to use of bio-fertilizers highly in place of chemical fertilizers to avoid/ minimize their environmental pollution and expensive cost effects. Because bio-fertilizers are cheap and eco-friendly also they have a nonpolluting effect on the environment (soil, water, and air) instead of improving soil fertility.

2. Role of rhizobial inoculation in the acquisition of plant nutrients

About eighteen elements have been acknowledged as important nutrients for plants, each of which has at least one specially defined role in plant growth. *Rhizobium* sp. can contribute to maintaining adequate nutrient uptake from soils by reducing the accumulation of nutrients in agricultural soils. Several studies can be cited about the associations with *Rhizobium* spp. and improvement of nutrient uptake: (Khokhar and Qureshi, 1998; Biswas *et al*., 2000; and Yanni *et al*., 2001) have reported that *rhizobium* inoculation

significantly increases the uptake of nutrients such as N, P, K, Ca, Mg, Zn, Na, Mo, and Fe by rice. Likewise, (Amara and Dahdoh, 1997) and (Hafeez *et al*., 2004) demonstrate that increased uptake of K and Ca by cotton is due to *R. leguminosarum bv. trifolii* and improved uptake of N, K, Zn, Na, Cu, and Fe by wheat are due to *Rhizobium* inoculation (Naveed *et al*., 2015). There is also the report that states the significant increase of K uptake is due to *Rhizobium* inoculation in Pigeon pea (*Cajanus cajan L. Millsp*) during harvest (Mfilinge *et al*., 2014).
When nutrients are found in the soil abundantly, their chance of uptake by plants increases (Stuart, 1980). Ndakidemi *et al*. (2011) reported that *Rhizobium* inoculation significantly enhances uptake of micronutrients such as Mn, Fe, Cu, Zn, B, and Mo in all organs (roots, shoots, pods, and whole plants) except the Mo uptake in roots. *Rhizobium* inoculation is also reported to show a significant increase in the soil Ca and Na (Fatima *et al*., 2007; Kisinyo *et al.*, 2012). *Bradyrhizobium* and *Rhizobium*on *Phaseolus vulgaris* inoculation improves the uptake of P, K, Ca, Mg, S, Mn, Fe, Cu, Zn, B, and Mo in leguminous plants (Makoi *et al*., 2013; Tairo *et al*., 2013).
Nutrient uptake of Sulla (Hydysarum coronarium) which is a forage crop is almost doubled compared to the control which is fertilized by nitrogen due to *rhizobial* inoculation in Northern Tunisia (Soumaya *et al*., 2016). *Rhizobial* inoculation also significantly increases the uptake of N, P, K, and Fe by rice plants compared with un-inoculated controls (Biswas *et al*., 2000).

Biological nitrogen fixation
Biological nitrogen fixation (BNF) is a process of converting atmospheric N into plant usable N such as ammonia through a cascade of reactions between microorganisms and plants with the use of complex enzyme systems (Gedamu *et al*., 2021). Legumes are BNF capable and meet their own N needs. The major part of N fixed by legumes is harvested as grains, while the soil and the succeeding crops also get benefit by N in the form of root and shoot residues. Legume crops substantially reduce the N requirement from external sources (Gopalakrishnan *et al*., 2015).
Biological nitrogen fixation by *rhizobia* occurs in the root or stem nodules (Lindstrom and Mousavi, 2019). It is a complex process, and successful symbiosis depends on the genetic background of both symbiotic partners and is strongly affected by environmental factors (Sogut, 2006; Emanoel *et al*., 2020). Biological N2 fixation is a symbiotic process during which plants provide shelter and carbon as an energy source to the symbiotic microorganisms in exchange for bacterially reduced nitrogenous compounds that can be readily assimilated by the host plant. Indeed, several published pieces of literature reported that BNF supplies legume plants with an average of 80% of N needs thus reducing the amount of mineral N needed especially in low inputs legume-based cropping systems (Daniel, 2019; Janati *et al* 2021).
Currently, the subject of BNF is of great practical importance because the use of nitrogenous fertilizers has resulted in unacceptable levels of water pollution (increasing concentrations of toxic nitrates in drinking water supplies) and the eutrophication of lakes and rivers. Further, while BNF may be modified to the needs of the organism, fertilizer is usually applied in a few large doses, up to 50% of which may be leached. This not only wastes energy and money but also leads to serious pollution problems, particularly in water supplies (Zahran 1999).

Phosphate solubilization
Microorganisms enhance the phosphorus availability to plants by mineralizing organic phosphorus in soil and by solubilizing precipitated phosphates (Tairo *et al*., 2013). Phosphate solubilizers mobilize fixed forms of phosphorus already present in the soil in the available form to the plant (Swarnalakshmi *et al*., 2020). Phosphate solubilizing microbial activities can enhance legume production by reducing reliance on fertilizer inputs (mainly N and P) (Ahmad *et al*., 2014). Therefore, for sustainable legume-based cropping systems, biological fertilization by the application of specific microbes can be promising for both crop productivity and economic-environmental sustainability (Janati *et al.*, 2021). Inoculation of legumes with *Rhizobium* increase the nodulation of legumes causing more nitrogen fixation and making it available for the plants and therefore, it is used as an alternative for urea to minimize the cost of production (Miriko, 2015). *Rhizobial* strains improve plant growth because of their phosphate solubilization ability in some legumes (Mebrahtu and Teklay, 2021).
Phosphate solubilization increases P nutrition in pulses (Swarnalakshmi *et al*., 2020). Phosphorus (P) is a major and key nutrient limiting plant growth. A large portion of applied and soil phosphorus becomes unavailable to plants because of its immobilization in either organic or inorganic form due to the low or high pH of soil. At low pH, P is fixed in the soil by free oxides and hydroxides of Al and Fe, while at high pH it is fixed by Ca. *Rhizobia* species that can solubilize or mineralize the bound phosphorus either by releasing enzymes, phosphatases, or organic acids include *B. japonicum*, *R. leguminosarum*, *S. meliloti*, and *Mesorhizobium mediterraneum* (Naveed *et al*., 2015; Gopalakrishnan *et al*., 2015). *Rhizobium leguminosarum bv. viciae* exhibited the highest inorganic P solubilization. Other inorganic P solubilizers include *Sinorhizobium meliloti*, *Rhizobium leguminosarum bv. phaseoli*, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum (*Alikhani *et al*., 2007)

