Detection and Identification of Plant Growth Promoting Bacteria from Sorghum (*Sorghum bicolor* L. Moench) Rhizosphere Soil in Northern Ethiopia

MSc. Thesis

By

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MSc Thesis Summited to the School of Graduate Studies of Jimma University, College of Agriculture and Veterinary Medicine in Partial Fulfillment of the Requirements for the Degree of Master of Science (MSc.) in Plant Biotechnology

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DEDICATION

I dedicate this work to my mother, Zertihun Mekonen for her great effort in making me successful; to my teachers who taught me at elementary, high school, and University levels, and to my late sister, Melesech Babiye.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my bona fide work, and that all sources of materials used in this thesis have been duly and accordingly acknowledged. The thesis has been submitted in partial fulfillment of the requirements for MSc Degree in plant biotechnology at College of Agriculture and Veterinary Medicine, Jimma University, and has been deposited in the University Library to be made available to users under the rules of the Library. I declare that this thesis has not been submitted to any other institution anywhere for the reward of any academic degree, diploma or certificate. Brief quotations from this thesis are allowed without special permission, provided that accurate acknowledgement of the source is made. Requests for permission for extended quotation or reproduction of this manuscript in whole or in part may be granted by the head of the department or the Dean of the college when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

Name: **Birhanu Babiye**

Signature: _____

Place: **Jimma University**

Date of Submission: __/__/__

ACRONMYS AND ABBREVEATIONS

PGP	Plant Growth Promoter
PGPRB	Plant Growth Promoting Rhizosphere Bacteria
SNNP	Southern Nations, Nationalities and Peoples
CSA	Central Statistical Agency
PS	Phosphate Solubilization
EPS	Exopolysaccharides
ISR	Induced Systemic Resistance
ROS	Reactive Oxygen Species
HNABRC	Holetta National Agricultural Biotechnology Research Center
IAA	Indole Acetic Acid
ICRISAT	International Crops Research Institute for The Semi-Arid Tropics
HGS	High Germination Stimulation
LGS	Low Germination Stimulation
PH	Plant Height
PFW	Plant Fresh Weight
PDW	Plant Dry Weight
RL	Root Length
RFW	Root Fresh Weight
RDW	Root Dry Weight
TCP	Tri- Calcium Phosphate
OD	Optical Density
MSD	Minimum Significance Differences

BIOGRAPHICAL SKETCH

The author of this thesis, Mr. Birhanu Babiye was born from his father Mr. Babiye Dametiwe and his mother Mrs. Zertehun Mekonen in 1992 G.C at 02 Kebele, Debatie Worda, Metekele Zone, and Western Ethiopia. When his age reached to education, he pursued his elementary school at Debatie elementary school, Debatie. After completing his primary school, he attended his secondary school at Debatie Secondary and Preparatory School at Debatie. Similarly, after completing his secondary school, he attended his preparatory school at the same town where he attended his secondary school at Debatie secondary and preparatory school. After completing his preparatory School education, he joined Aksum University in December 2012 G.C and graduated in July 2014 G.C with a Bachelor of Science degree in Biotechnology. After his graduation, he was employed at Ethiopian Institute of Agricultural research at National Agricultural Biotechnology research center and he served as a Biotechnology Researcher from February 2015 G.C to September 2018 G.C. Then in December 2018, G.C, he joined Jimma University College of Agriculture and Veterinary medicine to pursue studies leading to a degree in Master of science in Plant Biotechnology.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENIDCES

Detection and Identification of Plant Growth Promoting Bacteria from Sorghum (*Sorghum bicolor* L. Moench) Rhizosphere Soil in Northern Ethiopia

Abstract

Plant growth promoting rhizobacteria are the bacteria which subsist inside and outside of the plant tissue and promote plant growth through direct or indirect mechanisms. To increase sorghum production and productivity we utilize herbicides and chemical fertilizers to overcome sorghum production constraints, but those chemicals have negative side effects. The current study was conducted with the objective of isolation of PGPR from sorghum rhizosphere and screening for primary growth related trait, evaluation of potential PGPR at greenhouse for sorghum growth performance and identify through biochemical characterization. So that, in this study a total of 117 plant growth promoting rhizobacteria were isolated from the rhizosphere of 12 sorghum (Sorghum bicolor L. Moench) genotype by cultivating using 3 collected soil samples from the northern part of Ethiopia (Amhara and Tigray regional states) in greenhouse. Isolated bacteria were screened for primary growth promoting traits such as phosphate solubilization test, IAA production test at different concentration of L-tryptophan and ammonia production test. From the isolated bacteria 28% solubilized Phosphorous, 78% produced IAA at different concentration of tryptophan. The greatest IAA production was scored at 100 mg/L of tryptophan and the lowest production of IAA was scored at 150 mg/L of tryptophan, 69% of isolated bacteria produced ammonia. Hence, 15% of isolated bacteria fulfilled the above primary screening test and used for further greenhouse evaluation. Accordingly, eighteen bacteria were tested for greenhouse experiment using completely randomized design and all 18 isolates were significantly increased all the agronomic parameter as compared to the control such as plant shoot height, plant shoot fresh and dry weight, root length, root fresh and dry weight at $p < 0.01$ and $P \leq 0.001$. Two isolates G6E29 and G4E19 had significantly increased all the parameter but two isolates (G12E19 and G3E40) were statistically non-significant for root fresh weight compared to the control. These 18 potential isolates were characterized morphologically and biochemically. Eight isolates were grouped at Pseudomonas genera such as G43E29, G5E29, G6E29, G4E19, G6E19, G8E19, G9E19, and G10E19. Six isolates were grouped at Azotobacter such as, G8E29, G11E29, G12E29, G2E19, G3E19, and G3E40 and the rest four isolates G5E19, G12E19, G4E40, and G6E40 were grouped at Bacillus genera.Thus, the use of plant growth promoting rhizosphere bacteria could be useful to improve sorghum production and productivity. However, further molecular identification and evaluation of the isolates exhibiting multiple plant growths promoting traits on plant-microbe interaction for economic crop of Ethiopia is needed to uncover their efficacy as effective plant growth promoting rhizosphere bacteria.

Key word: - Plant Microbiome, Metabolites, Metagenomics and Phytohormones

1. INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) belongs to the family Poaceae (Gramineae) with a global ranking of fifth most important staple cereal food crop after wheat, rice, maize and barley. It is also a staple food for more than 500 million people in more than 30 countries of semi-arid area of the world (Idris *et al.*, 2009; FAOSTAT, 2011; Gottumukkala *et al*., 2016).

Sorghum domestication started at the north east quadrant of Africa, specifically in Ethiopian western part usually known as Ethio- Sudanese border region due to its unique adaptation to harsh and drought-prone environments. The total sorghum production in sorghum producing areas of the world is 55.6 million tons, and world average yield was 1.37 tons per ha in 2010. Sorghum is the second staple food next to maize for sub Saharan countries, were 18 million tons is produced annually from 27 million ha (Haussmann *et al*., 2000a; FAOSTAT, 2011; Gottumukkala *et al*., 2016).

FAO (2012) reported the United States of America is the top sorghum producer with about 9.7 million tons, followed by India, Nigeria, Sudan, and Ethiopia. In Ethiopia, sorghum is the fourth staple food crop both in area coverage and production after teff, maize and wheat. The crop is grown in almost all regions with estimated total land area of 1.8 million hectares (CSA, 2018).

The major sorghum producing regions of Ethiopia are Oromia, Amhara, Tigray, and southern nation, nationality and peoples. Compared to other African countries, Ethiopian sorghum productivity is very low with an average productivity of 2.7 tons per ha. This low productivity needs sorghum improvement to increase productivity to achieve food security (Geremew *et al*., 2004; Gottumukkala *et al*., 2016; CSA, 2018). Gebretsadik *et al.* (2014) and Hussein *et al.* (2016) described that both abiotic and biotic factors; such as drought, low soil fertility, insects, quelea bird and *Striga* weed are the major production constraints affecting sorghum productivity.

