International Journal of Research Studies in Science, Engineering and Technology
Volume 7, Issue 11, 2020, PP 23-34
ISSN : 2349-476X

Characterizing the Effectiveness of Nitrogen Fixing Bacteria to Optimize Chickpea (Cicer Arietinum L) Production

Fikadu Hailemichael*

Ethiopian Institute of Agricultural Research, National Fishery and Aquatic Life Research Center, Ethiopia

***Corresponding Author:** Fikadu Hailemichael, Ethiopian Institute of Agricultural Research, National Fishery and Aquatic Life Research Center, Ethiopia, Email: fikaduh.michael11@gmail.com*

ABSTRACT

Chickpea (Cicer arietinum L.) is one of the most important crops; well adapted in a wide range of agro-ecological circumstances and consumed by millions of peoples across the world. It is cultivated in tropical, sub-tropical and temperate regions of the world. Chickpea is originated from Asian country, particularly Turkey. But currently it is widely grown in over 50 countries in Asia, Africa, Americas, Australia and Europe. However, India is the largest producer of chickpea and contributes the major share to the global chickpea production area (69%). Ethiopia is the sixth (3%) chickpea producer in the world and largest producer in Africa (46%). Chickpea is one of the most important and widely consumed food crops across the world. It plays significant role in maintaining soil fertility through biological nitrogen fixation. Moreover, chickpea is enriched with good nutritional qualities. Thus it can improve the nutritional value of human diet and reduces malnutrition in the world. Among nutrients, nitrogen is the most important limiting macronutrients and required by plants in large quantities. It is a source of proteins, nucleic acids and plays a key role in the nitrogen cycle. But nitrogen in the soil is limited because more than 80 % of atmosphere nitrogen is present in the form of N2 which is not available to plants. However, nitrogen fixing bacteria has a crucial role in order to convert molecular nitrogen into available forms of nitrogen through biological nitrogen fixation at room temperature and lower pressure.

Keywords: *Agrochemicals; Biological nitrogen fixation; Nodules; Biofertilizer; Rhizobium; Chickpea*

INTRODUCTION

Chickpea (Cicer arietinum L.) is one of the most important cool season crops that is believed to have been originated in south-eastern Turkey and neighboring parts of Syria`. However, Turkey was the first Asian country who has grown chickpea in 7500 years ago. The crop later spread to India, Central Asia, Europe, Mediterranean, Africa, Latin and Central American countries (Rasool et al., 2015). Chickpea is one of the oldest legume crops and is consumed widely across the world.

It is mainly grown in warm climates of India, Pakistan, Iran, Ethiopia, Mexico, and Mediterranean region (Raza et al., 2019). Based on cultivation area, it is the world's third largest pulse crop which is widely grown in many countries such as India, Australia, Pakistan and Turkey which are considered as the major world producers (Ghribi et al., 2015).

Chickpea is an annual plant derived from the Fabaceae family. It is widely distributed being grown in temperate and semiarid regions such as South and North Asia, North and East Africa, Southern Europe, and North and South America and Australia (Fessehaie and Mohammed, 2016). Among these, the leading producer of chickpeas is India, which provides approximately 66% of global production, second country in order is Turkey (7.6%), followed by Pakistan (7%), Iran (3.5%), Canada (1.6%) and the United States (less than 1%), which contribute only to a small extent to total production of chickpea in the world. Chickpea is a plant known for a long time in Asia, mainly due to the wide possibilities of its application (Rachwa-Rosiak et al., 2015). South Asia is the leading chickpea producing region, 71.58%, where as Africa, Americas, Oceania, and West Asia regions each proportionally contribute 5.47% to 5.99% of the total chickpea production (Upadhyaya et al., 2016).

Chickpea is widely spread and cultivated in several parts of the world which covers an area of around11 million hectares with a total production of 11.6 million tons. However, 96% comes from developing countries, and it is mainly consumed in Indian Subcontinent, West Asia, North Africa, South-West Europe and Central America. It is grown in more than 50 countries, of which approximately 90% are located in Asia (Kumar, 2019). Thus, this paper aims to review about the effectiveness of nitrogen fixing bacteria in order to maximize the chickpea productivity.

Importance of Chickpea

Chickpea is one of the major food legumes belonging to the family leguminosae which is grown widely in tropical, subtropical, and temperate regions of the world, particularly in the semi-arid tropics of sub-Saharan Africa and South Asia. It is highly nutritive legume and an important source of the dietary protein consumed by different preparations as supplementary food.

Moreover, it also plays a significant role in maintaining soil fertility, through biological nitrogen fixation mechanisms (Cherinet Alem and Tazebachew Asres, 2016). Furthermore, chickpea is among the major export commodities with significant export market option among the field crops(Kumar, 2019). It is one of the most important cool season pulse crops of dry lands in the world. For a wide range of agro-ecological environments, chickpea is an alternative pulse crop for increasing the diversification of different cropping systems (Sabaghnia and Janmohammadi, 2014).

In Ethiopia, chickpea is widely produced by the majority of farmers and playing a crucial role in the farming systems and in the diets of the community. It is an ideal crop in order to reduce poverty, improve human health and nutrition, enhance ecosystem resilience (Tewodros Tefera, 2014) and source of cash income in Ethiopia's foreign exchange earnings through export to Asia and Europe (Lijalem Korbu et al., 2016).

It is also an important sources of diet, and consumed in Ethiopia in different preparations like snacks, curry, blend to bread/Enjera powder, green pea, and salads (Asnake Fikre, 2016). In Egypt, a chickpea grain is used to increase body weight, cure head and throat aches, and cough. Powdered grains are used for preparation of facial masks and added to antidandruff products. Cooked grains are a great addition to salads especially popular in Western Europe and the USA, while in the Middle East they are consumed surrounded by sugar or spices. Chickpea grains flour is also used as an addition to pasta, soups, and bread (Rachwa-Rosiak et al., 2015).