Mobilization of macro and micro plant nutrients
Most *rhizobial* strains produce siderophores, indole acetic acid (IAA), and organic acids in culture media. As with plants growing in low nutrient environments (Dakora & Phillips, 2002), *rhizobia* use these exuded compounds to enhance mineral nutrition. Siderophores are used to mobilize iron (Fe) whereas organic acids solubilize phosphorus (P) and manganese (Mn). There is also evidence that plant roots in a mixed cropping system can benefit directly from this pool of bacterially solubilized nutrients. Field studies have shown that inoculating groundnut with different *rhizobial* strains stimulates greater accumulation of calcium (Ca), P, magnesium (Mg), potassium (K), zinc (Zn), and other nutrient elements in seeds and nodules relative to that in un inoculated controls (Dakora, 2003; Soumaya *et al*.,2016). Alikhani *et al*. (2006) reported that many *rhizobia* isolated from Iran soil can mobilize P from organic to inorganic sources which can improve P plant nutrition.

Under aerobic environments, iron exists as insoluble hydroxides and oxy-hydroxides, which are not accessible to both plants and microbes. Bacteria can synthesize low molecular weight compounds termed siderophores (Naveed *et al*., 2015) capable of sequestering Fe^{3+}. These siderophores are water-soluble and have a high affinity for Fe^{3+} and thus making the iron available for plants. *Rhizobacteria* reduce Fe3+ ions to Fe^{2+} and release them into the cells (Gopalakrishnan *et al*., 2015). Siderophores can also form a stable complex with heavy metals such as Al, Cd, Cu, etc., and with radionucleides (Neubauer *et al*. 2000). Thus, the siderophore producing bacteria can relieve plants from heavy metal stress and assist in iron uptake. *Rhizobial* species, such as *R. meliloti*, *R. tropici*, *R. leguminosarum bv. viciae*, *R. leguminosarum bv. Trifolii* R. *leguminosarum bv. phaseoli*, *S. meliloti*, and *Bradyrhizobium* sp. are known to produce siderophores (Arora *et al*. 2001; Carson *et al*. 2000). Iron is one of the essential elements required by plants for growth and is a component of critical macromolecules such as leghaemoglobin and nitrogenase, both required for the N_2 fixation process (Jaiswal *et al*.,2021).

3. Role of rhizobial inoculation on growth and yield of grain legumes

The application of appropriate *rhizobia* together with plant growth-promoting rhizobacteria (PGPR) is considered an effective and environment-friendly approach that may greatly increase the crop yield by different mechanisms of action under variable conditions (Naveed *et al*., 2015). Besides the provision of symbiotic N, some *rhizobia* also exhibit physiologically desirable traits such as the production of plant growth-promoting phytohormones, which include indole-3-acetic acids (IAA), cytokinins, gibberellins, riboflavin, lumichrome, Nod factors, etc all of which play diverse roles in enhancing plant growth and productivity (Jaiswal *et al*.,2021).

Inoculation of legumes with *rhizobia* triggers plant growth, development, and yield and it is used as a substitute for mineral nitrogen fertilizer which is often costly. However, the *rhizobium* is host-specific as certain species can only infect specific legumes (Asante *et al*., 2020). Inoculation of soybean seed with a mixture of *bradyrhizobia* strains increases nodulation and total plant nitrogen. Several researchers have found that inoculation of soybean seeds before planting results in increases in nodulation, percentage N, plant growth, seed yield, and yield components compared to un-inoculated control (Miriko 2015).

Growth and yield of leguminous crops can be triggered by *rhizobial* inoculation directly by producing various metabolites/substances such as plant hormones, ACC deaminase enzymes, LCOs, siderophores, lumichrome, riboflavin, etc., and/or by fixing/solubilizing/increasing the uptake of mineral nutrients (Naveed *et al*., 2015). In a highly heterogenous hillside environment, and under a variety of traditional practices and cropping histories, *rhizobium* inoculants can benefit farmers by improving nodulation, plant growth, and grain yield even in the absence of co-interventions (Thilakarathna *et al*., 2019).

In Pakistan lentil (Lens culinaris) cultivation, inoculation with *rhizobia* offers the potential to increase yields by stimulating N2 fixation (Vienna, 1998). The application of P fertilizer and seed inoculation with effective *rhizobium* strains positively affects the nodulation and vegetative growth of the plants, which ultimately results in increased yield performance. There is a study that indicates that in addition to increasing grain yield, grain quality in terms of thousand seed weight also significantly improved because of *rhizobial* inoculation (Sisay Belete *et al*., 2019). Application of effective *rhizobia* strains alone or in combination with zinc significantly increases the grain yield of faba bean (Adissie *et al*., 2020). Grain and straw yields of rice increase consistently when inoculated with rhizobial strains (Biswas *et al*., 2000). Thus, decreasing the use of chemical fertilizers can reduce the impact of agriculture on the environment as well as contribute to the development of greater sustainable agriculture (Sogut, 2006).

