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Harnessing *Mesorhizobial* inoculants on the grain legume Chickpea (*Cicer arietinum L.*) in Ethiopia and contributions to promoting plant growth: Review

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Abstract

Chickpea is an important legume that serves as a vital source of nutrition in many regions of Ethiopia and sustain cropping system productivity due to its ability to fix atmospheric nitrogen. Legumes such as chickpea possess seeds with high protein content therefore, require high supply of nitrogen. To realize this requirement, the crop embraces nodule on its root where bacteria of the genus Mesorhizobium live with a specific function of converting the atmospheric nitrogen into plant available form called biological nitrogen fixation. However, the potential of the crop to accomplished the current demand of nitrogen for poor soils is limited by its association with symbiotic nitrogen fixing bacteria, poor understanding of the relationship between strain diversity and symbiotic performance. Therefore, the present review will be used as a baseline to understand Ethiopian chickpea Mesorhizobium genetic resources for better symbiotic nitrogen fixation in chickpea. Moreover, it allows to supporting research efforts to select efficient rhizobial inoculants in nutrient management for increasing the production and productivity of chickpea as well as maintaining soil health and to solve the future problems.

Keywords: Chickpea; Inoculants; *Mesorhizobium*; Symbiotic N2-fixation

1. Introduction

Nitrogen and phosphorus are the two most limiting elements for crop production. Although nitrogen is abundant in the atmosphere as N_2, it cannot be utilized directly unless it is converted into biologically available form of ammonia (NH_3). The nitrogen availability in the soil is affected by soil type, tillage, N-source, crop rotation, precipitation. The ability of the crops to recover applied Nitrogen chemical fertilizers is usually less than 50 because of their low fertilizer use efficiency (1); (2). Phosphorus is also the next most limiting element for plant growth for 95-99% of the phosphorus in the soil exists in the insoluble, immobilized and precipitated form which is attributed to pH-mediated sorption to the soil (3). This is because the readily available phosphate tends to react with calcium and magnesium under high pH; whereas iron or aluminum are fixed under low pH leading to its precipitation making it unavailable for plant uptake (4).

Naturally, microorganisms are playing a very important role in nutrient cycles of nitrogen and phosphorus by mineralizing them from organic matter into inorganic ones through their metabolic processes. Apart from that, inorganic nitrogen (N_2) from the atmosphere is converted into available form of ammonia through Biological nitrogen fixation (BNF) by prokaryotic microrganisms (Bacteria and Archea). It is estimated that prokaryotes annually fix upto 139 to 170 $\times 10^6$ tons of nitrogen in the terrestrial ecosystem, where more than 70% is fixed by the ensosymbiotic association of the root nodule bacteria with leguminous plants (5).

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Rhizobia effectively immunize the plants against different pathogens and confer resistance by enhancing the expression of different genes (6). Rhizobacterial strains is considered natural, ecofriendly and safe besides providing resistance against a broad spectrum of pathogens (7). Similarly, microorganisms enhance availability of phosphorus by solubilizing complex-structured (insoluble) phosphates viz. Tricalcium phosphate, rock phosphate, aluminum phosphate through chelating with organic acid production and mineralizing organic phosphorus by liberating enzymes such as phosphatases that ultimately enhance phosphate availability to plants (8); (4).

Other microorganisms in the rhizosphere are involved in several direct and indirect activities that enhance plant growth and development. The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root (9). Bacteria that colonize rhizosphere and plant roots that exert beneficial effects on plant growth are referred to as plant growth promoting rhizobacteria (PGPR) (10). PGPR include both free living microorganisms, endophytes colonizing plant tissues and bacteria that are able to establish symbiotic relationships with plants (11).

Mechanisms by which PGPR stimulate plant growth are broadly categorized as direct or indirect and some traits are considered as direct and at the same time as an indirect mechanism (12). The direct plant growth enhancement mechanisms include; nutrient acquisition (via N-fixation, phosphate solubilization and siderophore production) and modulating phytohormones. The indirect growth promoting mechanisms of PGPR include their ability to act as biocontrol against phytopathogens through various forms of antagonism like competition, production of antibiotics, siderophores and lytic enzymes, production of hydrogen cyanide, producing bacteriocin (13); (14), detoxifying virulent factors (15), pathogenic signal interference (16) and triggering induced systemic resistance (13); (12).