In Ethiopia, the most known biotic production constraint is Striga (*Striga hermonthica*) affecting by its association with the root of sorghum causing annual losses of up to 7 billion USD, which is considered to affect the livelihood of 300 million people due to a decrease in sorghum production and productivity (Atera and Itoh, 2011).

To increase sorghum growth and grain yield by decreasing the impact of striga on sorghum, farmers and researchers have been using herbicides and chemical fertilizers, but these chemicals, in addition to their positive effect in promoting plant growth and increasing sorghum grain yield, have negative side effects in that they pollute the environment and decrease soil microbial diversity by killing them through increasing soil pH (Hayat *et al*., 2010; Ahemad and Kibret, 2014; Souza *et al*., 2015).

In addition to utilization of herbicide and chemical fertilizer in an effort to reduce the impact of striga on sorghum productivity, several researches have been conducted with the goal of developing steriga tolerant varieties using conventional breeding practice. Despite these efforts, the problem still exist. The new approach to solve steriga constraint on sorghum production, these days, is on the interaction of steriga weed, sorghum and soil microbes (Atera and Itoh, 2011).

Beneficial bacteria which inhabit the soil rhizosphere of plant can manage soil environment to achieve attainable crop yield. Bacteria use exudates that are secreted by plant roots within the rhizosphere. They influence plant in a direct or indirect mechanism. Stimulation of plant growth is considered to be one of the influences on plants by soil bacteria. Rhizosphere bacteria that influence plant growth positively are referred to as plant growth promoting rhizobacteria, due to their effect on crop yield increase (Bloemberg and Lugtenberg, 2001; Cook, 2002).

There are a lot of factors that affect plant growth promoting rhizosphere bacteria; such as environmental condition, plant genotype, soil type, soil and filed condition and green house condition. The prominent factors that affect PGPR's function to promote plant growth are plant genotype and soil type. Genotype of plant secrete root exudates compound that differs among plant genotypes and the function of exudates compound also differs from soil to soil type and condition (Andreote *et al*., 2010; Glick, 2012; Vejan *et al*., 2016).

Plant growth promoting rhizobacteria can be helpful to plants either by increasing the availability of both macro and micro elements; such as nitrogen, phosphorus, iron and zinc in the rhizosphere producing plant growth promoting (PGP) substances; such as indole acetic acid and siderophore production (Cakmakci *et al*., 2006; Vivas *et al*., 2006; Hamdal *et al*., 2008 and Mayak *et al*., 2010).

Currently, there is an increasing interest on understanding the natural relationship between sorghum with PGPRs to develop growth promoting rhizobacteria as inoculants to supplement chemical fertilizers. In Ethiopia, there has been an attempt by Idris *et al.* (2009) and Tsegaye *et al*. (2019) regarding on the utilization of rhizosphere bacteria for promoting sorghum and teff growth as biofertilizer inoculants.

The growing interest in the use of plant growth promoting bacteria as inoculants for sorghum growth promoting was limited in Ethiopia, and had a little scientific justification and very limited studies on the potential role of PGPRs as plant growth promoting agents, which PGPRs are effectively associated with specific sorghum genotype are not studied well. Having those gaps about plant growth promoting rhizobacteria in Ethiopia, the current study; therefore, focused on the following objectives.

1.1. Objective of the Study

1.1.1. General Objective

- The general objective of this study was to isolate and identify plant growth promoting bacteria from sorghum (*Sorghum bicolor* L. Moench) rhizosphere soil in northern Ethiopia.

1.1.2. Specific Objectives

This study specifically attempted:

- To isolate PGPR bacteria from sorghum rhizosphere soil, and screen for growth prompting trait.
- To determine the effect of selected bacterial isolates on sorghum growth performance in Greenhouse.
- To identify effective growth promoting bacteria through biochemical characterization.

2. LITERATURE REVIEW

2.1. Global and Local Importance of Sorghum

Sorghum (*S.bicolor*) is a grain crop which originated in East Africa in the region along Ethio-Sudanese (Dahlberg *et al*., 2003). Globally sorghum is the fifth most important staple food crop after wheat, rice, maize and barley. It is commonly used for food, and stalks for fodder and building materials. In developed countries, sorghum is used primarily as animal food, and in the sugar, syrup, and molasses industry (Dahlberg *et al*., 2003; FAO, 2012).

The sub-Saharan Africa such as Nigeria, Sudan, Ethiopia and Burkina Faso produce about 18 million tons of sorghum annually making it the second important cereal crop after Maize (*Zea mays* L.). Nigeria is the leading Sorghum producer in Africa followed by Sudan, Ethiopia, and Burkina Faso. However, in terms of productivity, Egypt achieves the highest yields followed by Algeria, South Africa, Uganda, and Ethiopia (Haussmann *et al*., 2000a; FAOSTAT, 2006).

Sorghum is the fourth primary staple food crop in Ethiopia both in area coverage and production after teff, maize, and wheat. Cereals comprises 78.23% (8.8 million ha) of the field crops of which sorghum accounts for 14.41%. Sorghum is grown in almost all regions of the country. It occupies an estimated total land area of 1.8 million ha, and the major sorghum production regions of the country are Oromia at 38.5%, Amhara (32.9%), Tigray (14.1%), and SNNP region that accounts 7.6% (CSA, 2018).

Sorghum productivity in Ethiopia is low when compared to other African countries, and Ethiopians' average productivity is 2.7 tons ha^{-1}, ranking it fifth in Africa. The global average yield of sorghum stands 2^{nd} at 2.7 ton ha so the low national sorghum yield needs the necessity of sorghum improvement to enhance productivity and achieve food security (Geremew *et al*., 2004; FAOSTAT, 2006; CSA, 2018).

2.2. Constraints of Sorghum Production

In Ethiopia, the livelihoods of millions of farmers depend on sorghum production. However, its productivity is low (around 2.7 tons per ha) due to a number of abiotic and biotic factors. Among the abiotic factors are low soil fertility (nutrient deficiency) and drought, whereas biotic constraints include the parasitic weed- *striga* (*striga* species), foliar and panicle diseases, stem borers, and shoot fly (Geremew *et al*., 2004; CSA, 2018).

Among the major sorghum diseases anthracnose, smuts and rusts account for substantial yield losses in the country. Sorghum production constraints vary from region to region within Ethiopia. However, drought and *Striga* are the most important problems across regions (Sebnie and Mengesha, 2018). Consequently, the present research focuses on identifying sorghum growth promoting rhizosphere bacteria to enhance sorghum productivity in Ethiopia.

2.3. Striga: The Parasitic Weed of Sorghum

Plants belonging to genus *Striga* (Scrophulariaceae) comprise obligate root parasites of cereal crops that inhibit normal host growth by three processes. These are competition for nutrients, impairment of photosynthesis and a phytotoxic effect within days of attachment to its hosts (Joel, 2000; Gurney *et al*., 2006).

Striga are generally native to semi-arid tropical areas of Africa, but have been recorded in more than 40 countries. It is possibly originated from a region between the Semien Mountains of Ethiopia and the Nubian Hills of Sudan as this region is also the origin of domesticated sorghum (Ejeta, 2007; Atera and Itoh, 2011).

Approximately 30 *Striga* species have been described and most parasitize grass species (Poaceae). *Striga gesnerioides* (Willd) Vatke is the only Striga specie that is virulent to dicots, and among the 23 species of striga prevalent in Africa, *Striga hermonthica* is the most socio-economically important weed in eastern Africa. *Striga hermonthica* is particularly harmful to sorghum, maize and millet, but increasingly being found in sugarcane and rice fields (Mohamed and Musselman, 2008; Atera and Itoh, 2011).

2.4. Rhizosphere

Rhizosphere is the microbial storehouse and is defined as the narrow zone of soil approximately 7mm from the root and directly surrounding the root system where the biological and chemical features of the soil are influenced by the roots (Walker *et al*., 2003; Kundan *et al*., 2015).