Effects on growth of grain legumes: shoot and root growth; shoot and root biomass accumulation

Treating seeds with *rhizobium* significantly increases root length, nodule number on a plant root, and plant biomass (Jinwen *et al*., 2016). The biomass yield difference obtained from the inoculation of faba bean strains can be from the additional supply of nitrogen through biological nitrogen fixation by the inoculated strains (Adissie *et al*., 2020). The results of the study on chickpea under salt stress conditions by inoculating *rhizobial* strains show that salt-tolerant *rhizobial* strains stimulate root, shoot growth, and nodulation of chickpea affected by salt stress (Khaitov *et al*., 2016). *Rhizobium* inoculation is more effective for increased plant height, leaf area, shoot dry weight, and nodulation in terms of number and dry weight than supplying nitrogen fertilizers (Soumaya *et al*., 2016).

In Pakistan, inoculation of lentils has significant positive effects on nodulation, total biomass, grain yield, and N yield (Vienna, 1998). In the study by Nyoki and Ndakidemi (2014 a), *rhizobia* inoculation in cowpea significantly improves the plant height measured at four, six, and eight weeks after planting in both greenhouse and field experiments relative to the control treatment (Mfilinge *et al*., 2014). Bano *et al*. (2010) reported that bacterial strains adapted to drought stress are effective in the root-nodule symbiosis and also improve the low growth and yield of chickpea imposed by drought stress (Khaitov *et al*., 2016). Native *rhizobial* inoculation significantly affects the shoot dry weight of cowpea, this improvement is because *rhizobia* increase plant growth and improve the plant biomass by providing nitrogen through symbiotic fixation. Inoculated common beans have a higher shoot dry weight compared to the control indicating that inoculation with native isolates improves the growth of plants (Erana *et al*., 2020).

Effects on yield and yield components of grain legumes: seeds per pod; pods per plant; grain yield and straw yield

Biological nitrogen fixation using effective strains in the traditional cropping system is of vital importance for environmental sustainability and yield improvement which decreases the use of chemical fertilizers. Inoculation of efficient nitrogen-fixing *rhizobia* is most directly related to effective nodule formation for enhanced plant growth. The ability of inoculation to provide top yields over un-inoculated plants is a result of adequate inoculation of efficient *rhizobia* strain (Zerihun and Lijalem, 2020). Inoculating legumes with *rhizobia* is used to achieve substantial increases in legume nodulation, grain and biomass yield, nitrogen fixation, and post-crop soil nitrate levels. These gains are usually highest when the inoculated legumes are grown in nil-*rhizobia* or low-*rhizobia* soils, but minor in soils that already have a high number of compatible *rhizobia* (Abdullah *et al*., 2014).

A study by Fatima et al. (2008) investigates that inoculation of chickpea with *rhizobium* can improve N nutrition, promote vegetative growth, like root growth, and improve root uptake from soil. Therefore, inoculation of chickpea with *rhizobia* increases plant growth, ground dry matter, number of pods, seed yield, and nitrogen fixation under various climatic conditions (Khaitov *et al*., 2016). In Bangladesh, inoculation of groundnut with *bradyrhizobium* increase average nodule number, nodule dry weight, plant dry weight, pod, seed yield, and stover. In India, inoculation of chickpea results in at least a doubling of nodule number, a three-fold increase in nodule mass, and seed-yield increases ranging from 24 to 50% (Vienna, 1998).
There are studies on Mungbean (Vigna radiata L.) and soybean (Glycine max L.) that demonstrates *rhizobium* inoculation significantly affects the growth and yield components like number of pod bearing branches per plant, number of pods per plant, number of seeds per pod, and 1000-seed weight (Mfilinge *et al*., 2014). The study on the effects of *Rhizobium* inoculation in Vigna mungo and Vigna radiate shows that inoculated plants possess improved height, fresh weight, number of roots, nodules, number of leaves, shoots, pods, length of pods, seed weight, over the controls. Tairo and Ndakidemi (2013) on soybean found that plant height for field experiment increases with *rhizobium* inoculation for the entire interval of the soybean growth. Inoculated soybean plants show improvement in nodulation, growth, and yield (Zerihun and Lijalem, 2020).