Chickpea (*Cicer arietinum* L.) is second most important pulse crop after common bean grown in many countries worldwide (17). It is originated 10,000 years ago in the Mesopotamian region of South Eastern Turkey and spread to five recognized centers of diversity; the Mediterranean bassin, and Central and West Asia (18). It moved to India ~6,000 years ago and arrived in Ethiopia as early as 2,000-3,000 years ago (19). Ethiopia is one of the secondary centers of diversity of the crop (20) and the major chickpea growing country contributing to more than 40% of production in in Africa (18). In Ethiopia on conventionally farmed fields, the productivity of chickpea (both Desi and Kabuli types) is below 2 t/ha (21).In spite of its widespread cultivation in the country, the productivity of chickpea is low with an average of 1.9 t/ha compared to its potential 4-5 t/ha (22). The yield gap is attributed to many factors the most important ones are; low soil fertility, severe drought or erratic rainfall patterns, soil salinity/acidity, occurrence of diseases, pests and weeds (21). Its deficiency is also attributed to low biological nitrogen fixation (BNF) (23). It is one of the major protein sources of the majority of the population in the country due to its high protein content (17-22%) (24). It is grown as a rotation crop with other cereal crops to improve soil fertility and enhance yield for it derives 70% of its N through converting atmospheric dinitrogen (N_2) to ammonia (NH_3) through biological nitrogen fixation (25).

Thus, it improves the N content of soil which is available for the subsequent crop. The production of high content and quality proteins by chickpea is attributed to biological nitrogen fixation (26). Benefits of symbiotic nitrogen fixation (SNF) can be maximized by optimizing the amount of N_2 fixed by rhizobial bioinoculants for legume symbioses. The use of rhizobial inoculants in agricultural production is aimed at ensuring that the most effective microsymbiont occupies the largest proportion of nodules formed on the target host legume in the field (27). Although chickpea is capable of fixing inorganic nitrogen, its production is constrained by poor soil fertility mainly nitrogen and phosphorus. This is due to variation in effectiveness of the crop in biological nitrogen fixation. Effectiveness in symbiotic nitrogen fixation (SNF) in legumes is influenced by the existence of native rhizobia in the soils, genetic variation in bacterial strains, the number of infective cells applied and symbiotic response of the host cultivar (28); (29); (30); (31). For the several years now, there has been a lot of interest to study on phenotypic and symbiotic properties of different groups of chickpea rhizobia from different parts of Ethiopia (32); (33); (34); (35); (36); (37); and plant growth promoting properties of the rhizobia and rhizobacteria from the rhizosphere of chickpea (32).

Although few studies were undertaken on genetic diversity of the rhizobia and a symbiotic effectiveness trial of few isolates in Southern Ethiopia (35); (38); most of them were limited to studies of collecting large samples of cognate isolates of rhizobia from different chickpea growing regions and screening for their symbiotic properties in greenhouse trials. Moreover, chickpea rhizobial genetic diversity and its symbiosis has not been extensively explored. Similarly, the hitherto studies lacked to delve into chickpea rhizobial richness in terms of geographic distribution and relationship within strains diversity and their symbiotic performance in different soil and chickpea varieties has not been extensively tested. Recently the diversity of indigenous *Mesorhizobium* in Ethiopia, identification of effective and competitive strains being used to suggesting the potential strains for formulation of *Mesorhizobium* inoculants for chickpea were explore (39); (40).

2. The significance of chickpea production

Chickpea is originated in ~10,000 years ago in the Mesopotamian region of Southeastern Turkey; *thereafter spread to India ~6,000 years ago* and arrived in Ethiopia as early as 2,000-3,000 years ago (41); (18).Currently, chickpea is produced in more than 60 countries worldwide and the world's second most important grain legume after common bean with particular importance in the semi-arid tropics of sub-Saharan Africa and South Asia (17) and (42) estimated that the crop contributes to more than 20% of world pulse production covering 15% of the total land area.

It is grown on in South Asia and Sub-Saharan Africa, which accounts for more than 75% of the world chickpea area. Thus, the major chickpea producing countries are; Australia (629,400 tonnes), Myanmar (562,163 tonnes), Ethiopia (458,682 tonnes), Turkey (450,000 tonnes), Pakistan (399,030 tonnes) of the chickpea cultivation area. The crop has two types; the desi type characterized by brown to dark colored and rough surfaced seeds that account for up to 85% of production; whereas the cream to yellow colored seeded colour kabuli type controbutes to 15% of the production (43); (44); (42).