The root system in the rhizosphere serve for anchorage and uptake of water and nutrients from the soil as well as used as chemical factory because phenolic compounds are synthesized and released to numerous underground interactions. The compounds released by plant roots act as

chemical attractants for a huge number of heterogeneous microbial communities. The composition of these compounds depends upon the physiological status and species of plants and microorganisms (Kang *et al*., 2010).

Rhizosphere has three different component functions in soil fertility and plant growth support. These are rhizosphere soil - the soil zone that regulates by roots through release of root exudates substrates from the plant root for attraction of microbial activity; Rhizoplane - the root surface that strongly binds with soil particles and root - the plant root that is colonized by microorganisms specially growth promoting bacteria (Barea *et al*., 2005).

2.5. Plant Growth Promoting Rhizobacteria

Rhizobacteria is a group of symbiotic or non symbiotic rhizosphere bacteria that can compete to colonize the root environment which either their mode of action is directly beneficial to the plant or not. In the other way, rhizobacteria are called plant growth promoting rhizobacteria (Ahmad *et al*., 2008; Kundan *et al*., 2015; Parede *et al*., 2016). Hence, the term "plant growth promoting bacteria" refers to bacteria that have potential and function to enhance plant growth by making avail various growth promoting macro and micro elements to facilitate plant growth parameters (Ahmad *et al*., 2008).

2.6. Forms of Plant Growth Promoting Rhizobacteria

Based on the interactions with plants PGPR are grouped in to extracellular or intercellular plant growth promoting rhizobacteria. Extracellular or intercellular plant growth promoting rhizobacteria live outside the plant cell and inhabit the rhizosphere or the spaces between the cells of the root cortex (Viveros *et al*., 2010; Garcia *et al*., 2015). Examples of such bacteria are *Azotobacter, Serratia, Azospirillum, Bacillus, Caulobacter, Chromobacterium, Agrobacterium, Erwinia, Flavobacterium, Arthrobacter, Micrococcous, Pseudomonas, and Burkholderia*. Intracellular plant growth promoting rhizobacteria (iPGPR) are symbiotic or endophytic bacteria that inhabit inside the specialized nodular structures of root cells. *Allorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium and Frankia* are some examples of such group (Viveros *et al*., 2010; Bhattacharyya and Jha, 2012).

2.7. Mechanism of PGPR to Promote Plant Growth

Garcia *et al.* (2015) described that plant growth promoting bacteria enhance plant growth using two mechanisms, direct and indirect mechanisms. Specific bacterial genera are involved in enhancing plant physiological growth and resistance to different phytopathogens through various modes of actions. However, the mode of action by different PGPR vary depending on the type of host plant but can be influenced by a number of biotic factors such as plant genotypes, plant developmental stages, plant defense mechanisms, other members of the microbial community, and abiotic factors such as soil composition, soil management and climatic conditions (Garcia *et al*., 2015; Gupta *et al*., 2015).

2.7.1. Direct mechanism

In the case of direct mechanism, PGPR directly facilitate the growth and development of plants through direct contact to the plant for providing nutrient uptake or increases nutrient availability through nitrogen fixation, mineralization of organic compounds, and solubilization of mineral nutrients. The production of phytohormones and their effect on plants vary on the microbial strain and the plant species (Bhardwaj *et al*., 2014; Garcia *et al*., 2015). For example, Mishra *et al*. (2017) reported that the Phytohormone, IAA, produced by *Bacillus* and *Pseudomonas* spp affected plant cell function and division of *Zea mays* which can help the crop for stress tolerance; Indris *et al.* (2009) reported that phosphorus solubilizing by *Pseudomonas* spp increase sorghum growth and development as a biofertilizer; Ahmad *et al*. (2008) reported that increase the yield of rice through the use of potassium solubilize *Azotobacter* Spp are specific attempt.

PGPR directly affect plant metabolism by providing nutrients that are usually scarce in the rhizosphere, such as nitrogen, phosphorus and potassium which are important nutrients provided to plants. Inoculation of wheat with *Pseudomonas* sp. or *Bacillus* sp. resulted in significant increases in potassium, calcium, and magnesium uptake in a calcareous soil without fertilization (Öğüt and Neumann, 2011; Figueiredo *et al*., 2019).

2.7.1.1. Facilitating resource acquisition

This is one way that bacteria promote plant growth by using their direct mechanism. It is the best studied mechanisms of bacteria that are used for providing plants with nutrients that they lack such as fixed nitrogen, iron, and phosphorus. Because most of agricultural soils lack a

sufficient amount of one or more of these compounds. Now a day, agriculture increasingly dependent on chemical sources of nitrogen and phosphorus. However, besides the cost of these chemicals human and environmental hazards posed are tremendous. Thus, the best option now a days is the use of bacteria that have the potential to substitute nitrogen and phosphorus (Glick, 2012; Garcia *et al*., 2015). Hayat *et al.* (2010) *and* Nihorimbere *et al.* (2011) reported the genera of bacteria involved in facilitating resource acquisitions are *Rhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium, Sinorhizobium, Mesorhizobium, Azospirillum, Enterobacter, Klebsiella and Pseudomonas* the most important PGPR.

2.7.1.2. Modulating Phytohormone levels

For the survival of a plant in both biotic and abiotic stress factors, plants responded to their environment and for their growth and development, plant hormones play key roles. Plants are able to adjust their metabolism and the levels of their endogenous Phytohormone in order to decrease the negative effects of environmental condition to overcome the effects of growth limiting factor, so rhizobacteria have also produce or modulate Phytohormone under *in vitro* conditions and many PGPB can alter Phytohormone levels and affect plant hormonal balance and its response to stress (Davies and Bockus, 2001; Glick *et al*., 2007). Some example of the previous work, Khan *et al.* (2017) state regulation of IAA production for *Vinca rosea* stress, Park *et al.* (2017) regulation of GA and ABA *Glycine max* (L.) Merr for Heat stress and Shahzad *et al.* (2017) reported that regulation of ABA in *Oryza sativa* L for Salt stress are the latest work on modulating Phytohormone levels.

2.7.2. Indirect Mechanisms

An indirect mechanism is a mechanism in which the soil bacteria involve in prevention or neutralizing effects of phytopathogens on plants by producing repressive substances that increase natural resistance of the host. It helps plants grow actively under environmental stress or protect plants from infections. The contributions of PGPR in indirect mechanism are production of hydrolytic enzymes (chitinases, cellulases, proteases, etc.), various antibiotics in response to plant pathogen, induction of systematic resistance against various pathogen and pests, and production of siderophore (Akhgar *et al*., 2014; Gupta *et al*., 2014; Nivya, 2015). For example, Frankowski *et al.* (2001) reported that Chitinase produced by *S. plymuthica* C_{48} inhibited spore germination and germ-tube elongation in *Botrytis cinerea* and used as indirect mechanism, Singh *et al.* (2017) reported that β-1, 3-glucanase synthesized by *Paenibacillus*

spp. strain 300 and *Streptomyces* Spp. strain 385 lyse fungal cell walls of *F. oxysporum* f. Spp, *cucumerinum* are example of the indirect mechanisms.

2.8. Application PGPR Trait in Crop Improvement

Most of the soil deplete with a number of microscopic lives such as bacteria, fungi, actinomycetes, protozoa, and algae. Bacteria are the most common (95%) and the soil hosts a large number of bacteria around 10^8 to 10^9 cells per gram of soil but only about 1% bacterial cells in soil are generally culturable. Biotechnological tools have a great role to identify such uncultivable bacteria present in the soil and the number and type of bacteria that are found in different soils are affected by the soil conditions such as temperature, moisture, salt, chemicals, number and types of plants found in those respective soils (Schoenborn *et al*., 2004).

As described by Glick (2012), bacteria affects crops in three ways based on the interaction between soil bacteria and plants beneficial (bacteria that have a positive effect on plant), harmful (bacteria that have a negative effect on plant) or neutral (bacteria that have nether positive or negative effect on plant). Following are the detail description of the impact of both direct and indirect mechanisms to improve crop productivity.