4. Contribution of rhizobial inoculation in disease suppression in grain legumes

Micro-organisms in the rhizosphere react with many metabolites released by plant roots. Their interactions with roots help in nutrient uptake of plants, the adaptation of plants to adverse soil chemical conditions, and disease susceptibility. Soil beneficial microorganisms have been studied intensively because of their potential impact on agricultural productivity. The study on the middle salinated soils of Uzbekistan indicates that the effective indigenous *rhizobial* strains isolated from chickpeas have the characters of broad host range, high nodulation efficiency, N fixation, and salt tolerance. Selected strains enhance chickpea yield and reduce the percentage of disease plants caused by *Fusarium* pathogens in salinated soil (Khaitov *et al*., 2016).
Rhizobia show several indirect mechanisms of plant growth promotion which involve antibiosis, parasitism, competition for nutrients, and induction of systemic resistance. Antibiosis is one of the effective mechanisms of *rhizobia* to prevent the proliferation of phytopathogens by synthesizing antimicrobial compounds known as antibiotics. Many *rhizobial* species have been reported to secrete antibiotics and cell wall degrading enzymes that can inhibit/kill the phytopathogen (Gopalakrishnan *et al*., 2015; Naveed *et al*., 2015).
Parasitism is a type of symbiosis in which one organism which is the parasite is benefited and the other organism which is a host is harmed from the association which may lead to the injury or death of the host. Some *rhizobial* species such as *R. leguminosarum*, *S. meliloti*, and *B. japonicum*, attack fungal pathogens *Fusarium*, *Macrophomina*, and *Rhizoctonia* using the association (Naveed *et al*., 2015).
The other way of inhibiting pathogens by *rhizobial* inoculation is by using a biocontrol process in which one organism avoids the growth or proliferation of other pathogenic organisms through the production of antibiotics, cell wall degrading enzymes, siderophores, and competition for nutrients (Gopalakrishnan *et al*., 2015). There are *rhizobial* strains that are biocontrol agents. For example, in iron competition, the biocontrol agent produces siderophores that sequester iron in the rhizosphere and make it less available to certain pathogenic microorganisms in the rhizosphere. In this case, since the pathogens cannot get adequate iron, they can be inhibited (Naveed *et al*., 2015). Some siderophore producing *rhizobia* are *R. leguminosarum bv. viciae*, *R. leguminosarum bv. trifolii*, *R. leguminosarum bv. phaseoli*, *R. meliloti*, and *R. tropici* (Carson *et al*. 2000).
Making the host plant more resistant to pathogens by inducing a change in its susceptibility is another way of disease suppression using *rhizobia* inoculation. Because, some *rhizobia* species can produce bio-stimulatory agents (Peng *et al*., 2002; Singh *et al*., 2006). *Rhizobium leguminosarum bv. Phaseoli R. leguminosarum bv. trifolii* and *R. etli* have been reported to protect pathogenic stresses through induction of enhanced systemic resistance in potato and rice plants (Mishra *et al*., 2006; Reitz *et al*., 2000). Generally, *rhizobia* can indirectly help the growth of legumes by inhibiting the phytopathogens by producing antibiotics, fungal cell wall-degrading enzymes, and through starvation by siderophore production (Naveed *et al*., 2015).

Root rot; chocolate spot; insects

Bradyrhizobium japonicum, *R. meliloti*, and *R. leguminosarum* are controlling agents of pathogens that infect okra and sunflower, such as *Fusarium solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani* (Ozkoc and Deliveli 2001; Shaukat and Siddqui 2003). Whereas *R. leguminosarum bv. Phaseoli* and *R. leguminosarum bv. trifolii* are controlling agents of sheath blight of rice (Chandra *et al*., 2007; Mishra *et al*., 2006). In addition, *R. leguminosarum viciae* controls Pythium root rot of sugar beet (Bardin *et al*., 2004); *R. etli* controls cyst nematode of potato (Reitz *et al*., 2000), *M. loti* controls white rot disease in Brassica campestris. Co-inoculation of *Pseudomonas* with *Rhizobium* reduces the incidence of collar rot in chickpea. (Sindhu and Dadarwal 2001). Bradyrhizobium controls the infection of *M. phaseolina* in peanuts. *Sclerotinia sclerotiorum*, *M. phaseolina*, *F. oxysporum*, *F. solani*, *R. solani* and *Colletotrichum sp*. are inhibited by the use of *R. leguminosarum B. subtilis* and *Pseudomonas* isolated from root nodules and rhizosphere of common bean, as dual culture or as cell-free culture filtrate (Gopalakrishnan *et al*., 2015).
Siderophores produced *by Sinorhizobium meliloti* suppress *Macrophomina phaseolina* which is the cause of charcoal rot in groundnut (Arora *et al*., 2001). Co-inoculation of groundnut with *Rhizobium* and *Trichoderma harzianum* effectively inhibits *Sclerotium rolfsii*, the fungal pathogen that causes stem rot disease (Ganesan *et al*., 2007). A *Rhizobium* is also found to protect soybean from root rot caused by *Phytophthora megasperma*, while a *Sinorhizobium sp.* inhibits plant infection by *Fusarium oxysporum* (Deshwal *et al*., 2003). Rhizobitoxine-producing strains of *Bradyrhizobium japonicum* are also able to successfully block infection of soybean by *Macrophomina phaseolina*, the causal pathogen of charcoal rot (Jaiswal*et al*.,2021).

5. Effects of rhizobial inoculation in soil fertility enhancement

Soil amendment with beneficial microorganisms is gaining popularity among farmers to improve the decline of soil fertility and to increase food production and maintain environmental quality. (Gitonga *et al*., 2021). Benefits provided by BNF associated with legume trees in tropical environments include improvements to efficiency of nitrogen (N) use, an increase of soil carbon sequestration, stabilization of soil organic matter, a decrease of soil penetration resistance, and improvement of soil fertility. So BNF is a crucial ecosystem service to the sustainability of tropical agriculture (Emanoel *et al*., 2020).

The fixation of nitrogen provides the plant with available ammonium, whereas the plant provides the rhizobia with simple sugars. Estimates have shown that this symbiotic relationship contributes 40 million tons of nitrogen yearly to grain legumes. This makes BNF an important source of fixed nitrogen to productivity directly by increasing the production of legumes and indirectly improving soil fertility (Nyaguthii, 2017). The expanded interest in ecology has drawn attention to the fact that BNF is ecologically concerned and that its greater exploitation can reduce the use of chemical fertilizers and can be helpful in the reforestation and restoration of misused lands to productivity (Zahran 1999).

The efficiency with which atmospheric N is fixed by legumes, as well as the total amount of N, incorporated into the soil system can be considerably increased by inoculating the seeds with effective strains of *rhizobium*. Studies have shown that inoculation with effective *rhizobium* strains and a small amount of P fertilizer significantly increases grain yields of legumes. This practice appears to play an important role in the sustainable intensification of smallholder systems in Sub-Saharan Africa because of its potential to enhance both soil fertility and crop yields at a low cost (Sisay *et al*., 2019).