Due to the presence of different important characteristics, chickpea made it the most cultivated pulse crop and appreciated nutritional source particularly in the diets of the poor peoples and vegetarians all over the world. It has the ability to fix more than 70% of atmospheric nitrogen via symbiotic association with rhizobia which makes it a capable crop for "alternative agriculture" and it is now attracting substantial attention in the industrialized world (Minaei et al., 2015).

In Ethiopia, chickpea is widely grown across the country and serves as a multi-purpose crop; It provides a cheap and high nutritional quality, plays significant role in maintaining soil fertility through biological nitrogen fixation and saves fertilizer costs, it reduces malnutrition and improves human health (Cherinet Alem and Tazebachew Asres, 2016).

Chickpea is enriched with good nutritional qualities and considered as a functional food which has the potential beneficial effects on human health. It is an important source of zinc, folate, protein, fiber, lipid and complex carbohydrates for persons who are insulin sensitivity. The total content of carbohydrate, fat and sugar in chickpea is higher than in other pulses (Rasool et al., 2015). The consumption of chickpea provide consumers with valuable nutrition and potential health benefits such as it is helpful for lowering blood cholesterol level and reduce the risk of chronic diseases and optimize health. In addition, chickpea oil contains many medicinal and nutritionally important tocopherols, sterols and tocotrienols. Chickpea has medicinal application on some of the important human diseases like cardiovascular disease, type 2 diabetes, digestive diseases and cancer (Khan et al., 2015). Furthermore, it is used to treat aphrodisiac, bronchitis, cholera, constipation, diarrhea, dyspepsia, flatulence, snakebite, sunstroke and warts (Kabuo et al., 2015).

Production of Chickpea in Ethiopia

Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent‘s production during 1994-2006 (Wondafrash Mulugeta and Dejene Girma, 2016) . It covers about 239,512 hectares of land

and more than 409,733 tons of grain is produced annually with average productivity of 1.71tons/ha. In 2011, Ethiopia produced 400,208 tons of chickpea from a total area of 231,300 hectares, while in 2012, the chickpea seeded area in Ethiopia increased by 8200 hectares from the previous year and totally about 239,500 hectares with the total production of about 409,733 tons.

The consistent increase in cultivated area and production implies the importance of the crop to the country (Legesse Hidoto et al., 2017). Currently, there are major challenges to chickpea production in Ethiopia, such as soil nutrient deficiency of both macro and micronutrients. In Ethiopia, chickpea is produced under wide spectrum of altitude ranging from 1,500 to 2,600 meter above sea level, mainly with residual moisture from the main rainy season and in a very few cases using irrigation (Dagnachew Bekele et al., 2016).

It is also the leading consumer and seller of chickpea in Africa, and is among the top ten most important producers in the world. Chickpea is among important commodities accounting for more than 15% of Ethiopian legumes with a total area of 239000ha and about one million households are engaged in its production. It is adapted to cooler agro-ecological environments and vertisols, which are located in the central highlands of Ethiopia. The crop is known for soil nitrogen enrichment, rotational advantages and less cost of production.

The crop production is mainly concentrated in four political regional states with Amhara regional state shares more that 50% and followed by Oromia (Asnake Fikre, 2016). In Amhara region, chickpea covers an area of 130,381hectares with annual production estimated to 225,080 tons. In terms of production, chickpea is the second most important crop after faba bean (Cherinet Alem and Tazebachew Asres, 2016).

Nutritional Composition of Chickpea

From the nutritional point of view, chickpea is an excellent source of macronutrients (energy, protein, fat carbohydrate, fiber, sugar), lipids (saturated, monounsaturated, polyunsaturated) minerals (phosphorus, calcium, magnesium, iron, zinc, potassium, sodium, copper and manganese) and vitamins ((vitamin C, Thiamin, riboflavin, niacin, panthotenic acid, folate, vitamin B6, choline, vitamin K, vitamin E and vitamin A) and β-carotene (Singh and Pratap, 2016, Hoskem et al., 2017, Wallace et al., 2016). In addition contains several essential amino acids such as lysine, methionine, threonine, valine, isolucine and leucine which are the major components of seed protein (Khan et al., 2015).

Chickpea seeds are characterized by high levels of monosaccharides (ribose, fructose and glucose), disaccharides (sucrose and maltose), and Oligosaccharides (Raffinose, Ciceritol, Stachyose, and Verbascose). There are four major proteins found in chickpea. These are albumins, globulins, gluteins, and prolamines. Among these, globulin is the dominant protein and followed by albumin.

Prolamines are soluble in alcohol and have a high content of proline and glutamine, whereas gluteins are soluble in dilute solutions of acids and bases, detergents, and chaotropic and reducing salts. Moreover, gluteins contain higher concentrations of methionine and cystine than globulins, and thus are a more important nutrient. But there is chemical composition and amount of protein differences found in chickpea and other legumes may be due to the variety, environmental conditions, as well as geographic location, plant growing season and method of analysis used by the authors (Rachwa-Rosiak et al., 2015).

Chickpea improves the nutritional value of human diet and can reduce malnutrition in the world since it is a good source of protein (18-29%) with its high digestibility (53 to 89%), carbohydrates (59-65%), fiber (3-17%), lipids (4.5-6.6%), and ash (2.48-3.50%). Besides on the medicinal level, chickpea seed is used for its anti-helminthes properties as well as for the treatment of bronchitis, leprosy, skin diseases, and liver infections. It is considered to be the most cholesterol-lowering food compared to other legumes (Raza et al., 2019).