2.8.1. Nutrient Fixation

Nutrient fixation is among the effect of plant growth promoting soil Bacteria that increases the accessibility and concentration of nutrient by fixing their supply for plant growth and productivity. The most fixing nutrient by PGPB are nitrogen and used by plant in the form of nitrate (NO^{3-}), ammonium (NH^{4+}), solubilize phosphate, a siderophore production, IAA production for different plant species (Kumar, 2016; Paredes and Lebeis, 2016). Rilling *et al.* (2018) reported that Proteobacteria (*Bosea* and *Roseomonas*), Actinobacteria (*Georgenia, Mycobacterium, Microbacterium, Leifsonia*, and *Arthrobacter*), Bacteroidetes (*Chitinophaga*) and Firmicutes (*Bacillus* and *Psychrobacillus*) taxa are involved in nitrogen fixation, Suleman *et al. (*2018) reported that *Pseudomonas* and *Bacillus* strains have shown an increase in yield of wheat and other crops due to phosphorus solubilization and organic acid production, Sharma *et al.* (2003) reported that Mung bean plants, inoculated with the siderophore-producing *Pseudomonas* strain GRP3 and grown under iron-limiting conditions, showed reduced chlorotic symptoms and an enhanced chlorophyll level compared to uninoculated plants. Fageria (2014) reported that plant growth promoting microbes enhances beneficial nutrients approximately by 40%–70% N, 80%–90% P, and 50%–70% K per utilized area to promote plant growth.

2.8.2. Nitrogen Fixation

Nitrogen is the most vital nutrient for plant growth and productivity. Although 78% of nitrogen presents in the atmosphere, it remains a limiting factor in agriculture since nitrogen remains unavailable to the plants (Gaby and Buckley, 2012). Biological nitrogen fixation is a direct mechanism effect of PGPR process that accounts approximately two-thirds of global nitrogen fixation and fixation process that are carried out either by symbiotic or non-symbiotic interaction with plants (Ahemad and Kibret, 2014). For example, Rilling *et al.* (2018) and Islam *et al.* (2013) reported that Proteobacteria (*Bosea* and *Roseomonas*), Actinobacteria (*Georgenia, Mycobacterium, Microbacterium, Leifsonia*, and *Arthrobacter*), Bacteroidetes (*Chitinophaga*) and Firmicutes (*Bacillus* and *Psychrobacillus*) taxa are involved in nitrogen fixation.

FAO (2012) report indicated that countries which used nitrogen fertilizer with their amount. Europe (western and eastern Europe) use 54 isolates, America (North and Latin) use 11 isolates, Africa use 3 isolates, Asia use 5 isolates and Japan used 13 isolates, which shows the research output in the world about PGPR as Nitrogen fixation. Symbiotic PGPR bacteria can enter plant root and form nodule which have the potential to fix atmospheric N_2. These are rhizobacterial strain *Rhizobium sp*, *Azoarcus Spp*, *Beijerinckia Spp*, *Pantoea agglomerans*, and *K. pneumonia* to improve soil quality and enhance nodule formation of plant. N_2 fixation process are regulated and carried out by specific gene called *nif* gene (Ahemad and Kibret, 2014; Damam *et al*., 2016).

2.8. 3. Phosphate Solubilization

Phosphorus is the second most essential nutrient next to nitrogen required by plants with adequate amount for optimum plant growth. It plays an important function in all major metabolic processes such as energy transfer, signal transduction, respiration, macromolecular biosynthesis, and photosynthesis. Approximately 95–99% of phosphorus present in the form of insoluble, immobilized, or precipitated, difficult for plants to absorb (Torri *et al*., 2017).

Plants absorb phosphate only as monobasic (H_2PO^{4-}) and dibasic (HPO^{4-2}) ions. Hence, soil bacteria are used for solubilization and mineralization of phosphorus using important trait of phosphate solubilizing bacteria that can be done by potential phosphate solubilizing PGPR are

in the genera *Arthrobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Microbacterium Pseudomonas, Erwinia, Rhizobium, Mesorhizobium, Flavobacterium, Rhodococcus,* and *Serratia* that have attracted the attention of agriculturists as soil inoculants to improve plant growth and yield (Sherathia *et al*., 2016).

2.8. 4. Potassium solubilization

Potassium is the third major essential macronutrient used for plant growth. Concentrations of soluble potassium in the soil are very low because of more than 90% of potassium in the soil exists in the form of insoluble rock and silicate minerals. Without adequate potassium, the plants will have poorly developed roots, slow growth, produce small seeds and have lower yield. Plant growth promoting rhizobacteria are able to solubilize potassium rock through production and secretion of organic acids that have the potential to solubilize potassium. The most known potassium solubilizing rhizobacteria are *Acidothiobacillus ferrooxidans, Bacillus edaphicus, Bacillus mucilaginosus, Burkholderia*, *Paenibacillus* and *Pseudomonas* (Liu *et al*., 2012; Parmar and Sindhu, 2013).

Maurya *et al. (*2014) and Meena *et al*. (2015$_b$) reported that potassium solubilization can bear potassium minerals and release K for improving the growth and yield of plant. It is generally believed that microorganisms contribute to the release of K^+ from K bearing minerals by several mechanisms. Released H^+ can directly dissolve the mineral K as a result of slow releases of exchangeable K, readily available and exchangeable K. As occurs in the case of P solubilization, the major mechanism of K mineral solubilization is by production of the organic and inorganic acids and production of protons (acidolysis mechanism).

The types of various organic acids such as oxalic acid, tartaric acids, gluconic acid, 2-ketogluconic acid, citric acid, malic acid, succinic acid, lactic acid, propionic acid, glycolic acid, malonic acid, fumaric acid, etc. have been reported in KSB, which are effective in releasing K from K-bearing minerals (Keshavarz *et al*., 2013; Prajapati *et al*., 2013; Saiyad *et al.*, 2015) .

2.8. 5. Siderophore Production

As described by Cornelis (2010), Siderophores are used for plants in both direct and indirect enhancement mechanisms of plant growth by PGPR. Iron is an essential micronutrient for all organisms that can live in the biosphere based on the fact that iron is the fourth most abundant element on earth but in aerobic soil iron is not readily assimilated by either bacteria or plants because ferric ion (Fe^{+3}) is only sparingly soluble so that the amount of iron available for

assimilation by living organisms is extremely low. Microorganisms have evolved specialized mechanisms for the assimilation of iron by producing low molecular weight iron-chelating compounds known as siderophore, plant growth promoting rhizobacteria including *Aeromonas*, *Azadirachta*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhizobium*, *Serratia* and *Streptomyces* sp (Arora *et al*., 2013; Sujatha and Ammani, 2013).

2.8.6. Phytohormone Production

A wide range of microorganisms found in the rhizosphere are able to produce Phytohormone substances that can regulate plant growth and development. Plant growth promoting rhizobacteria produce Phytohormone such as auxins, cytokinins, gibberellins, ethylene and Indole Acetic Acid (IAA) can affect cell proliferation in the root architecture by overproduction of lateral roots and root hairs with a subsequent increase of nutrient and water uptake (Kang *et al*., 2010; Miransari and Smith, 2014). The type of hormones produced by PGPR are listed along with their functions in table 1 below.