Legume- *Rhizobium* symbiotic nitrogen (N) fixation is an important biological character and also the base of improving soil fertility. There is evidence that certain strains of bacteria in the root nodules of leguminous crops can help tolerate toxic levels of salinity. The salt tolerance abilities of *rhizobia* may have an important effect on the successful *rhizobium*-legume associations under salinity conditions. It aims at obtaining ecologically safe food and higher yield without disturbance of the environment and simultaneously, improves the soil quality. For example inoculation of chickpea seed with *Rhizobium* bacteria has beneficial effects for both crop production and soil fertility (Khaitov *et al*., 2016).

6. Economic benefits of rhizobial inoculation for grain legume growers

Biological nitrogen fixation (BNF) has been widely used as a replacement for nitrogen fertilizers in legume production because of its economic efficiency in the provision of sustainable agro-ecosystem services (Koskey *et al*., 2017). The exploration of BNF as a key ecological service can bring economic, ecological, and agronomic benefits to assist in the process of sustainable intensification of agriculture in the humid tropics (Emanoel *et al*., 2020). The N2 fixed by legume crops in Australia has an economic value, in terms of both the N itself and rotational benefits (Vienna, 1998).

The symbioses between *rhizobium* or *Brady rhizobium* and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume-based crop and pasture production than the application of fertilizer-N (Zahran, 1999). Inoculants are low-cost but increase legume yield. This means, the use of seed inoculation economically reduces the purchase and application costs of chemical fertilizers (Vanlauwe *et al*., 2019). Inoculation improves grain yield and grain quality. It offers alternative low-cost and low-input options to sustainably increase yield performances of grain legumes. This has an important economic significance (Sisay Belete *et al*., 2019).

7. Contribution of rhizobial inoculation in mitigating climate change

Global warming and climate change are expected to cause land degradation (Gopalakrishnan *et al*., 2015). Phytohormones produced from rhizobia-legume symbiotic associations may promote plant adaptation to various edapho-climatic stresses (Jaiswal*et al*.,2021). Salinity is one of the most brutal among abiotic stresses limiting groundnut (Arachis hypogaea L.) productivity. The nitrogen-fixing bacteria play a vital role in crop production and soil health due to their ability to fix atmospheric nitrogen and prove to be environmentally friendly by minimizing pollution problems concerned with the application of chemical fertilizers even under stress conditions (Khalid *et al*., 2020).

Applications of chemical fertilizers are a big cause of climate change as they have a strong connection with greenhouse gas (GHG) emissions. It has been reported that 10–20% of global GHG (Carbon dioxide, Methane, and Nitrous oxide) emissions are from the agricultural sector because of nitrogen (N) fertilizer use in crop production. Nitrous oxide (N_2O) is a potent greenhouse gas that contributes to the destruction of the stratospheric ozone which causes climate change. Hence it is essential to minimize these losses by replacing chemical fertilizers with inoculants. Thus, the replacement of chemical fertilizers with inoculants can be a good source to mitigate the ill effects of climate change (Khalid *et al*., 2020).

There is a study that demonstrates that nodulation, growth, and N2 fixation in alfalfa can be improved by inoculating plants with competitive and drought-tolerant rhizobia. This could be an economically feasible way to increase alfalfa (M. sativa) production in water-limited environments. Strains of *rhizobia*, like *R. leguminosarum bv. phaseoli* are recently reported to be heat tolerant and to form an effective symbiosis with their host legumes. These associations will be relevant for cultivation in arid climates (Zahran 1999).

8. Conclusions and future direction

Biological nitrogen fixation is a better alternative N source, through the use of *rhizobia* inoculants. It is a nonpolluting and more cost-effective way to improve soil fertility. The application of *rhizobial* inoculants in legume production has great economic, environmental, and ecological benefits. Increasing and spreading the role of biofertilizers such as *rhizobium* can decrease the use of chemical fertilizers and decline adverse environmental effects. Therefore the use of *Rhizobium* inoculants for improvement in N-fixation and productivity of grain legumes is so advisable since inoculants are cost-effective and eco-friendly.

9. Acknowledgment
The Author thanks Getahun Mitiku, Holota Agricultural Research Center, and Dr. Kenatu Angassa, Addis Ababa Science and Technology University, for the edition of the manuscript.