Characteristics of the Rhizobia and Their Roles in Chickpea Production `

Rhizobia are one of the most efficient bacteria which associate symbiotically with the roots of leguminous plants that can fix atmospheric nitrogen by the process of biological nitrogen fixation. They are soil microorganisms that can live in association with the plant roots and able to fix atmospheric nitrogen and convert it into plant usable form in specialized structures called nodules where aerobic condition are maintained by leghaemoglobin (Makkar and Jangra, 2017). Rhizobia are a group of bacteria that are well known for their ability to colonize root surfaces and form symbiotic associations with legume plants. They are distinguished from other prokaryotes by the ability to form nodules on roots and stems of leguminous plants and fixing atmospheric nitrogen effectively. Another characteristic property of rhizobia is their host specificity. At genetic level host specificity is determined by nodulation and nitrogen fixation (Sahgal and Jaggi, 2018).

They are gram negative bacteria which includes both fast-growing Rhizobium) and slow-growing (Bradyrhizobium) spp. Further, they produce white colonies, motile rode, do not form endospore, small to medium in size (0.5-0.9 X 1.2 – 3.0 µm) and have a thread like structure called flagella. When the culture become old, their cells going to be unstained and produce large amounts of polyhydroxybutyric acid (PHBA). These biopolymers are very important in order to produce different types of environmentally friendly bioplastics and easily degraded in the environment. Rhizobia are predominantly aerobic, which can grow well in the presence of oxygen. Therefore, oxygen is their final electron acceptor during metabolic reaction they possess (Datta et al., 2015).

However, they rapidly lose their viability in water and relatively easy to culture. Even though their usual metabolism is aerobic, many strains are able to grow well under microaerophilic conditions at oxygen stresses of less than 0.01 atm. Rhizobia can grow on a wide range of carbohydrates, but usually grow best on glucose, amino compounds, mannitol, or sucrose. However, some strains require vitamins for their growth. The optimum temperature and pH requirements for the growth of most Rhizobial strains are at ranges of 25-30°C and 6.0-7.0, respectively. Most rhizobia weakly absorb congo red dye. Moreover, fast-growers produce an acid reaction while slow growers produce an alkaline reaction when they grow in the standard YM medium containing bromthymol blue (Somasegaran and Hoben, 2012).

They are also very important to improve plant growth and reduce disease incidence in various crops. Rhizobia are known to control the growth of many soilborne plant pathogenic fungi like Fusarium, Rhizoctonia, Sclerotium, and Macrophomina. Antagonistic activity of Rhizobia is mainly attributed to the production of antibiotics, hydrocyanic acid (HCN), mycolytic enzymes, and siderophore under iron limiting conditions (Das et al., 2017).

Rhizobia have the ability to fix atmospheric nitrogen in symbiotic association with chickpea roots. Absence of suitable strains, small population size and poor survival of Rhizobia cause problems in nodules formation. Chickpea fixes atmospheric nitrogen (about 140 kg/ha) through bacteria (Rhizobium) present in their roots, which in turn improves the soil fertility. Hence the presence of appropriate nodule forming bacteria in the soil is essential for management and utilization of atmospheric nitrogen.

Chickpea yield can be increased by applying effective and competitive Rhizobial isolates because they are an economical promising microorganism to increase chickpea production. Artificial seed inoculation of chickpea in those soils lacking native effective rhizobia is a very useful practice for improving root nodulation and yield of the crop. In general, nitrogen fixers benefit the plant by providing them atmospheric nitrogen, which contributes to the development of plant growth and biomass production (Gul et al., 2014).

Importance of Biological Nitrogen Fixation (BNF) In Legumes

Nitrogen is one of the primary macronutrients that are required by plants in large quantities for their growth and productivity. It forms an integral part of proteins, nucleic acids (RNA and DNA) and other essential biomolecules, and plays a key role in the nitrogen cycle. However, plants absorb most of nitrogen (N) in the form of nitrates, ammonium and sometimes urea for their proper growth and development (Ju et al., 2018, Santoyo et al., 2019).

But more than 80 % of nitrogen is present in the atmosphere which is unavailable to plants. Therefore, unavailable form of nitrogen should be converted into an available form of ammonia through different pathways. For this activity, biological nitrogen fixation has a crucial role that involves the conversion or reduction of molecular nitrogen into ammonia by microorganisms using nitrogenase enzyme system. The reduced form of nitrogen is then utilized by the plants for the synthesis of various proteins, vitamins, and other nitrogen-containing compounds (Kumar et al., 2015).

Nitrogen (N2) can be reduced to ammonia (NH3) through different mechanisms such as fixation by symbiotic micro-organisms, by free-living micro-organisms and by industrial -Bosch Process. However, currently, chemical fertilizers are produced through industrial Haber-Bosch process and applied in different agricultural lands worldwide to supply usable forms of nitrogen for plants.

But the industry can furnish NH3 from N2 and natural gas at high temperatures and pressures. During this process, approximately 1.87 tons of carbon dioxide is released for each ton of NH3 and it consumes more than 1% of the global annual energy generation. On the other hand, biological nitrogen fixation is the process that can convert atmospheric nitrogen to ammonia or nitrate at room temperature and lower pressure (Deng et al., 2018).

The major portion of biological nitrogen fixation is carried out by symbiotic nitrogen fixers such as Rhizobium which is mediated by nitrogenase enzymes, a biological catalyst which is present in the bacteriod. Biological nitrogen fixation is the cheapest and environment friendly procedure in which rhizobia interacting with leguminous plants and fix aerobic nitrogen into soil. The presence of rhizobia increases plant productivity without any harm to human health and environments (Temam Abrar and Alemayehu Letebo, 2017).

Available forms of nitrogen limits the productivity of leguminous crops in agricultural systems since it has a major role in food production. BNF is restricted to bacteria and archaea and does not occur in eukaryotes. It is cost effective and ecologically sound source of nitrogen which helps to decrease the dependence on chemical fertilizers. However, application of nitrogen fixing bacteria as biofertilizer is one of the upcoming agricultural technologies throughout the world since it is the most efficient and environmentally sustainable methods for increasing the growth and yield of crop plants (Mus et al., 2016).