Chickpea is a vital source of carbohydrates, protein, minerals (calcium, phosphorus, magnesium, zinc, iron), vitamins (riboflavine, niacin, thiamin), unsaturated fatty acids (linoleic, oleic acids), dietary fibre and lipids; of which, carbohydrates account to about 60%, protein content (17-22 %), dietary fibre 17.4%, total Sugar (10.4%) and 6% fat (Jukanti et al.2012). It is also the most important commodity crop in the agricultural economy in advanced developing economies (Turkey) and developed countries (USA, Australia and Canada). Chickpea is sown with low water inputs, largely reliant on stored residual moisture following wet seasons and can grow well even in the marginal soil and soils of varying textures (43). Consequently, it is a relatively drought tolerant grain legume grown in semi-arid regions may act as an insurance crop in poor seasons when the main crop fails due to drought (45).

Chickpea obtains its nitrogen from conversion of atmospheric nitrogen into ammonia, by a process known as biological nitrogen fixation through a symbiotic relationship with Mesorhizobium (46). Nitrogen fixation increases the nutritional protein content of the seeds and enhance soil fertility and benefits both chickpea host and its following crops there by reducing the use of fertilizer (25), When chickpea is grown in a rotation, it can reduce rate of weeds and diseases and pests (47). As reported by (39) estimated that up to 181 kg N per hectare is obtained when chickpea pods are harvested and crop residues are tilled into the soil. Studies conducted in Turkey indicated that inoculation with Mesorhizobium ciceri increased the average shoot dry weight by 12% and nitrogen % by 7.9% (48).

3. The process of biological nitrogen fixation (BNF)

The biological nitrogen fixation is a process converting inert atmospheric dinitrogen (N_2) to biologically available form ammonia (NH_3) by prokaryotic micro-organisms. Prokaryotic organisms (bacteria and the archaea) (diazotrophs) have the enzymatic nitrogenase, encoded by the *nif* gene; to break the strong triple bond between the two N atoms and make them reactive with hydrogen atoms to form ammonia (49); (50). BNF is categorized into two categories; nonsymbiotic nitrogen fixing group that are either free living, associative or endophytic and endo-symbiotic nitrogen fixation. A symbiotic nitrogen fixation is mediated by bacteria such as *Cyanobacteria*, *Azotobacter*, *Azospirillum*, *Acetobacter diazotrophicus*, *Azoarcus* (51). The second category includes; nodule forming bacteria (*Rhizobiaceae* family*)* with Leguminous plants and the actinomycete *Frankia with* non-leguminous plants. It is estimated that Biological nitrogen fixation (BNF) contributes ~139 to 170 × 10^6 tons of nitrogen per year to the terrestrial ecosystem compared to the ~65 ×10^6 tons of nitrogen added in the form of synthetic fertilizer per year (5).

3.1 Rhizobia legume Symbiosis

Rhizobium-legume symbiosis is a host specific association and the need to identify specific strains and the diversity of rhizobia associated with legumes is vital for better exploitation of the benefits of the rhizobia as biofertilizers (52). The efficiency of nitrogen fixation varied between strain either due to genomic background of the rhizobia and/ or the combinations between strains, plant varieties and soil factors (53). It is, therefore, of paramount importance to understand the major phases of the general symbiotic process involved such as plant infection, nodulation and nodule maturation, senescence, release of rhizobia and persistence of rhizobial populations in soil (54). The developmental stages start with the attachment of the bacteria within root hairs followed by root hair curling, epidermal invasion and crack entry for the formation of root nodules that provide an environment suitable for nitrogen fixation by rhizobia (55). The success of the symbiosis depends on the recognition of rhizobia by the legume host of to activate the expression of a group of bacterial nodulation (*nod*) genes leading to initiation of bacterial infection (56).