References
Abdullahi A. A., J. Howieson, G. O' Hara, J. Tepolilli, R. Tiwari, A. VivasMarfisi, A. A. Yusuf (2014). History of rhizobia inoculants use for grain legumes improvement in Nigeria – the journey so far.
Adissie S., Adgo E. & Feyisa T. (2020). Effect of rhizobial inoculants and micronutrients on yield and yield components of faba bean (Viciafaba L.) on vertisol of Wereillu district, South Wollo, Ethiopia, *Cogent Food & Agri*., **6**:1, 1747854, DOI: 10.1080/23311932.2020.1747854
Ahmad, E., Zaidi, A., and Khan, M. S. (2014). "Response of PSM inoculation to certain legumes and cereal crops," in *Phosphate Solubilizing Microorganisms*, ed. M. S. Khan, A. Zaidi and J. Musarrat (Cham: Springer), 175–205. doi: 10.1007/978-3-319-08216-5_8
Alikhani H.A., Saleh-Rastin N., Antoun H. (2006). Phosphate solubilization activity of rhizobia native to Iranian soils. *Plant and soil*, **287** (Suppl **1-2**): 35-41
Alikhani H.A., Saleh-Rastin N., Antoun H. (2007). *Proceedings of the First International Meeting on Microbial Phosphate Solubilization.* Springer; Dordrecht, The Netherlands. Phosphate solubilization activity of rhizobia native to Iranian soils; pp. 35–41.
Amara MAT, Dahdoh MSA (1997). Effect of inoculation with plant growth promoting rhizobacteria (PGPR) on yield and uptake of nutrients by wheat grown on sandy soil. *Egypt J. Soil Sci*. **37**:467–484
Arora NK, Kang SC, Maheshwari DK (2001). Isolation of siderophore producing strains of Rhizobium meliloti and their biocontrol potential against Macrophomina phaseolina that causes charcoal rot of groundnut. Curr Sci 81:673–677
Asante M., Ahiabor B. D. K., and Atakora W. K. (2020). Growth, nodulation, and yield responses of groundnut (Arachis hypogaea L.) as influenced by combined application of rhizobium inoculant and phosphorus in the Guinea Savanna Zone of Ghana. *International Journal of Agronomy*. Volume 2020, Article ID 8691757, 7 pages.
Bahati L. S. (2015). Effect of rhizobia inoculant on soybean nodulation and arbuscular mycorrhiza fungi colonization under greenhouse and field conditions. A thesis submitted in partial fulfillment of the requirements for the award of Master of Science Degree in Integrated Soil Fertility Management, Department of Agricultural Resource Management, Kenyatta University, Kenya.
Bano, A., Batool, R., Dazzo, F., (2010). Adaptation of chickpea to desiccation stress is enhanced by symbiotic rhizobia. *Symbiosis* **50**: 129-133.
Bardin SD, Huang HC, Pinto J, Amundsen EJ, Erickson RS (2004) Biological control of Pythium damping-off of pea and sugar beet by Rhizobium leguminosarum bv. viceae. *Can. J. Bot*. **82**:291–296
Biswas JC, Ladha JK, Dazzo FB (2000). Rhizobial inoculation improves nutrient uptake and growth of lowland rice. *Soil Sci. Soc. Am.* J. 64:1644–1650
Carson K.C., Meyer J.M., Dilworth M.J. (2000). Hydroxamate siderophore of root nodule bacteria. *Soil Biol Biochem*. 32:11–21
Chandra S, Choure K, Dubey RC, Maheshwari DK (2007) Rhizosphere competent Mesorhizobium loti MP6 induces root hair curling, inhibits Sclerotinia sclerotiorum and enhances growth of Indian mustard (Brassica campestris). *Braz. J. Microbiol*. **38**:128–130
Dakora F.D. (2003). Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. Research review. *New Phytologist* (2003) 158: 39–49. doi: 10.1046/j.1469-8137.2003.00725.x
Daniel Keskse Bahru (2019). Review on: Response of Legume Crops to Rhizobium Inoculation and Inorganic Fertilizers (N, P, K and S) Application in Ethiopia. *Intern. J. Res. and Innov. in Earth Sci.* **6**(**4**): 2394-1375 ISSN (Online)
Deshwal, V. K., Pandey, P., Kang, S. C., and Maheshwari, D. K. (2003). Rhizobia as a biological control agent against soil borne plant pathogenic fungi. *Indian J. Exp. Biol*. **41**: 1160–1164. Available online at: http://nopr.niscair.res.in/handle/ 123456789/23372
Datta A., Singh R. K., Kumar S., Kumar S. (2015). An effective and beneficial plant growth promoting soil bacterium "*Rhizobium*": A Rev. *Annals of Plant Sciences*, 2015, **4** (**01**), 933-942.
Díaz-Valle A, López-Calleja, A.C. and Alvarez-Venegas R. (2019). Enhancement of pathogen resistance in common bean plants by inoculation with rhizobium etli. *Front. Plant Sci*. 10:1317. doi: 10.3389/fpls.2019.01317
Emanoel G. Moura, Cristina S. Carvalho, Cassia P. C. Bucher, Juliana L. B. Souza, Alana C. F. Aguiar, Altamiro S. L. Ferraz Junior, Carlos A. Bucher and Katia P. Coelho. (2020). Diversity of rhizobia and importance of their interactions with legume trees for feasibility and sustainability of the tropical agrosystems. Review. *Diversity*, **12**:206
Erana K., Berhanu A., Anteneh A. & Solomon T. (2020). Symbiotic effectiveness of cowpea (Vignaunguiculata (L.) Walp.) nodulating rhizobia isolated from soils of major cowpea producing areas in Ethiopia, *Cogent Food & Agriculture*, **6**:1, 1763648, DOI: 10.1080/23311932.2020.1763648
Fatima, Zarrin, Muhammad Zia and M Fayyaz Chaudhary (2007). Interactive effect of Rhizobium strains and P on soybean yield, nitrogen fixation and soil fertility. *Pakistan J.Botany* **39**: 255.
Fatima, Z., Bano, A., Sial, R., Aslam, M., (2008). Response of chickpea to plant growth regulators on nitrogen fixation and yield. *Pakistan J. Botany* **40**(**5**): 2005-2013.
Ganesan, S., Kuppusamy, R. G., and Sekar, R. (2007). Integrated management of stem rot disease (Sclerotium rolfsii) of groundnut (Arachis hypogaea L.) using Rhizobium and Trichoderma harzianum (ITCC - 4572). *Turkish J. Agric. For*. **31**: 103–108. doi: 10.3906/tar-0609-15
Gedamu S. A., Tsegaye E. A. & Beyene T. F. (2021). Effect of rhizobial inoculants on yield and yield components of faba bean (Viciafaba L.) on vertisol of Wereillu district, South Wollo, Ethiopia, *CABI Agri. and Biosci*. **2**: 8 (2021)