Biological nitrogen fixation is a natural means of providing nitrogen for plants or it is the complex biochemical reactions in which atmospheric nitrogen is enzymatically reduced into usable form for the plant by the nitrogenase enzyme complex of the bacteroids. Biological nitrogen fixation is one of the critical components of many terrestrial, as well as aquatic ecosystems across the biosphere. In agriculture, about 65% of N consumption is produced by BNF (Bhowmik and Das, 2018).

Legumes are one of the most important crops which have the ability to fix nitrogen in association with rhizobia. Rhizobia inoculants are inexpensive source of biofertilizers, used for sustainable legume supply to the growing population because it is environmental friendly. Biological nitrogen fixation contributes about 100 million tons of nitrogen for terrestrial ecosystems, 30 to 300 million tons for marine ecosystems and 20 million tons from chemical fixation due to atmospheric phenomena. Nitrogen-fixing rhizobia can play an important role in the life of host plant because it ensures their nitrogen supply, defense against pathogens and pests as well as adaptation to various environmental stresses (Simon et al., 2014). In agriculture, approximately 80% of the biologically fixed nitrogen comes from symbiosis involving leguminous plants and bacteria of family Rhizobiaceae. They play a major role in the nitrogen supply of most soil ecosystems through their ability to fix nitrogen in symbiosis with legumes (Makkar and Jangra, 2017).

Mechanism of Biological Nitrogen Fixation

The Rhizobia make a symbiotic association with legumes to convert atmospheric di-nitrogen (N2) into ammonia through their enzymatic mechanisms, because plants cannot directly utilize nitrogen in organic form. The interaction between root nodules and rhizobia is takes placed through proteins which are produced by both partners during their signal exchange and growth of symbiosome (Howieson and Dilworth, 2016).

Due to the presence of specific genes, rhizobial strains can easily infect the roots of various species of leguminous plants and leading to the formation of nodules. These genes can determine the compatibility between specific rhizobial strains with the particular leguminous plants. The nodulation is regulated by highly complex chemical signaling in between both the plant and the bacteria. The interaction between the host plant and rhizobia leads to the release of chemicals by the root cells into the soil, and some of these chemicals encourage the growth of the bacterial population in the area around the roots. Each root nodule consists of thousands of living Rhizobium bacteria, collectively called bacteroids (Kumar, 2019).

The rhizobia should colonize and bind to the plant surface in order to establish a symbiotic relationship with the host plant. Bacterial infection and nodule development are coordinated by molecular signals, exchanged by legumes and rhizobia in which legumes release flavonoids which signal to rhizobia that the plant is seeking symbiotic bacteria (Andrews and Andrews, 2017). In response, the rhizobia releases nodulation factor which stimulates the plant to create deformed root hairs. Signaling molecules are very important in the host recognition and initiation of the nodulation process. Rhizobia then form an infection thread that allows them to enter into the legume root cells through root hairs (Unay and Perret, 2019).

When the rhizobia are inside the root cells, the cells divide rapidly to form nodule. Within a week after infection, nodules are formed and enclose the bacteriod, which are actual sites for nitrogen fixation. After all, the rhizobia are released from the tip of the infection thread into the cytoplasm of the host cells and nitrogen fixation starts within the nodules via nitrogenase enzyme. Then the rhizobia convert atmospheric nitrogen into ammonia that is directly used by the plant for synthesis of amino acids and nucleotides, while the plant provides the rhizobia with sugars as a carbon source and shelter to create conducive environment for multiplication (Simon et al., 2014).

Role of Nitrogenase in Biological Nitrogen Fixation

Nitrogenase is a complex enzyme that is responsible for the conversion (reduction) of atmospheric nitrogen gas into ammonia by nitrogen-fixing organisms, and is synthesized in the cytosol of the bacteroids. Then legumes can utilize this ammonia to convert certain precursor metabolites (alpha-ketoglutarate and phosphoenopyruvate) into amino acids and proteins. It has two protein components, Fe-containing protein and Fe-Mo protein. First Mo-Fe protein is involved in the conversion of dinitrogen to ammonia, and then Fe protein assists Mo-Fe protein by providing electrons for the reduction of dinitrogen (Somasegaran and Hoben, 2012). The important feature of nitrogenase enzyme is its sensitivity towards oxygen; as a result the protein becomes damaged. The MoFe protein is relatively insensitive towards oxygen than Fe protein because conformational changes are take placed in catalytic unit (Bhowmik and Das, 2018).

In order to protect this condition, there is presence of oxygen scavenging operating process for which high respiratory activity takes place; as a result enzyme becomes modified to catalytically active form. However, in free-living organisms and anaerobic nitrogen-fixing bacteria, such type of problems doesn't occur. Although such problem is mainly occurred in aerobic bacteria, they have a number of mechanisms such as metabolic activity for protecting the nitrogenase complex.

Rhizobium controls the levels of oxygen by producing iron protein containing leghaemoglobin in the root nodules of leguminous plants. Hence the presence of this protein determines the effectiveness of symbiosis. Because it provides sufficient oxygen for the metabolic processes of symbiotic bacteroids, but it prevents the accumulation of free oxygen which will destroy the nitrogenase activity. However, the synthesis of leghemoglobin requires genetic information from the legume and the rhizobia (Kumar, 2019).

Factors Affecting Biological Nitrogen Fixation

Nitrogen fixing rhizobia cannot express their full potential in nitrogen fixation when the environment and the plant are in poor conditions. The process of nitrogen fixation depends much on the functional state of the legume plant and the optimum environmental conditions supporting the macro and micro symbionts.

Therefore, major environmental factors can affect the tolerance ability of both the host plant and symbiotic rhizobia (Simon et al., 2014). However, there are several environmental stresses which may affect the nitrogen fixation in plants. It includes salinity, soil pH and temperature, greatly influence the growth, survival and metabolic activity of Rhizobia (Patil et al., 2014).