Table 1. Phytohormones producing bacteria and their function on different host plant

Phytohormone	Producing bacteria	Function	Host plant	stress	Reference
IAA	*Pseudomonas sp., Bacillus sp.*	cell division, elongation, and differentiation	*Sulla carnosa (Desf)*	Salt stress	Hidri *et al*., 2016
IAA	*Serratia sp.*	cell division, elongation, and differentiation	*Cicer arietinum L*	Nutrient stress	Zaheer *et al*., 2016
IAA	*Bacillus subtilis Pseudomonas sp.*	cell division, elongation, and differentiation	*Zea mays,*	Salt and heat stresses	Mishra *et al*., 2017
GA	*Azospirillum lipoferum*	seed dormancy, floral and lateral shoot growth	*Triticum aestivum L.,*	Drought stress	Creus *et al*., 2004
GA	*Phoma glomerata, Penicillium sp.*	seed dormancy, floral and lateral shoot growth	*Cucumis sativus*	Drought stress	Waqas *et al*., 2012
GA	*Aspergillus fumigates*	seed dormancy, floral and lateral shoot growth	*Glycine max (L.) Merr*	Salt stress	Khan *et al*., 2011
ABA	*Bacillus amyloliquefaciens*	stress responses and adaptation	*Oryza sativa L*	Salt stress	Shahzad *et al*., 2017
ABA	*Bacillus licheniformis, Pseudomonas fluorescens*	stress responses and adaptation	*Vitis vinifera L.,*	Water stress	Salomon *et al*., 2014
ABA	*Bacillus aryabhattai*	stress responses and adaptation	*Glycine max (L.) Merr.,*	Heat stress	Park *et a*l., 2017
CK	*Micrococcus luteus*	cellular proliferation and differentiation, prevention of senescence	*Zea mays*	Drought stress	Raza and Faisal, 2013

Were, IAA = indole acetic acid, GA = Gaberillic acid, ABA = Abscisic acid and CK = cytokinin

2.8.7. Exopolysaccharide Production (EPSs)

Exopolysaccharides (EPSs) are high molecular weight, biodegradable polymer that is produced biologically from monosaccharide residues and their derivatives by diverse type of bacteria, algae, and plants. EPSs used for traits that are directly responsible for plant growth and development by maintaining water potential, aggregating soil particles, ensuring obligate contact between plant roots and rhizobacteria. *Rhizobium leguminosarum*, *Azotobacter vinelandii*, *Bacillus drentensis*, *Enterobacter cloacae*, *Agrobacterium sp*., *Xanthomonas sp*., and *Rhizobium* are good examples (Mahmood *et al*., 2016; Pawar *et al*., 2017).

2.8.8. Production of Protective Enzymes

Enzyme production is the indirect mechanism that uses PGPR bacteria to promote plant growth by producing effective enzyme that control phytopathogenic agents that attack plant. The most enzyme that PGPR bacteria produce and capable of lysing cell walls and neutralizing pathogens are β-1,3-glucanase, ACC-deaminase, and chitinase. Bacteria such as *Pseudomonas fluorescens* and *Paenibacillus sp and Streptomyces sp* can produce the enzyme β-1,3-glucanase- and chitinase to control fungal growth and promote plant growth (Goswami *et al*., 2016; Meena *et al*., 2016; Ramadan *et al*, 2016).

2.8.9. Disease Resistance Antibiosis

As described by Ulloa *et al.* (2015), disease resistance antibiosis is the utilization of microbial antagonists against plant pathogens by producing antibiotic and control pathogen for agricultural crops and currently they can substitute chemical pesticides. PGPR, like *Bacillus spp*. and *Pseudomonas sp*., play a major role inhibiting pathogenic microorganisms by producing antibiotics. For example, Olanrewaju *et al.* (2017) reported as the production of antibiotics such as Tas A, sublancin, subtilosin, bacilysin, chlorotetain, subtilin, bacillaene, surfactin, iturin, fengycin, Ecomycins, 2,4-Diacetyl Phloroglucinol (DAPG), Pseudomonic acid, Phenazine-1-carboxylic acid (PCA), Pyoluteorin, Pyrrolnitrin, OomycinA, Cepaciamide A, Viscosinamide, Butyrolactones, Zwittermycin A, Aerugine, Azomycin, Rhamnolipids, Cepafungins, Kanosamine, and Karalicin derived from *Bacillus* and *Pseudomonas* genera and used against several plant pathogens has become one of the most effective and most studied bio-control mechanisms.

2.8.10. Induced Systemic Resistance (ISR)

Induced resistance are defined as a plant physiological state that enhance defensive capacity in response to specific environmental stimuli most of for biotic challenges. Biopriming plants with some plant growth promoting rhizobacteria can also provide systemic resistance against a broad spectrum of plant pathogens such as fungal diseases, bacterial diseases and viral diseases. In some instances even damage caused by insects and nematodes can be reduced after application of plant growth promoting rhizobacteria (Doornbos *et al*., 2012; Glick, 2012; Naznin *et al*., 2012).

2.8.11. Stress Management

Stress includes any kind of factors that have a negative effect on plant growth and development in different condition and increases the formation of Reactive Oxygen Species (ROS) like (H_2O_2, $O2^-$, and OH^-). ROS production causes oxidative stress that damages plants by oxidizing photosynthetic pigments, membrane lipids, proteins, and nucleic acids. Plants are frequently subjected to various environmental stresses such as both biotic and abiotic type stress (Table 1). Plants have the nature to develop specific response mechanisms for type of stress and PGPR bacteria help the plant to give the response to stress (Ramegowda and Senthil, 2015; Foyer *et al*., 2016).

2.8. 11.1. Abiotic Stress Tolerance

Abiotic stress can happen suddenly without the intervention of mankind that cannot be controlled at a time. Abiotic stress that occurs by drought, salinity, and high temperature is the most dominant stress limiting both plant growth and productivity so that PGPR bacteria have their contribution to remove the effect of abiotic stress on plant. The use of PGPR effect in plant abiotic stress management is by means of neutralizing the toxic effect and improved leaf water status, particularly under salinity and other abiotic stress conditions through bacterial strains such as *Pseudomonas putida* and *Pseudomonas fluorescens* (Naveed *et al*., 2014; Vejan *et al*., 2016).

2.8. 11.2. Biotic Stress Tolerance

Biotic stress is caused by different kind of living pathogens such as bacteria, viruses, fungi, nematodes, protists, insects, and viroids those have a results in a significant reduction in agricultural yield, but these problems can be solved by using PGPR bacteria like *Paenibacillus polymyxa strains*, *Bacillus amyloliquefaciens strain*, *B. licheniformis strain*, *B. thuringiensis*

strain, *P. favisporus strain*, and *B. subtilis strain* (Gusain *et al*., 2015; Haggag *et al*., 2015; Ngumbi and Kloepper, 2016).

2.9. Biotechnology to Access the Function of Unknown Microbes

The earth biosphere is dominated by microorganisms that have various function and contains about $4 - 6 \times 10^{30}$ prokaryotic cells. Thus, microorganisms are highly diverse group of organisms and constitute about 60% of the Earth's biomass (Singh *et al*, 2009; Ghazanfar *et al*., 2010).

Microorganisms have the key function in ecological processes such as soil structure formation, promoting plant growth, decomposition of organic matter and xenobiotic, and recycling of essential elements as mentioned in the above such as carbon, nitrogen, phosphorous, and sulfur and nutrients (Ghazanfar *et al*., 2010). Thus, microbes play a critical role in modulating global biogeochemical cycles and influence all lives on Earth and all organisms in the biosphere either directly or indirectly depending on microbial activities (Singh *et al*, 2009; Ghazanfar *et al*., 2010). However, due to their general unculturability it is believed that only a small percentage of bacteria in nature can be cultured and in the present era of biotechnology new culture independent technology, metagenomic is the culture independent analysis of a mixture of microbial genomes (Schloss *et al*., 2003; Riesenfeld *et al* ., 2004).

Metagenomic is the application of modern genomics techniques to study communities of microbial organisms by isolation of DNA directly in their natural environments by passing the need for isolation and laboratory cultivation of individual species of bacteria (Riesenfeld *et al* ., 2004. Metagenomic can answering questions commonly asked in microbiology such as 'Which species inhabit a given environment?' and 'What are these microbes doing and how are they doing it?' (Beja *et al*., 2000).

3. MATERIALS AND METHODS

3.1. Soil Sampling for Isolation of Growth Promoting Bacteria

A total of 46 soil samples were collected randomly from the northern part of Ethiopia (Tigray and Amhara regions) in which sorghum is frequently cultivated for daily consumption of people which inhabited in the area. Lists of areas from which the samples are collected is presented in (Table 2).