3.2 Rhizobial infection and nodulation genes

Rhizobium-legume symbioses are species-specific and each host plant may be nodulated by one or a few microsymbiont species (57). For this reason, the nodulation process begins with an intricate signal exchange and recognition by the symbionts, the control of the plant defense responses, nodule organogenesis, and efficient N_2 fixation and ammonium assimilation (58). The plant initiates by releasing signal molecules such as flavonoids (secondary plant metabolites), phenolics, sugars, dicarboxylic acids and amino acids. The rhizobia detect the compounds and respond by aggregating and attaching them around the root hair of the host (59).

The signal molecules in the rhizosphere define specificity, competitiveness, infectivity and effectiveness of the legume rhizobium symbiosis because different legumes produce different types and mixes of compounds (60). Flavonoid signal molecules activate nodulation protein D (NodD) in rhizobia by stimulating the binding of the transcriptional regulator NodD of the nodulation genes promoter (61). Implicating bacterial NodD protein triggers the transcription of a range of genes within the rhizobia (62). The nodulation genes are important for the synthesis and secretion of nod factors (lipochitin-oligosaccharides) that are receptors for the plant flavonoid signal to induce structural and functional alterations within the plant root (63). Apart from the Nod factors, various bacterial cell structures such as the lipopolysaccharides, β-glucans, exopolysaccharides, capsular proteins and K antigen are also recognized by the plants to determine the host specificity (64).

More than 30 different *nod*, *nol* and *noe* genes are involved in the synthesis and secretion of Nod factors (53). The common nodulation genes (*nodABC*, *nodD*, *nodIJ*) exist in all symbiotic rhizobia except some *Bradyrhizobium* strains. Among these genes *nodABC* encode the enzymes required for the synthesis of the core Nod factor structure of an *N*-acetyl glucosamine oligosaccharide backbone with a fatty acyl chain at the non-reducing end (65). Nod factors also trigger curling of the root hair towards the attached bacterium and generating a shepherd hook structure that entraps the bacterial microcolony attached to the tip of the root hair (66). At this point, the root hair plasma membrane invaginates and start formation of intracellular infection thread (ITs) in which the bacterium enters into the plant root interior (67). In this way, cell division along the infection thread into the root cortex initiate the formation of the nodule primordium or the infected cells (59).

The bacteria released from tip of the infection thread into root cortical cells via endocytosis. Subsequently, the bacteria continue to divide and fully internalized by the host cell to become an intracellular organelle surrounded by a host-derived symbiotic membrane (68). The enveloping membrane (symbiosomes) controls provision of energy, sequestration of free oxygen molecules and nutrient exchange between the symbionts to create suitable environment for nodule development (61). The type of nodules formed by legumes is classified into determinate and indeterminate Nodules. Nodules can be determinate (which grows up to maturity, stops the development and starts N_2 fixation), such as in soybean, common beans and indeterminate nodules (which holds a meristematic zone that guarantees a continuous growth of the organ concomitantly with N_2 fixation), such as in *Medicago truncatula*, alfalfa and pea (50).

Within the symbiosome (nitrogen-fixing unit of the nodule) rhizobia undergo cell division and differentiation to form the nitrogen fixing entity known as the bacteroids. Bacteroid is a metabolic switch that converts free-living rhizobia into N_2-fixing organelles, that express the nif and fix genes clusters required for nitrogenase enzyme complex assembly and functioning (Unay and Perret 2019). They are also sites for transport (exchange) of reduced carbon compounds from the plant to the nodule and of fixed nitrogen from the bacteroids to the host plant cytoplasm (69).

The Nitrogenase enzyme requires a FeS-cluster and other metal-dependent cofactors for electron transduction; thus, nitrogenase complex consisted of two metallo protein componets; an iron-protein coded by dinitrogenase reductase *(nifH)* structural gene and iron-molybdenum encoded by α and β subunits dinitrogenase (*nifD, nifK)* (70). Both the Fe and MoFe components of nitrogenase are O_2 labile. Bacteria fix nitrogen inside root nodules in which low oxygen and abundant carbon sources are provided (58). Commonly, the nitrogenase reduces atmospheric nitrogen through a series of energy-demanding metabolic steps. The rhizobia require reduced carbon essential for bacterial physiology as well as to produce (16 ATPs) required to fuel symbiotic N_2 fixation (71). The nitrogenase catalyzes the following reaction;

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

Nitrogen fixation requires the construction of specialized symbiotic nodules structures and protection against excess oxygen. For this reason, the internal layer of the nodules acquires thick mucilaginous layer and a high concentration of hemoglobin that is essential to control oxygen homeostasis and protect the rhizobial nitrogenase enzyme complex from oxidation (31). In general, the rhizobium legume symbiosis contains a set of nod, nif and fix genes which is important

to entice the formation of proficient nodules on roots of legume crops for the continual function of nitrogenase or symbiotic nitrogen fixation (60).