Salt Stress

Salinity is a serious agricultural problem to the production of grain legumes especially in the semi-arid and arid regions of the world. About 40 % of the world's land surface is affected by salinity-related problems. It is known that higher concentration of ions in saline soils gets accumulated in the plant cells and inactivate enzymes that inhibits protein synthesis and photosynthesis. Most of the leguminous plants are more sensitive to salinity and they require slightly acidic soil for N2 fixation (Patil et al., 2014).

Salinity doesn't affect free living Rhizobia but it influences on bacterial infection process (by decreasing the number and the deformation of root hairs), nod factor production, nodule growth and functioning (by limiting the nutrient supply via photosynthesis products and oxygen consumption) and BNF (by reducing the nodule metabolism, leghemoglobin content and atmospheric nitrogen diffusion) (Gopalakrishnan et al., 2015). High concentrations of soluble salts affect microbes via osmotic effect and specific ion effects. Soluble salts increase the osmotic potential (more negative) of the soil water, drawing water out of cells which may kill microbes and roots through plasmolysis. Low osmotic potential also makes it more difficult for roots and microbes to remove water from the soil. Salinity reduces microbial activity, microbial biomass and changes microbial community structure (Yan et al., 2015).

Salt concentration negatively affects the overall growth and development and yield of legume plants by its harmful effects on biological nitrogen fixation, reducing the supply of photosynthesis to nodule of plant (Chaudhary and Sindhu, 2015).

Salinity stress also severely affects germination, growth, nodulation and yield of chickpea, however, the reproductive processes are considered the most salt sensitive. But the mechanism(s) how the salinity affects reproductive processes in chickpea yet not known (Kotula et al., 2015).

Legumes are highly sensitive to high salt concentration during vegetative and reproductive phases.

Salinity affects vital physiological functions, nutritional imbalances, and hormonal regulation, osmotic effects, reduced carbon fixation, flower abortion, and reduce numbers of flowers and decrease crop production. It is becoming one of the major problems to threat crop productivity. Generally, about 43-72% of chickpea yield can be lost when the salt concentration increased to 2.5- 3.8 dsm-1 (Nadeem et al., 2019).

Soil pH

Most leguminous plants and rhizobia prefer neutral or slightly acidic conditions for their better growth and activity. In most of the cases, the Rhizobium attachment to root hairs is affected by acidic conditions in soils because a pH sensitive stage occurs early in the infection process. Plant growth and most soil processes, including nutrient availability and microbial activity, are favoured by a soil pH range of 5.5 – 8. When the pH of the soil is below 4.8, aluminium solubility in soil solution increases and reduces root growth while manganese disrupts photosynthesis and other functions of growth, resulting in the reduction of nitrogen fixation by rhizobia. Moreover, the top layer of soil pH is low and it may affect the microbial activity and decrease legume nodulation (Singh and Singh, 2018).

Soil acidity restricts biological nitrogen fixation by reducing the survival mechanisms of Rhizobium in soils, and ultimately reducing nodulation in legumes. But the bacterial community structures can be changed when the pH is in acidic or alkaline conditions. Therefore, soil pH is one the most important factor which can control the microbial activity (Zhang et al., 2017). When the pH is below 6.0, it adversely affects nitrogen fixation by rhizobia (Thapa and Binita, 2015).

Temperature Stress

Temperature is one of the most critical factors which can affect the survival of free rhizobia as well as the symbiotic association between the host plant and rhizobia especially in arid and semi-arid regions. For most of rhizobia, optimum temperature range for growth is 28 to 31oC and majority of them are unable to grow at 38oC.

When the temperature increase, the number of nodule as well as nodule dry mass decreases (Singh and Singh, 2018). Further, it is responsible to decrease the amount of photosynthesis and chlorophyll content from leguminous crops.

Hence the supply of photosynthates to Rhizobium becomes reduce (Basu et al., 2016). Therefore, the root hair, nodule structure and function are highly affected. The best temperature for nodule functioning in common beans is between 25˚C - 30˚C. But some legume species can fix nitrogen at different critical levels of temperature such as 20˚C - 35˚C, 35˚C - 40˚C, and at maximum of 50˚C (Simon et al., 2014).

On the other hand, low root zone temperatures inhibit the synthesis and secretion of plant-to-bacteria signals; such as it reduces root exudation and nod factor synthesis. These signal exchange effects combine to delay nodulation onset and reduce the nodule growth rate, leading to smaller nodules (Junior and Andrade, 2015).

Effects of Agrochemicals in Chickpea Production

Currently modern intensive agriculture is practicing in the worldwide to increase agricultural productivity by the application of external chemical inputs including fertilizers, pesticides, fungicides, and herbicides (Hassen et al., 2016) but it deteriorates the environment and cause harmful impacts on living beings. Because plants couldn't uptake all chemical inputs sufficiently. In this case, they reach into water bodies through rain water, cause eutrophication in water bodies and affect living beings (Youssef and Eissa, 2014).

Agrochemicals are commonly used in agricultural production systems to control diseases, pests and weeds in order to maintain high quality of agricultural products and reduce yield losses to feed the world population sufficiently. But these chemical inputs have a negative impact on the nutritional value of farm product, adversely affect soil, plant and consumers' health, impact on living organisms and depreciate the environment.