Table 2. Soil sampling area with passport data

N0	Code	Date	Region	Zone	Woreda	Kebele	Altitude	Longitude	Latitude
1	ES19	18/02/2011	Amhara	North shoa	Bekewot	Abayatir	1373	09.55.11.0	040.01.42.3
2	ES19	18/02/2011	Amhara	North shoa	Bekewot	Abayatir	1371	09.55.09.51	040.01.41.9
3	ES19	18/02/2011	Amhara	North shoa	Bekewot	Abayatir	1376	09.55.09.9	040.01.42.2
4	ES19	18/02/2011	Amhara	North shoa	Bekewot	Abayatir	1375	09.55.11.5	040.01.42.4
5	ES29	20/02/2011	Tigray	West Tigray	Haftayhumera	Maykedira	635	14.10.26.8	036.36.11.3
6	ES29	20/02/2011	Tigray	West Tigray	Haftayhumera	Maykedira	635	14.10.27.4	036.36.11.3
7	ES29	20/02/2011	Tigray	West Tigray	Haftayhumera	Maykedira	634	14.10.27	036.36.10.1
8	ES29	20/02/2011	Tigray	West Tigray	Haftayhumera	Maykedira	633	14.10.27.1	036.36.10.3
9	ES40	22/01/2011	Amhara	Oromiya	Xumakarsi	Jarakichini	1453	10.30.53.3	039.58.47.7
10	ES40	22/01/2011	Amhara	Oromiya	Xumakarsi	Jarakichini	1457	10.30.54.2	039.58.47.7
11	ES40	22/01/2011	Amhara	Oromiya	Xumakarsi	Jarakichini	1458	10.30.54.4	039.58.47.9
12	ES40	22/01/2011	Amhara	Oromiya	Xumakarsi	Jarakichini	1457	10.30.53.3	039.58.47.2

3.2. Rhizosphere Soil Sampling

Plant growth- promoting rhizosphere bacteria were isolated from 12 sorghum genotypes (Table 3) using 3 soil samples from a total of 46 random soil samples. The selection was based on their PGPR bacterial diversity using metagenomics tool in which both cultivable and uncultivable soil microbes by DNA extraction directly from their environmental sample. All the 12 sorghum genotypes were cultivated in the NABRC greenhouse at Holeta in the three 3 soil samples by adding 700g soil to 800g capacity plastic pot. All sorghum genotypes were grown in 4 replications by sowing two seeds per pot.

Sorghum seeds were first surface sterilized by adding 5% local bleach (sodium hypochlorite) for 30 seconds followed by 1.5% Tween 20. The seeds were then washed by sterilized water five times and germinated on Whatman paper on a plate. Finally, the seedlings were transferred to pots in the greenhouse and allowed to grow for 40 days.

Table 3. Sorghum genotype used to isolate PGPR

Sorghum genotype	Source/Region	Character	Selection Criteria
Degalit	Tigray Region	Local landrace	Landrace and widely used
ETWS 90754	Amhara Region	Wild type	Wild type
ETWS 91242	Beneshangul Region	Wild type	Wild type
Framida	Purdue University	Striga resistance	Striga resistant and widely used
Hora_Doldy2	Landrace	LGS	Landrace and LGS
Jigurti	Landrace	HGS	Landrace, widely used and HGS
Misikir	Drought Score	Drought tolerant	Drought tolerant
S35	ICRISAT	Stay green	Stay green or Drought tolerant
Shanquired	China	Striga susceptible	HGS and model for striga susceptible
SR5-Ribka	IBC	Striga resistant and Fusarium compatibility	Striga resistant and Fusarium compatibility
SRN39	Purdue University	Striga resistance	Striga resistant and widely used
Teshale	ICRISAT	Best released varieties	Widely used

Were, LGS = low germination stimulant, HGS = High germination stimulant and IBC = International Biodiversity Center

3.3. Isolation of PGPR Bacteria

To isolate PGPR bacteria, all cultivated 12 sorghum genotypes were harvested at the same time after 40 days in greenhouse and the roots were cut from the stem using a sterilized surgical blade. Then, all roots were put into falcon tubes which had 35 ml of sterilized 85% saline water. The Falcon tube was shaken on a shaker for 30 minutes to wash the rhizosphere bacteria. Then, the samples were centrifuged at 10,000 rpm for 10 min, and roots were transferred to another falcon tube which contained 35 ml sterilized saline water. After that, the second tube was centrifuged, and the roots were put into another falcon tube. Finally, the two-round pellets were mixed by removing the supernatant. The mixed pellets were used to isolate PGPR bacteria.

One gram (1g) of pellet suspension was taken and transferred to 9 ml of sterilized 85% saline solution. The serial dilution continued up to 1×10^{-8} by taking 1000 µl of diluted sample and was poured to the nutrient agar plate media from the dilution factor of 1×10^{-4}, 1×10^{-5} and 1×10^{-6} by taking 100 µl of diluted sample and by spreading plate method in 3 replications for each.

The plates were then incubated at 28^{O}C for 2 days. Individual bacterial colonies were selected and subculture on nutrient agar seven times for purification. Hence, a total of 117 pure bacterial isolates were obtained by sub culturing.

Then for each isolate, two copies were made; one copy for long term preservation in 40% glycerol at - 80^{o}c and another copy stored in 4^{o}C refrigerators for the active work. All the 117 isolates were tested for primary screening of related trait as followed.

3.4. Detection of Plant Growth Promoting Traits

3.4.1. Phosphate Solubilization Test

Phosphate solubilization activity of plant growth promoting rhizosphere bacterial isolates were detected in plate assay method using Pikovaskaya (PVK) agar following method described in Pikovaskaya (1948). A loop full pure fresh overnight culture isolate was streaked on the Pikovaskaya (PVK) agar media in three replications. PVK agar medium contained: glucose = 10 g; $Ca_3(PO_4)_2$ = 5 g; $(NH_4)SO_4$= 0.5 g; NaCl = 0.2 g; $MgSO_4.7H_2O$ = 0.1 g; KCl = 0.2 g; NaCl = 0.2 g; $MnSO_4.H_2O$ = 0.002 g; $FeSO_4.7H_2O$ = 0.002 g and yeast extract = 0.5 g per liter of a media.

The plates were incubated for 18 days at 28^{o}C after which the isolate that could make a clear hallo zone was selected. Plates without streak of isolates were used as a control. The clear hallo zone of the isolate was measured using a ruler. The isolate differentiation was made using phosphate solubilization index calculated with the following formula.

$$\textbf{Phosphate solubilization index} = \frac{colony\ diameter + clearhallozone\ diameter}{colony\ diameter}$$

3.4.2. IAA Production Test

Isolates that have the potential to solubilize the phosphate were selected and tested for the Production of IAA by using the method described by Thakuria *et al*. (2004) and Sawar and Kremer (1995). With a replication of 3 for each isolate, 100 µl of overnight fresh bacterial cell suspension was added to 20 ml of sterile peptone yeast extract broth (which contained per litter peptone = 10 g; beef extract= 3 g; NaCl= 5 g; L-tryptophan= 50 mg; distilled water= 1L; p^H = 7) in to 50 ml sterilized falcon tubes, and was incubated for 72 h at 28^{o}C in the dark by wrapping with aluminum foil.

After 72 h of incubation, cultured isolates were taken and centrifuged at 10,000 rpm for 10 min, and 10 ml of the supernatant was withdrawn and put in 15 ml test tube, and then added 5 ml of Salkawaski reagent which contained a 1:1 ratio of (50 ml, 35% perchloric acid, and 1 ml per 1.5 M of $FeCl_3$ solution. The culture falcon tubes were incubated at 37^{o}C in the dark for 1h. Formation of red color in the medium was then considered as the ability of IAA production of isolates.

Produced IAA was quantified by measuring their optical density (OD) at absorbance of 530 nm with the standard of produced IAA and the results for each isolates were recorded and repeat the test for positive isolate was conducted at 3 concentrations of tryptophan (25 mg/L; 100 mg/L and 150 mg/L) and the OD was measured at 530 nm and compared at which high concentration IAA was produced.

3.4.3. Test for Ammonia Production

Isolates which had the potential to solubilize phosphorus and able to produce IAA were further tested for Ammonia (NH_3) production following the method described by Cappuccino and Sherman (1992).