3.3 The genome and phylogeny of symbiotic rhizobia

Rhizobial genomes are considered to have two main components of core and accessory genes. Core genome is mostly chromosomal, essential for cellular function, shared across all members of a species contain higher GC. The accessory genome is the one located on chromosomal islands including symbiosis genes, increase adaptive potential of host through provision of phenotypic advantageous for various niches and contain lower GC (72); (73); (53). A common feature of the rhizobial genomes is that genes involved in nodulation and N_2-fixation are clustered on symbiotic plasmid (pSym) or incorporated into the chromosome as symbiotic islands (74). The symbiosis genes in rhizobia refer to nodulation genes (nod, noe and nol) which are responsible for nodulation and nitrogen fixation genes (nif and fix) that are involved in atmospheric nitrogen fixation (75).

Rhizobia typically have large and complex multipartite genomes compared to most bacteria, ranging in size from ~5-10 Mb that enable them to adapt to different habitats (76). Rhizobia have one chromosome and several plasmids and/or megaplasmids that may represent 50% of the genome (77), that contribute to an evolutionarily dynamic genome through the process of horizontal gene transfer (56). In many cases, the phylogenetic positions of the symbiosis genes, especially the nodulation genes, are different from those of the chromosomal (housekeeping) genes (53).

Rhizobial symbiosis genes are often carried on symbiotic islands or plasmids (pSym) that can be transferred (horizontal transfer) between different bacterial species within and across genera (65). Integrative and conjugative elements (ICEs) are generally regarded as regions of contiguous DNA integrated within a bacterial genome that are capable of excision and horizontal transfer via conjugation (78). As ICEs are universal mobile genetic elements present as "genomic islands" within bacterial chromosomes; symbiosis islands are the largest documented ICEs and their transfer converts nonsymbiotic *Mesorhizobia* into nitrogen N_2- fixing symbionts of leguminous plants (79).

3.4 Taxonomy and diversity of nitrogen fixing rhizobia

The geographic distribution, diversity and phylogenetic relatedness of rhizobia could highlight their evolutionary origin as well as their unique characteristics which can be utilized for manipulation of symbiotic nitrogen fixation in legumes (52). This suggests that representative strains from the large genetic diversity of rhizobial species can be selected for developing elite rhizobial bioinoculants for precision agriculture (80). Subsequently, (53) suggested that rhizobial diversity depends on four factors; long evolutionary history, environmental selection for their survival (chromosome genes), host selection for nodulation (symbiosis genes) and lateral transfer of symbiosis gene (creating novel combinations of chromosome and symbiosis genes). Strain competition and cohabitation in the vicinity enables the horizontal transfer of genetic material between bacteria which may enrich the genetic pool of individual strains and increase intrinsic population diversity (57).

Traditionally, taxonomy of rhizobia was based on the host specificity of the rhizobial strains, or cross inoculation group concept. However, this criterion was not useful to classify species of rhizobia due to the possibility of natural transfer of symbiotic plasmids among bacterial strains in the soil and occurrence of some rhizobial strains which are capable of nodulating a wide range of legumes. The location of symbiotic genes was also used as a genotypic tool to differentiate between the fast and slow growing legume nodulating bacteria, they are typically chromosome located for the slow growing *Bradyrhizobium* and on plasmids for fast growing *rhizobium* strains (81).

Later on, phenotypic features (morphological, physiological, biochemical) of bacteria have been used for the description of new bacterial species in numerical taxonomy at 80% phenotypic similarity. Phenotypic analysis is used for preliminary classification of bacteria into the genus as well as selection of metabolic, physiological, ecological and plant growth promoting characteristics of rhizobial strains that may beneficially influence plant growth and development and for providing information about ecological competence of the rhizobia in the organism's habitat (82); (11).