Furthermore, they promote the accumulation of toxic compounds in soils and then absorbed by most crops from soil (Alori and Babalola, 2018). Chemical fertilizers directly affect bacterial biomass and diversity by increasing nutrient availability and indirectly by altering soil pH in to acidic conditions. As a result of this, it leads to impact on microbial community structure (Zhang et al., 2017). Pesticides are chemically synthesized compounds that are routinely utilized in agriculture to control a number of plant diseases. They are highly resistant to degradation by biotic and abiotic factors, leach into the lower strata of the soil and absorbed by plant roots. Hence, a large input of pesticides has an adverse effect on the quality of agricultural crops, diversity of the microbial flora and their enzymes. Although the impacts of pesticides on agricultural products vary according to the type of pesticide used, all pesticide residues cause nutrient imbalance, reduction in the quality of agricultural products and have a great threat to human health (Bhandari, 2014). In addition, the application of pesticides leads to a reduction in nodule size and total nitrogen fixation (Roy et al., 2014).

Importance of Bio-Fertilizers in Agriculture

Presently modern agriculture becomes dependent on chemical fertilizers of nitrogen and phosphorus to increase the availability of essential nutrients in the soil and maximize the production of crops as well. But it leads to several environmental problems related to greenhouse effect, soil deterioration, and air and water pollution.

Hence, it is essential to reduce the consumption of chemical fertilizers and pesticides in agriculture without having any adverse effect on crop production by incorporating harmless fertilizers. However, biofertilizer is the most suitable alternative to replace chemical fertilizers, for large-scale agricultural practices which is eco-friendly, environmentally and economical. It also maintains soil structure, biodiversity of agricultural land and promotes plant growth by increasing efficient uptake or availability of nutrients for the plants (Thomas and Singh, 2019).

Bio fertilizers are products containing living cells of different microorganisms like bacteria, fungi and algae which have the ability to convert nutritionally important elements from unavailable to available form through biological processes. When applied to seed or plant surface, it starts to colonize the rhizosphere of the plant and promotes growth by increasing the availability of primary nutrients to the host plant (Youssef and Eissa, 2014). Furthermore, biofertilizer is formulated by effective living microorganisms in order to regulate the growth, development and physiology of plants for better crop production (Vejan et al., 2016). Biofertilizer is an essential part of organic farming in modern era since it is important for general agricultural production and economy on global scale (Maurya et al., 2019).

It plays a very important role to maintain and improve the soil environment via nitrogen fixation, phosphate and potassium solubilization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil. In agricultural farms', 60% to 90% of the total applied fertilizer is lost and the remaining 10% - 40% is absorbed by plants.

Hence effective microbial inoculants have a crucial role in integrated nutrient management systems to sustain agricultural productivity and promoting a healthy environment (Ju et al., 2018). Increasing and extending the role of biofertilizers can reduce the need for chemical fertilizers, decrease adverse environmental effects and maintain soil fertility. In addition to this, they are also cost effective and since anyone can afford to increase the productivity of different crops. Therefore, in the development and implementations of sustainable agriculture techniques, biofertilization has a great importance in order to mitigate environmental pollution and deterioration of nature (Kumar et al., 2015).

Conclusion And Future Perspectives

To date, production of chickpea is decreasing throughout the world due to several factors. Among these, temperature, salinity, pH, root rot and agrochemicals are the most important factors that can reduce the growth and productivity of chickpea. In order to solve these problems, effective nitrogen fixing bacteria plays a vital role by making a symbiotic association with chickpea roots. But nitrogen fixing bacteria are highly susceptible for chemical inputs because it can affect the biological nitrogen fixation mechanism between Rhizobium and the host plant.

In modern agricultural practice, effective nitrogen fixing bacteria have the tendency to maintain soil fertility and promote the productivity of chickpea. In addition, nitrogen fixing bacteria have positive impact on the growth, symbiotic parameters, yield and yield attributes, nutrient uptake and quality in chickpea. From this point of view, it is highly recommended to characterize and formulate effective nitrogen fixing bacteria as biofertilizer to maximize the productivity of chickpea. Furthermore, awareness should be created throughout the community in order to replace chemical fertilizers with biofertilizer.

References

[1] Alori, E. T. & Babalola, O. O. 2018. Microbial inoculants for improve crop quality and human health. Frontiers in microbiology, **9,** 2213.

[2] Andrews, M. & Andrews, M. E. 2017. Specificity in Legume-Rhizobia Symbioses. International Journal o f Molecular Sciences.

[3] Asnake Fikre. Progresses of chickpea research and development in Ethiopia. Harnessing Chickpea Value Chain for Nutrition Security and Commercialization of Smallholder Agriculture in Africa, Proceedings of a Workshop, 2016. 25-39.

[4] Basu, P., Singh, U., Kumar, A., Praharaj, C. & Shivran, R. 2016. Climate change and its mitigation strategies in pulses production. Indian J. Agron, **61,** S71-S82.

[5] Bhandari, G. 2014. An overview of agrochemicals and their effects on environment in Nepal. Applied Ecology and Environmental Sciences, **2,** 66-73.

[6] Bhowmik, S. N. & DAS, A. 2018. Biofertilizers: A Sustainable Approach for Pulse Production. Legumes for Soil Health and Sustainable Management. Springer.

[7] Chaudhary, D. & Sindhu, S. S. 2015. Inducing salinity tolerance in chickpea (Cicer arietinum L.) by inoculation of 1-aminocyclopropane-1-carboxylic acid deaminase-containing Mesorhizobium strains. African Journal of Microbiology Research, **9,** 117-124.

[8] Cherinet Alem & Tazebachew Asres 2016. 12. Participatory evaluation and selection of chickpea varieties at Debre Mawi and Debre Yakob watersheds, Western Amhara Region, Ethiopia. Harnessing Chickpea Value Chain for Nutrition Security and Commercialization of Smallholder Agriculture in Africa**, 163**.

[9] Dagnachew Bekele, Fikre, A., Eshete, M., Korbu, L. & Girma, N. 2016. 8. Genetic progresses achieved in Ethiopian chickpea (Cicer arietinum L.) breeding program based on grain yield and seed size. Harnessing Chickpea Value Chain for Nutrition Security and Commercialization of Smallholder Agriculture in Africa**, 119**.