Then, 100 µl of pure overnight culture of fresh bacterial cell suspensions were inoculated in 30 ml of peptone broth (4%) in triplication and were incubated at 28^{o}C for 72 hours. After the incubation, 2 ml Nessler's reagent which contained (potassium iodide= 50 gm; saturated mercuric chloride= 35 ml; distilled water= 25 ml; potassium hydroxide (40%) = 400 ml) was added using serological pipette.

The formation of yellow to brown precipitate showed the presence of NH_3. For the control, Nessler's reagent was added to the broth without inoculums. Then, the produced $NH_3$was quantified by reading the OD at 530 nm comparing the potential of isolate with the standard of produced ammonia.

3.5. Evaluation of Bacterial Isolates for Sorghum Growth Promotion

3.5.1. Inoculum Preparation

The isolates which have the potential to pass the screening test were considered for greenhouse evaluation by following the method described by Idris *et al*. (2009). Flasks which have the capacity of 250 ml were selected and filled with 150 ml of nutrient broth and were sterilized with steam sterilization method, and cooled down overnight by putting at the hood. Then, 200 µl of pure overnight suspension culture was added to the broth and incubated at incubator shaker for 72 h by adjusting rpm 150 per minute and temperature 28^{o}c. After 72 h of incubation, the standard concentration was adjusted at 1×10^{-9}

3.5.2. Greenhouse Evaluation

Growth promoting potential of the isolated PGPR bacteria was evaluated with completely randomized design with 3 replications using Teshale sorghum genotype which has low growth or higher Striga susceptible trait. The seeds were surface sterilized by the following procedure, washing the seed by distilled water 3 times and then washing it with 1.5 % of 5 % bleach by adding 2 drops of Tween 20. Finally, the seeds were rinsed five times in sterile water and germinated by soaking them at the plate with Whatman paper and with 3 ml of distilled sterilized water.

Pots with the capacity of 1.5 kg were filled with 1 kg of sterilized soil (steam sterilization for 20 minute) and planted with three germinated seeds, with three replications for one genotype. Therefore, each test isolate pot had 9 plants in a completely randomized design. The bacterial inoculums 100 ml with the standard concentration of 1×10^{-9} were applied after the first and the second leaf appeared and developed.

The temperature of the greenhouse was maintained at 28 ^{o}C and watering was done (500 ml regularly at evening time with 3 days gap). The plants were harvested 5 weeks after the first inoculation. For the control, only distilled water was used instead of the bacterial suspension. The growth-promoting ability of microbial isolates were determined based on the data recorded on plant shoot height, plant shoot dry and fresh weight, and root length, root dry and fresh weight.

Data on plant shoot height and root lengths were recorded by measuring the height and length using ruler. Data on plant soot and root fresh weight of both plant shoot height and root length were recorded by measuring the weight by sensitive electronic balance in the unit of gram. Data for dry weight of shoot and the roots were recorded by made dry the sample using dry heat oven at 65^{o}c for 4 hours and measured the weight using sensitive electronic balance in the unit of gram. The percent (%) of bacterial performance for all agronomic parameters compared to the control was determined using the following formula.

$$\textbf{Increased \%} = \frac{\boldsymbol{Treatment\ value - control\ value}}{\boldsymbol{control\ value}} \times \mathbf{100}$$

3.6. Biochemical and Morphological Characterization

The ability of the isolates in gram staining, sugar utilization with or without gas production, and catalase tests were determined according to the methods described in detail below.

3.6.1. Sugar Utilization Test

The ability of the isolates to utilize carbohydrates and sugars as a carbon source was determined according to the following protocol (Prescott. 2002). One litter basal media was prepared. It contains (10 g peptone broth, 5 g sodium chloride, 1 g beef extract, 7.2 ml phenol red, 10 g each tested carbohydrate (glucose, lactose, and sucrose) and 1 L sterilized water). Then, autoclaved and dispensed to 2 ml basal media to sterilized ELISA plate, and was added 100 µl of pure culture bacterial suspension of tested isolate, and was incubated for 24 h at 28^{O}C. The color changed from purple to yellow was the positive indicator for utilizing the carbon source.

3.6.2. Catalase Reaction Test

Overnight culture of PGPR was thoroughly mixed with 3% H_2O_2 on microscopic slides (Prescott, 2002). The slides were examined for the bubble formation and showed catalase positive but did not form bubble catalase negative.

3.6.3. Gram Staining

The gram staining procedure was carried out according to the method described in Prescott (2002). As briefly described, 100 µl overnight culture of bacterial cell suspension was added to surface sterilized microscopic slide, and it was smeared gently. Then, the slides were inserted into crystal violate and washed by sterilized water. Again, the slides were inserted to iodine solution and washed by sterilized water. Then, the slides were inserted into 97% of ethanol and washed by sterilized water. Finally, the slides were inserted into safranin solution and washed by sterilized water and examined using the 100x objective lens microscopy and purple colored bacteria were gram positive, whereas read colored or colorless bacteria were gram negative.

3.6.4. Morphological characterization

A loop full active cell suspension of the isolates were streaked on nutrient agar media and incubated for 24 hours at 28^{O}c then the colony morphology was recorded.

3.6.5. Classification of Bacterial Genera

Based on the above chemical test the bacterial genera was classified in to different bacterial genera which was based on the characteristics of the bacterial genera which fulfilled the test result.

3.6.6. Statistical Analysis

The significance effect of PGPR isolates on sorghum growth promoting potential were determined by using ANOVA table in a completely randomized design (CRD) based on the factor used. *F* values and means were made by using the Tukey men separation model at *P*=0.01 probability levels and the correlation analysis for agronomic parameters were done.

4. RESULTS AND DISCUSSION

4.1. Isolation of PGPR Bacteria

In the current study a total of 117 PGPR isolates were isolated. Out of the 117; 33(28%) isolates solubilized phosphate, out of the 33; 26(78.78%) isolates produced IAA, out of the 26; 18(69.23%) isolates produced ammonia. From the total of 117; 18(15%) isolates solubilized phosphate, produced IAA and ammonia and selected as a potential PGPR. These might be due to potential of each isolates depending on their individual sources plant genome and taxonomic genera. However, those 18 isolates (Table 4), had different potential in primary growth promoting trait. These might be due to the potential of each isolate depend on their source genotype and environmental condition (Dinesh *et al*., 2015). Ahmad *et al*. (2008) described that, due to nutrient availability, plant rhizosphere has heterogeneous and functional microbes. As indicated in previous research such as rice (Mehnaz., *et al.*, 2001; Thakuria *et al.*, 2004), Wheat (Khalid *et al*., 2004); Sorghum (Indris *et al*., 2009), Mung bean (Anjum *et al*., 2011,); Ginger (Dinesh *et al*., 2015) and Maize (Abedinzadeh *et al*., 2019), plant growth promoting rhizosphere bacteria can promote or increase plant growth, particularly cereal and horticultural tuber crops either through direct or indirect mechanisms.

Eighteen isolates were compared for their potential for phosphate solubilization, IAA production and ammonia production tests and greenhouse evaluation was conducted to check whether they promote sorghum growth or not using Teshale sorghum genotype. It was the most Striga susceptible sorghum variety with low growth rate compared to other sorghum genotypes as described by Andreote *et al*. (2010) and Vejan *et al.* (2016). The purpose of using different sorghum genotypes to isolate PGPR was that most of PGPR are plant genotype and soil environmental condition dependent according to Dinesh *et al.* (2015) who isolated Ginger growth promoting bacteria from different Ginger genotype and those isolates were classified as under different genera and species.

Table 4. Selected eighteen potential isolates with their soil sources, sources genotype along with their trait.