Gitonga N. M., Koskey G., Njeru E. M., Maingi J. M. and Cheruiyot R. (2021). Dual inoculation of soybean with Rhizophagus irregularis and commercial Bradyrhizobium japonicum increases nitrogen fixation and growth in organic and conventional soils. *AIMS Agriculture and Food*. **69**(**2**): 478–495.
Gopalakrishnan S., Sathya A., Vijayabharathi Vijayabharathi Rajendran, Rajeev Kumar Varshney, C. L. Laxmipathi Gowda, Lakshmanan Krishnamurthy. (2015). Plant growth promoting rhizobia: challenges and opportunities. Review article. 3 *Biotech* **5**:355–377.
Hafeez FY, Safdar ME, Chaudhry AU, Malik KA (2004). Rhizobial inoculation improves seedling emergence, nutrient uptake and growth of cotton. *Aust. J. Exp. Agric*. 44:617–622
Jaiswal, S. K, Mohammed, M. Ibny F. Y. I. and Dakora, F. D. (2021). Rhizobia as a source of plant growth-promoting molecules: potential applications and possible operational mechanisms. *Front. Sustain. Food Syst.* **4**:619676. doi: 10.3389/fsufs.2020.619676
Janati W., Benmrid B., Elhaissoufi W., Zeroual Y., Nasielski J. and Bargaz A. (2021). Will Phosphate Bio-Solubilization Stimulate Biological Nitrogen Fixation in Grain Legumes? Review. *Frontiers in Agronomy*. **3**: 637196
Jinwen H., Afshar R. K., and Chen C. (2016). "Lentil response to nitrogen application and rhizobia inoculation." *Communications in Soil Science and Plant Analysis* **47**(**21**): 2458-2464. DOI: 10.1080/00103624.2016.1254786.
Khaitov B., Kurbonov A., Abdiev A. and Adilov M. (2016). Effect of chickpea in association with Rhizobium to crop productivity and soil fertility. *Eurasian J Soil Sci* 2016, **5** (**2**) 105 – 112.
Khalid R., Zhang X. X., Rifat H. and Ahmed M. (2020). Molecular characteristics of rhizobia isolated from arachis hypogaea grown under stress environment. *Sustainability* **12**: 6259.
Khokhar S.N., Qureshi A. (1998). Interaction of azorhizobium caulinodans with different rice cultivars for increased N 2 fi xation. In: Malik KA, Mirza MS, Ladha JK (eds) Nitrogen fi xation with non-legumes. Kluwer Academic, London, pp. 91–93
Kisinyo, PO, SO Gudu, CO Othieno, JR Okalebo, PA Opala, JK Maghanga, JJ Agalo, WK Ng'etich, JA Kisinyo and RJ Osiyo (2012). Effects of lime, phosphorus and rhizobia on Sesbania sesban performance in a Western Kenyan acid soil.
Koskey G, Mburu SW, Njeru EM, Kimiti JM, Ombori O and Maingi JM (2017) Potential of Native Rhizobia in Enhancing Nitrogen Fixation and Yields of Climbing Beans (Phaseolus vulgaris L.) in Contrasting Environments of Eastern Kenya. *Front. Plant Sci*. **8**:443. doi: 10.3389/fpls.2017.00443.
Lennox JA, John GE, Akwarandu C. (2020). Potential growth enhancement of crops using pure culture of rhizobium Spp. *J. AgriSci Food Res*; **11**:278. doi: 10.35248/2593-9173.20.11.278
Lindstrom K. and Mousavi S. A. (2019). Effectiveness of nitrogen fixation in rhizobia. *Microbial Biotechnology* (**2019**) **0**(**0**): 1–22.
Makoi, Joachim HJR, Sylvia Bambara and Patrick A Ndakidemi (2013). Rhizobium inoculation and the supply of molybdenum and lime affect the uptake of macroelements in common bean ('P. Vulgaris L.') plants. *Australian J. Crop Sci*. **7**: 784.
Mebrahtu G.and Teklay T. (2021). Effect of P application rate and rhizobium inoculation on nodulation, growth, and yield performance of chickpea (Cicer arietinum L.) *Review Article*. *International Journal of Agronomy*. Volume 2021, Article ID 8845489, 14 pages.
Mfilinge A., Mtei K. and Ndakidemi P. A. (2014). Effects of rhizobium inoculation and supplementation with P and K, on growth, leaf chlorophyll content and nitrogen fixation of bush bean varieties. *American J.Res. Commun.* **2**(**10**): 49- 87. www.usa-journals.com, ISSN: 2325-4076.
Miriko M.C. (2015). (BSc. Horticulture) (2015). Effect of phosphorus and rhizobia inoculation on growth and yield of desmodium and lucerne in Kiambu Country, Kenya. MSc Thesis. Kenyatta University, Kenya.
Mishra RPN, Singh RK, Jaiswal HK, Kumar V, Maurya S (2006) Rhizobium-mediated induction of phenolics and plant growth promotion in rice (Oryza sativa L.). *Curr. Microbiol*. **52**:383–389
Naveed M., Mehboob I., Hussain M. B., and Zahir A. Z. (2015). Perspectives of rhizobial inoculation for sustainable crop production. N.K. Arora (ed.), *Plant Microbes Symbiosis*: *Applied Facets*, 209 DOI 10.1007/978-81-322-2068-8_11, © Springer India 2015. Chapter 11.
Ndakidemi, PA, FD Dakora, EM Nkonya, D Ringo and H Mansoor (2011). Yield and economic benefits of common bean (Phaseolus vulgaris) and soybean (Glycine max) inoculation in northern Tanzania. *Animal Prod. Sci.* **46**: 571-577.
Nyaguthii M. C. (2017). Soybean (*Glycine max*) response to rhizobia inoculation as influenced by soil nitrogen levels. MSc Thesis. Kenyatta University, Kenya.
Nyoki, Daniel and Patrick A Ndakidemi (2014a). Effects of phosphorus and Bradyrhizobium japonicum on growth and chlorophyll content of cowpea (Vigna unguiculata (L) Walp). *American J. Experimental Agri*. **4**: 1120-1136.
Ozkoc I, Deliveli MH (2001). In vitro inhibition of the mycelial growth of some root rot fungi by Rhizobium leguminosarum biovar phaseoli isolates. *Turk. J. Biol*. **25**:435–445
Peng S, Biswas JC, Ladha JK, Gyaneshwar P, Chen Y (2002). Influence of rhizobial inoculation on photosynthesis and grain yield of rice. *Argon J*. **94**:925–929
Reitz M, Rudolph K, Schroder I, Hoffmann-Hergarten S, Hallmann J, Sikora RA (2000). Lipopolysaccharides of Rhizobium etli strain G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode Globodera pallida . *Appl. Environ. Microbiol*. **66**:3515–3518
Sessitsch, J.G. Howieson, X. Perret, H. Antoun, and E. Martínez-Romero. (2002). Advances in rhizobium research. *Critical Reviews in Plant Sciences*, **21**(**4**):323–378.
Shaukat SS, Siddqui IA (2003). The influence of mineral and carbon sources on biological control of charcoal rot fungus, Macrophomina phaseolina by fluorescent pseudomonads in tomato. *Lett. Appl. Microbiol*. **36**:392–398
Sindhu SS, Dadarwal KR (2001). Chitinolytic and cellulolytic Pseudomonas sp. antagonistic to fungal pathogen enhances nodulation by Mesorhizobium sp. Cicer in chickpea. *Microbiol. Res*. **156**:353–358