[10] Das, K., Prasanna, R. & Saxena, A. K. 2017. Rhizobia: a potential biocontrol agent for soilborne fungal pathogens. Folia microbiologica, **62,** 425-435.

[11] Datta, A., Singh, R. K., Kumar, S. & Kumar, S. 2015. An effective and beneficial plant growth promoting soil bacterium "Rhizobium": a review. Ann Plant Sci, **4,** 933-942.

[12] Deng, J., Iñiguez, J. A. & Liu, C. 2018. Electrocatalytic nitrogen reduction at low temperature. Joule, **2,** 846-856.

[13] Fessehaie, R. & Mohammed, Z. 2016. 16. Status and Future Prospects of Chickpea Weed Research in Ethiopia. Harnessing Chickpea Value Chain for Nutrition Security and Commercialization of Smallholder Agriculture in Africa**, 203**.

[14] Ghribi, A., Maklouf, I., Blecker, C., Attia, H. & Besbes, S. 2015. Nutritional and compositional study of Desi and Kabuli chickpea (Cicer arietinum L.) flours from Tunisian cultivars. Adv Food Technol Nutr Sci Open J, **1,** 38-47.

[15] Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R. K., Gowda, C. L. & Krishnamurthy, L. 2015. Plant growth promoting rhizobia: challenges and opportunities. 3 Biotech, **5,** 355-377.

[16] Gul, R., Khan, H., Khan, N. & Khan, F. U. 2014. Characterization of chickpea germplasm for nodulation and effect of rhizobium inoculation on nodules number and seed yield. J Anim Plant Sci, **24,** 1421-1429.

[17] Hassen, A. I., Bopape, F. & SangeR, L. 2016. Microbial inoculants as agents of growth promotion and abiotic stress tolerance in plants. Microbial inoculants in sustainable agricultural productivity. Springer.

[18] Hoskem, B. C. S., Da Costa, C. A., Nascimento, W. M., Santos, L. D. T., Mendes, R. B. & De Campos Menezes, J. B. 2017. Productivity and quality of chickpea seeds in Northern Minas Gerais, Brazil. Revista Brasileira de Ciências Agrárias, **12,** 261-268.

[19] Howieson, J. & Dilworth, M. 2016. Working with rhizobia, Australian Centre for International Agricultural Research Canberra.

[20] Ju, I., Wj, B., Md, S., Ia, O. & Oj, E. 2018. A review: Biofertilizer-A key player in enhancing soil fertility and crop productivity. Journal of Microbiology and Biotechnology Reports,**2**.

[21] Junior, L. & Andrade, M. 2015. Legume-rhizobia signal exchange: promiscuity and environmental effects. Frontiers in microbiology, **6,** 945.

[22] Kabuo, N. O., Dialoke, S. A., Omeire, G. C., Bedi, E. N., Peter-Ikechukwu, A. I. & Irekpita, T. E. 2015. Comparison of proximate composition of some cultivars of chickpea (Cicer arietinum L.) cultivated in Owerri, Imo State, Nigeria. Food Science and Quality Management, **37,** 103-109.

[23] Khan, M. A., Ammar, M. H., Migdadi, H. M., El-Harty, E. H., Osman, M. A., Farooq, M. & Alghamdi, S. S. 2015. Comparative nutritional profiles of various faba bean and chickpea genotypes. International Journal of Agriculture and Biology, **17**.

[24] Kotula, L., Khan, H. A., Quealy, J., Turner, N. C., Vadez, V., Siddique, K. H., Clode, P. L. & Colmer, T. D. 2015. Salt sensitivity in chickpea (C icer arietinum L.): ions in reproductive tissues and yield components in contrasting genotypes. Plant, cell & environment, **38,** 1565-1577.

[25] Kumar, A. 2019. Plant Growth Promoting Rhizobacteria for Agricultural Sustainability: From Theory to Practices, Springer.

[26] Kumar, A., Bahadur, I., Maurya, B., Raghuwanshi, R., Meena, V., Singh, D. & Dixit, J. 2015. Does a plant growth-promoting rhizobacteria enhance agricultural sustainability. J Pure Appl Microbiol, **9,** 715-724.

[27] LEGESSE HIDOTO, TAR'AN, B., WORKU, W. & MOHAMMED, H. 2017. Towards zinc biofortification in chickpea: performance of chickpea cultivars in response to soil zinc application. Agronomy, **7,** 11.

[28] Lijalem Korbu, Damte, T. & Fikre, A. 2016. Harnessing Chickpea Value Chain for Nutrition Security and Commercialization of Smallholder Agriculture in Africa.

[29] Makkar, K. & Jangra, S. 2017. Isolation and Characterization of Rhizobium from Chickpea (Cicer arietinum). Int. J. Curr. Microbiol. App. Sci, **6,** 2880-2893.

[30] Maurya, R., Verma, S. & Bahadur, I. 2019. Advances in the Application of Plant Growth-Promoting Rhizobacteria in Horticulture. Plant Growth Promoting Rhizobacteria for Agricultural Sustainability. Springer.

[31] Minaei, H., Kahrizi, D. & Zebarjadi, A. 2015. Effect of plant growth regulators and explant type upon cell dedifferentiation and callus induction in chickpea (Cicer arietinum L.). J Appl Biotechnol Rep, **2,** 241-244.

[32] Mus, F., Crook, M. B., Garcia, K., Costas, A. G., Geddes, B. A., Kouri, E. D., Paramasivan, P., Ryu, M.-H., Oldroyd, G. E. D., Poole, P. S., Udvardi, M. K., Voigt, C. A., Ané, J.-M. & Petersa, J. W. 2016. Symbiotic Nitrogen Fixation and the Challenges to Its Extension to Nonlegumes. Applied and Environmental Microbiology, **82**.