The advent of molecular analyses, together with the isolation and characterization of more root nodule from many legumes has revolutionaized rhizobial taxonomy. These include; DNA base composition (G + C content), DNA-DNA hybridization, sequences of the 16S ribosomal RNA genes and Fatty Acid Methyl Ester (FAME) profiles (83). DNA-DNA hybridisation (DDH) technique has been applied as the gold standard method and strains classified in the same species should have 70% DDH relatedness among each other (84). However, DDH results vary between different laboratories and this incurs inconsistent classification of the same species.

Apart from the 16S rRNA gene, PCR-based RFLP analysis of 16S-23S rRNA gene intergeneric spacer have been widely used at a faster rate than the 16S rRNA, thus adding valuable information to the analysis. Despite, the 16S rRNA gene is highly conserved to allow separation of closely related species; the genomes of rhizobia may lose or gain plasmids or genomic islands (HGT) and genetic recombination in 16S rRNA genes leading to insufficient rhizobial taxonomy (85). Later, Multilocus Sequence Analysis (MLSA) of housekeeping protein coding genes including 16S rRNA, *atpD* (ATP synthase F1, beta subunit), *recA* (recombinase A) and *rpoB* (RNA polymerase, beta subunit) was established to discriminate between closely related species at 96-97% similarity ((85); (83); (86). These genes have a faster rate of evolution than the 16S rRNA gene but are conserved enough to retain genetic information useful for taxonomic purposes (83).

Several genomic approaches have been employed to define and demonstrate the involvement of *rhizobial* genomes in the symbiotic events. Recently genomic data analysis tools based on whole genome sequences such as Average Nucleotide Identity (ANI) have provided scientifically valid taxonomic standards for classifications; that accommodate the rapidly expanding field of genomics to classify microbial diversity (87). The standard ANI value of 95-96% has been applied for species threshold, while 75 and 70% could be the thresholds for genus and family, respectively (53). Generally, whole genome sequencing offers the ability to explore rhizobial diversity using culture independent methods (39) and to use genomic data to assess species relationships using methods such average nucleotide identity (ANI) and pan-genome enumeration (88); (89). Whole genome tools also provide opportunities to discriminate more nuanced strain-level diversity, which may facilitate prediction and selection of rhizobial diversity better suited for use as legume biofertilizers (as postulated by (82).

The use of polyphasic approach (combination of phenotypic, genotypic and symbiotic characters together with the exploration of more legumes for their endosymbionts) contributes to the advancement of rhizobial classification for several decades. Thus, the root nodule bacteria that are classified under the family *Rhizobiaceae* have been classified into two classes, *Alphaproteobacteria* and *betaproteobacteria*, 13 genera and more than 98 species ((90); (91). The well-known members of the Alphaproteobacteria are the genera *Rhizobium*, *Mesorhizobium*, *Ensifer* (former *Sinorhizobium*), *Azorhizobium*, *Bradyrhizobium*, *Methylobacterium*, *Devosia*, *Ochrobactrum*, *Phyllobacterium*, *Shinella*, *Microvirga.* The two genera belonging to the class *betaproteobacteria* are the genera *Burkholderia* and *Cupriavidus* (11).

3.5 Chickpea rhizobia

Chickpea establish symbiosis with root nodule bacteria belonging to the specific genus *Mesorhizobium* with an intermediate growth rate between the genera *Rhizobium* and *Bradyrhizobium* (46). Although chickpea rhizobia are considered for long as very host specific to *M ciceri* and *M mediterraneum* (92). Later studies have shown that chickpea is able to establish symbioses with several species of *Mesorhizobium*, such as *Mesorhizobium amorphae*, *Mesorhizobium loti*, *Mesorhizobium tianshanense*, *Mesorhizobium muleiense*, *Mesorhizobium abyssinicae* and *Mesorhizobium shonense* (93); (94). Recently, (39) reported that the *Mesorhizobium* genus encompasses 36 distinct species; of which eight *Mesorhizobium* species were cosmopolitant that nodulate chickpea globally; and 20 chickpea symbionts had not been previously recognized.