Singh RK, Mishra RPN, Jaiswal HK, Kumar V, Pandev SP, Rao SB, Annapurna K (2006) Isolation and identifi cation of natural endophytic rhizobia from rice (Oryza sativa L.) through rDNA PCR-RFLP and sequence analysis. *Curr. Microbiol*. **52**:345–349
Sisay B., Melkamu B., Birhan A., Adugna T., Kindu M. and Endalkachew W. (2019). Inoculation and phosphorus fertilizer improve food-feed traits of grain legumes in mixed crop-livestock systems of Ethiopia. *Agriculture, Ecosystems and Environment* **279**: 58–64.
Sogut T. (2006). Rhizobium inoculation improves yield and nitrogen accumulation in soybean (Glycinemax) cultivars better than fertiliser, *New Zealand J. Crop Horti. Sci.*, **34**:2, 115-120, DOI: 10.1080/01140671.2006.9514395.
Somasegaran, P. and Hoben, H.J. (1985). Methods in Legume-Rhizobium Technology. NifTAL project and MIRCEN. Department of Agronomy, 2nd Soil Science Hawaii Institute Tropical Agriculture Human Research, University of Hawaii at Manoa, Honolulu, 1-52.
Soumaya T.H., Sana D. F., Faysal B. J. and Imran H. (2016). Effect of rhizobium inoculation on growth and nutrient uptake of Sulla (Hedysarumcoronarium L.) grown in calcareous soil of Northern Tunisia. *Romanian Biotechnological Letters*, **21**(**4**): 2016.
Stuart C. F. (1980). The mineral nutrition of wild plants. Annual review of ecology and systematics: 233-260.
Swarnalakshmi K, Yadav V, Tyagi D, Dhar DW, Kannepalli A, Kumar S. (2020). Significance of plant growth promoting rhizobacteria in grain legumes: growth promotion and crop production. *Plants*. **9**(**11**):1596. https://doi.org/10.3390/plants9111596
Tairo E. V., Ndakidemi P.A. (2013). Possible benefits of rhizobial inoculation and phosphorus supplementation on nutrition, growth and economic sustainability in grain legumes. *American J. Res. Commun*., **1**(**12**):532-556 www.usajournals.com, ISSN: 2325-4076
Thilakarathna M. S., Chapagain T., Ghimire B., Pudasaini R., Tamang B. B., Gurung K., Choi K., Rai L., Magar S., Bishnu BK, Gaire S. and Raizada M. N. (2019). Evaluating the effectiveness of rhizobium inoculants and micronutrients as technologies for Nepalese common bean smallholder farmers in the real-world context of highly variable hillside environments and indigenous farming practices. *Agriculture*, 9: 20
Vanlauwe B., Hungria M., Kanampiu F. and Giller K.E. (2019). The role of legumes in the sustainable intensification of African smallholder agriculture: Lessons learnt and challenges for the future. *Agriculture, Ecosystems and Environment* **284** (**2019**): 106583
Vienna (1998). Improving yield and nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia. Printed by the IAEA in Austria.
Yanni YG, Rizk RY, Abd El-Fattah FK, Squartini A, Corich V, Giacomini A, De Bruijn F, Rademaker J, Maya-Flores J, Ostrom P, Vega-Hernandez M, Hollingsworth RI, Martinez-Molina E, Mateos P, Velazquez E, Wopereis J, Triplett E, Umali-Gracia M, Anarna JA, Rolfe BG, Ladha JK, Hill J, Mujoo R, Ng PK, Dazzo FB (2001). The beneficial plant growth promoting association of Rhizobium leguminosarum bv. trifolii with rice roots. *Aust. J. Plant Physiol*. **28**:845–870
Zahran H. H. (1999). Rhizobium-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. *Microbiol Mol Biol Rev*. **63**(**4**): 968–989.
Zerihun G. G. and Lijalem A. D. (2020). Response of soybean to Rhizobial inoculation and starter N fertilizer on nitisols of Assosa and Begi areas, Western Ethiopia. *Environ Syst Res* **9**:14.