[33] Nadeem, M., Li, J., Yahya, M., Wang, M., Ali, A., Cheng, A., Wang, X. & Ma, C. 2019. Grain legumes and fear of salt stress: Focus on mechanisms and management

strategies. International journal of molecular sciences, **20,** 799.

[34] Patil, S., Patil, D., Patil, M., Gaikwad, P., Bhamburdekar, S. & Patil, P. 2014. Isolation, characterization and salt tolerance activity of Rhizobium sp. from root nodules of some legumes. International Journal of Current Microbiology and Applied Sciences, **3,** 1005-1008.

[35] Rachwa-Rosiak, D., Nebesny, E. & Budryn, G. 2015. Chickpeas—composition, nutritional value, health benefits, application to bread and snacks: a review. Critical reviews in food science and nutrition, **55,** 1137-1145.

[36] RasooL, S., Abdel Latef, A. & Ahmad, P. 2015. Chickpea: role and responses under abiotic and biotic stress. Legumes under Environmental Stress: Yield, Improvement and Adaptations**, 67**-79.

[37] Raza, H., Zaaboul, F., Shoaib, M. & Zhang, L. 2019. An Overview of Physicochemical Composition and Methods used for Chickpeas Processing International Journal of Agriculture Innovations and Research, **7**.

[38] Roy, A., Singh, S. K., Bajpai, J. & Bajpai, A. K. 2014. Controlled pesticide release from biodegradable polymers. Central European Journal of Chemistry, 12**,** 453-469.

[39] Sabaghnia, N. & Janmohammadi, M. 2014. Evaluation of selection indices for drought tolerance in some chickpea (Cicer arietinum L.) genotypes. Acta Technologica Agriculturae, **17,** 6-12.

[40] Sahgal, M. & Jaggi, V. 2018. Rhizobia: Culture Collections, Identification, and Methods of Preservation. Microbial Resource Conservation. Springer.

[41] Santoyo, G., Sánchez-Yáñez, J. M. & De Los Santos-Villalobos, S. 2019. Methods for Detecting Biocontrol and Plant Growth-Promoting Traits in Rhizobacteria. Methods in Rhizosphere Biology Research. Springer.

[42] Simon, Z., Mtei, K., Gessesse, A. & Ndakidemi, P. A. 2014. Isolation and characterization of nitrogen fixing rhizobia from cultivated and uncultivated soils of northern Tanzania. American Journal of Plant Sciences, **5,** 4050.

[43] Singh, N. & Pratap, A. 2016. Food legumes for nutritional security and health benefits. Biofortification of Food Crops. Springer.

[44] Singh, Z. & Singh, G. 2018. Role of Rhizobium in chickpea (Cicer arietinum) production-A review. Agricultural Reviews, **39**.

[45] Somasegaran, P. & Hoben, H. J. 2012. Handbook for rhizobia: methods in legume-Rhizobium technology, Springer Science & Business Media.

[46] Temam Abrar & Alemayehu Letebo 2017. Isolation and Characterization of Rhizobia from Rhizospher and Root Nodule of Cowpea, Elephant and Lab Lab Plants. International Journal of Novel Research in Interdisciplinary Studies**, 4,** 1-7.

[47] Tewodros Tefera 2014. Analysis of chickpea value chain and determinants of market options choice in selected districts of southern Ethiopia. Journal of Agricultural Science, 6**,** 26.

[48] Thapa & Binita 2015. Soil Fertility. Soil Science Division**, 1**.

[49] Thomas, L. & Singh, I. 2019. Microbial Biofertilizers: Types and Applications. Biofertilizers for Sustainable Agriculture and Environment. Springer.

[50] Unay, J. & Perret, X. 2019. Synthetic Plasmids to Challenge Symbiotic Nitrogen Fixation Between Rhizobia and Legumes. Methods in Rhizosphere Biology Research. Springer.

[51] 51. Upadhyaya, H., Dwivedi, S., Sharma, S., Singh, S., Lalitha, N. & VARSHNEY, R. 2016. 6. Chickpea germplasm for use in crop improvement: Approaches and way forward. Harnessing Chickpea Value Chain for Nutrition Security and Commercialization of Smallholder Agriculture in Africa**, 52**.

[52] 52. Vejan, P., Abdullah, R., Khadiran, T., Ismail, S. & Nasrulhaq Boyce, A. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules, **21,** 573.

[53] 53. Wallace, T., Murray, R. & Zelman, K. 2016. The nutritional value and health benefits of chickpeas and hummus. Nutrients, **8,** 766.

[54] 54. Wondafrash Mulugeta & Dejene Girma 2016. Genotype x environment interaction and stability analysis for yield and yield related traits of Kabuli-type Chickpea (Cicer arietinum L.) in Ethiopia. Harnessing Chickpea Value Chain for Nutrition Security and Commercialization of Smallholder Agriculture in Africa**14,** 1564-1575.

[55] 55. Yan, N., Marschner, P., Cao, W., Zuo, C. & Qin, W. 2015. Influence of salinity and water content on soil microorganisms. International Soil and Water Conservation Research, **3,** 316-323.

[56] 56. Youssef, M. & Eissa, M. 2014. Biofertilizers and their role in management of plant parasitic nematodes. A review. Journal of Biotechnology and Pharmaceutical Research, **5,** 1-6.

[57] 57. Zhang, Y., Shen, H., He, X., Thomas, B. W., Lupwayi, N. Z., Hao, X., Thomas, M. C. & Shi, X. 2017. Fertilization shapes bacterial community structure by alteration of soil pH. Frontiers in microbiology, **8,** 1325.

Citation: *Fikadu Hailemichael" Cost Characterizing the Effectiveness of Nitrogen Fixing Bacteria to Optimize Chickpea (Cicer Arietinum L) Production", International Journal of Research Studies in Science, Engineering and Technology, vol. 7, no. 11, pp. 23-34, 2020.*