The symbiosis genes of *Bradyrhizobium* and *Mesorhizobium* species are located in chromosomal symbiosis islands, the exceptions described to date being *Mesorhizobium amorphae* (95) and *Mesorhizobium huakuii* (75). The phylogenetic analysis of symbiosis genes *(nifH* and *nodC)* has been used for determining the symbiovar of rhizobial strains; all chickpea-nodulating rhizobia should be assigned to the symbiovar *ciceri* that is *Mesorhizobium* spp. sv. *ciceri* (53). Symbiovar represents a group of bacterial strains distinguishable from other strains of the same species on the basis of physiological or biochemical characters, which can be shared by different species due to lateral gene transfer (30).

4. Plant growth promoting rhizobacteria (PGPR)

Rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. Bacteria that colonize rhizosphere and plant roots that exert beneficial effects on plant growth are referred to as plant growth promoting rhizobacteria (PGPR) (10). PGPR include both free living microorganisms, endophytes colonizing plant tissues, and bacteria that are able to establish symbiotic relationships with plants (11). Based on the degree of association with root cells, PGPR are also grouped into extracellular plant growth promoting rhizobacteria (ePGPRs) and internal plant growth promoting rhizobacteria (iPGPRs) (96).

The extracellular plant growth promoting rhizobacteria (ePGPRs) exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex; that include the bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*

and *Pseudomonas* (3). The intracellular plant growth promoting rhizobacteria(iPGPR) are located inside the specialized nodular structures of root cells belongs to the family of Rhizobiaceae; that includes *Allorhizobium, Bradyrhizobium, Mesorhizobium* and *Rhizobium* endophytes and Frankia species, both of which can symbiotically fix atmospheric nitrogen with some groups of higher plants (97). PGPRs benefit not only the growth of plants but the entire community, including bacteria, plants and soil fauna; such benefits include an increase in growth and N- and P-uptake by plants, increased photosynthesis and decreased carbon costs, through the inoculation of elite strains (98). The application of PGPR in diverse crops has been reported to approximately increase the yield by 20-40% (6). They promote plant growth through direct and indirect mechanisms.

4.1 Plant growth promoting mechanisms

PGPR play great role in facilitate nutrient uptake or increase nutrient availability and regulating plant hormone levels. PGPR directly improved plant growth by providing the plants with bacterial synthesized organic molecules such as (amino acids, carbohydrates, enzymes, inorganic substances), promoting plant growth through production of plant growth regulators (Auxins, abscisic acid, cytokinins, gibberellic acid) and produce the siderophore for solubilize and sequestering of iron (Fe) from the soil and supply it to the plant cells and synthesis of hydrogen cyanide (99); (5). They produce several types of organic molecules that include; amino acids, carbohydrates, and enzymes and inorganic substances (99).

This mechanism includes biofertilizer activity through nutrient fixation (biological nitrogen fixation, nutrient solubilization (phosphate solubilization, potassium solubilization), biostimulators (production of phytohormones) and siderophores to enhance growth and Fe uptake by plants, respectively (91). PGPR can promote plant growth through indirect mechanisms through production of antagonistic substances and promotion of host Induced Systemic Resistance (ISR), against pathogens (1). They produce HCN (98), Antibiotics, Bactreiocin, HCN, NH_3, Hydrolytic enzymes, ExoPolysaccharides, Growth substance, polysaccharides and produces protecting enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity (100) that indirectly benefit the host plants.

4.1.1 PGPR as biofertilizer (provision of nitrogen, phosphorus and iron)

Biofertilizer is a substance containing living microorganisms (organic compounds), when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (1). Mode of action of PGPR as biofertilizers to enhance the nutrient status of host plants included; biological N_2 fixation by free living, associative and symbiotic nitrogen fixing bacteria, increasing the availability of nutrients (P, Fe, etc) in the rhizosphere, inducing increases in root surface area, enhancing other beneficial symbioses of the host and combination of modes of action (8). PGPR are involved in both solubilization of inorganic (insoluble) phosphates and mineralizing organic phosphorus by liberating enzymes such as phosphatases to make phosphorus available for plants (64). Microorganisms mineralize organic phosphorus in soil by solubilizing complex-structured phosphates viz. Tricalcium phosphate, rock phosphate, aluminum phosphate; that turns organic phosphorous to inorganic form ultimately aiding the phosphate availability to plants (4). Phosphorus is taken up in soluble forms (H2PO4- and HPO4 2-); this bioavailable fraction constitutes only the 0.1% of the total phosphorus content in soils and phosphorus deficiency imposes an important constraint on chickpea production (101). Phosphorus is required for many plant functions including vital nucleic acid and adenosine triphosphate (ATP) production; a decrease in phosphorus availability to the plant causes a decrease in nodule formation and nitrogen fixation due to lack of ATP metabolic actions in the associated plant cell (102).

Reductions in the concentration of ATP and energy charge in inorganic phosphate deficient nodules results in significant declines in nitrogenase activity, symbiotic nitrogen fixation (SNF) capacity and the growth and productivity of legume crops (29). PGPR are also capable of solubilizing iron by the production of iron binding molecules like siderophores which can form Fe-siderophore complex, readily available to plants under iron deficiency conditions. Many bacteria produce organic compounds called siderophores that chelate Fe^{3+} and increase its availability for plant uptake after the reduction of Fe^{3+} to Fe^{2+} or directly as siderophore-Fe^{3+}complex (8); 101).

4.1.2 PGPR as plant growth regulators

PGPR also produce multitudes of phytohormones such as auxins (Indole acetic acid; IAA), gibberellins, cytokinins, abscisic acid and ethylene ammonia (64). Indole acetic acid (IAA) is a common product of L- tryptophan metabolism produced by PGPRs helps in the production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake (10). It been estimated that more than 80% of the soil bacteria are able to produce auxins, especially IAA, indolebutyric acid or similar compounds derived from tryptophan metabolism (90). The naturally occurring auxin indole-3-acetic acid (IAA) promote root elongation lateral root development and is involved

in the early steps of nodule organogenesis (69). Cytokinins mediate the rhizobial infection in legumes and increase chlorophyll content.

Abscisic acid (ABA) is an important plant hormone related to plant response to drought; Abscisic acid could inhibit nodulation, rhizobial infection and gene expression of several nodule associated genes (50). The production of gibberellins stimulates the root system and few bacterial species such as *Bacillus pumilus* and *Bacillus licheniformis* are capable of producing the hormone (9). In rhizobia, cytokinin leads to nodule development by regulating the different Nod factors pathway and initiating the cortical cell division (6).

4.1.3 PGPR as biocontrol agents (Antagonism)

PGPR protect plants from the attack of plant pathogens by different direct and indirect mechanisms that create conducive environment for normal plant growth (Kour *et al*.2019). Siderophores bind the soluble form of iron from soil to make it available to plants; thus, siderophore-Fe complex is up taken by plant roots making the soil environment Fe-deficient for pathogenic fungi (100). Thus, siderophore producing rhizobacteria improve plant health by improving iron nutrition and inhibiting the growth of other microorganisms with the release of their antibiotic molecule and hinder the growth of pathogens. Iron deficiency causes growth inhibition, decrease in nucleic acid synthesis inhibition of sporulation, and causes changes in cell morphology of the pathogen (9). The ability of PGPR to synthesize antibiotics and other extracellular metabolite such as subtilin, sublancin, chlorotetain, rhizoctinins, surfactins hinders the growth of phytopathogens even at low concentration (102). PGPR also induce systemic resistance to plants against different pathogens by the activation of plant defense mechanisms and directly suppress broad spectrum of pathogens pathogesn by competition, antagonism and producing antipathogenic compounds (7); (6); (99). PGPR also modulate a wide range of environmental stresses like high temperature, cold, drought, salinity, alkalinity UV, and pathogen infection; abiotic stress is the primary cause of crop loss worldwide by more than 30% (4). Under acidity, salinity, drought, plants produce ethylene to overcome the stressful conditions. The PGPR possess the ability to produce an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme to decrease the ethylene synthesis by competing with plant ACC oxidase (90).

5. Conclusion

The present review revealed the existence of wide distribution of chickpea Mesorhizobium genetic resources in different chickpea growing agroecological regions of Ethiopia for better symbiotic nitrogen fixation in chickpea. High symbiont diversity could permit local adaptation to soil and microbial factors and it could impart variation in symbiotic performance. Exploring the existence of naturally occurring chickpea rhizobia provide an important complement further to evaluate their potential as inoculants under and field conditions to validate their feasibility for inoculant development. In addition, could help to fill the gap of information for those concerned, to study further screening of competent and symbiotically effective strains potentially improve chickpea production in the country.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

ZM selected the scope of the article and did primary literature review writing, FA conceptualization and editing the manuscript.

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