Isolate code	Soil source	Source genotype	Genotype trait
G4E29	Humera	Framida	Striga resistance
G5E29	Humera	Hora - Doldy2	LGS and Landrace
G6E29	Humera	Jigurti	HGS and Landrace
G8E29	Humera	S35	Stay green
G11E29	Humera	SRN39	Striga resistance
G12E29	Humera	Teshale	Best released varieties
G2E19	Shoa Robit	ETWS 90754	Wild type
G3E19	Shoa Robit	ETWS 91242	Wild type
G4E19	Shoa Robit	Framida	Striga resistance
G5E19	Shoa Robit	Hora - Doldy2	LGS
G6E19	Shoa Robit	Jigurti	HGS
G8E19	Shoa Robit	S35	Stay green
G9E19	Shoa Robit	Shanquired red	Striga susceptible
G10E19	Shoa Robit	SR5-Ribka	Fusarium compatibility
G12E19	Shoa Robit	Teshale	Best released varieties
G3E40	Kemise	ETWS 91242	Wild type
G4E40	Kemise	Framida	Striga resistance
G6E40	Kemise	Jigurti	HGS

Were, LGS = low germination stimulant, HGS = High germination stimulant

4.2. Detection of Plant Growth Promoting (PGP) Traits

4.2.1. Phosphate Solubilization Test

Out of 117 isolates; 33 isolates solubilized phosphate. However, from 33 isolates 18 isolates produced IAA and Ammonia in addition to solubilizing the phosphate, but all 18 isolates had statistically a significance different phosphate solubilization potential at (P = 0.01).

Figure 1. Phosphate solubilizing test on the pleat

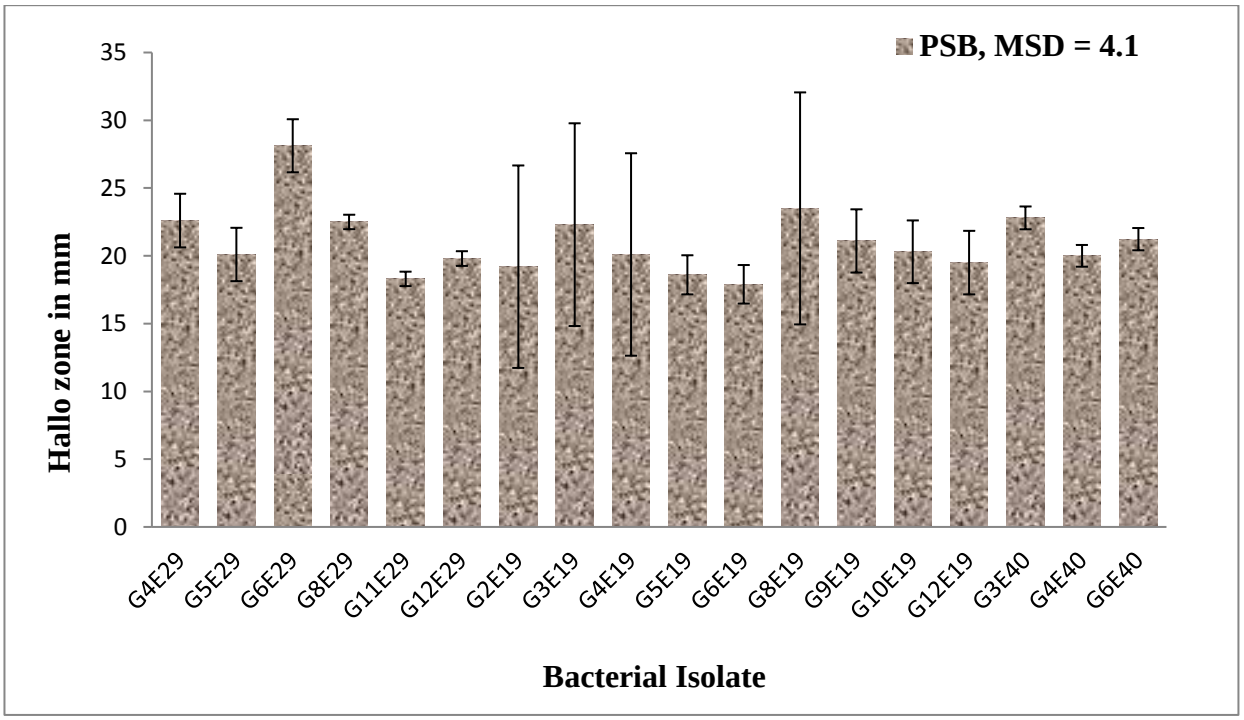

Figure 2. Diameter of phosphate solubilization test clear hallo zone

As the result showed, isolate G6E29 resulted in greatest hallow zone with 28.12 mm diameter clear hallo zone and followed by G3E40, G4E29 and G8E29 resulted 22.8 mm, 22.6 mm and 22.5 mm diameter clear hallow zone and isolated from the soil at Kemisse, Humera as well as Jigurti (landrace), ETWS 91242 (Benishangul Region), Framida (Purdue University), S35 (ICRISAT) and Hora-Doldy sorghum genotype respectively. However, isolate G5E29, G4E19, G9E19, G10E19, G4E40 and G6E40 resulted 20.1 mm, 20.1 mm, 21.1 mm, 20.3 mm, 20 mm and 21.23 mm diameter clear hallo zone isolated from Hora - Doldy2 (landrace), Framida (Purdue University), Shanquired (China),SR5-Ribka(IBC) and Jigurti(landrace) sorghum genotype using soil sample from Shoa Robit, Humera and Kemise area. The isolate G11E29 which was isolated from SRN39(Purdue University) genotype and Haftay Humera soil; G5E29 which was isolated from Hora-Doldy2 (landrace) genotype and Haftay Humera soil; G6E19 which was isolated from Jigurti(landrace) genotype and shoa Robit soil scored lowest 18.3 mm, 18.6 mm, and 17.9 mm diameter clear hallo zone compared to the other isolate.

Tri-calcium phosphate (TCP) is used in phosphate solubilization test as a source of phosphate in an insoluble form as described by Gouda *et al*. (2018). These significance difference might be due to the isolates which had production potential of phosphatase enzyme can solubilize insoluble phosphate into a solubilized and usable form directly by plants or Phosphate solubilizing bacteria reduces pH of rhizosphere soils by releasing organic acids which dissolve phosphate mineral through anion exchange (Sherathia *et al*., 2016). This process increases the availability of phosphorus for plant uptake; but isolates which can't produce organic acid have low phosphate solubilization potential compared isolates capable of production of organic acid (Figure 2). No isolates were solubilized TCP which are isolated from the bulk soil, this might be due to PGPR needs root exudates molecule which secretes from the plant to the rhizosphere soil and used as a carbon source that makes to colonize the root by PGPR which can solubilize TCP. But in the bulk soil, there is no root exudates molecule.

In general, the isolate from soil at Humera along with landrace sorghum genotype resulted in higher phosphate solubilizing PGPR relative to other genotype and soil type belongs to *Pseudomona* bacterial genera. However, the isolate from Kemise had the medium phosphate solubilizing bacteria associated with all sorghum genotype and belongs to *Bacillus* bacterial genera. Isolate from the Soil at Shoa Robit and all sorghum genotypes had low phosphate solubilizing PGPR association belongs to *Azotobacter* bacterial genera; these might be due to the environmental condition, the soil type and the source Sorghum genotype affect the association of phosphate solubilizing bacteria with the rhizosphere of sorghum (Glick, 2012;

Vejan *et al*., 2016).The current study is contradicting with Indris *et al*. (2009) who scored 10 mm clear hallo zone isolates from sorghum and wailed grass. However, the current study scored 28.12 mm clear hallo zone, and also differ from the result of Agbodjato *et al*. (2016) who scored that the highest clear hallo zone diameter was 5 mm, isolates from maize rhizosphere soil. However, based on the current study 5 mm diameter clear hallo zone was the lower hallo zone, but in these studies even the lower hallo zone with 17.9 mm of clear hallo zone.

4.2.2. IAA production Test

Twenty six of the isolates were found to be able to produced IAA at 50 mg/L Tryptophan concentration out of 33 tested isolate by converted the yellow color broth to red-pink color (Figure 3). However, 18 isolates (Table 4) were the most potential isolates for IAA production and highly significant (P=0.01).

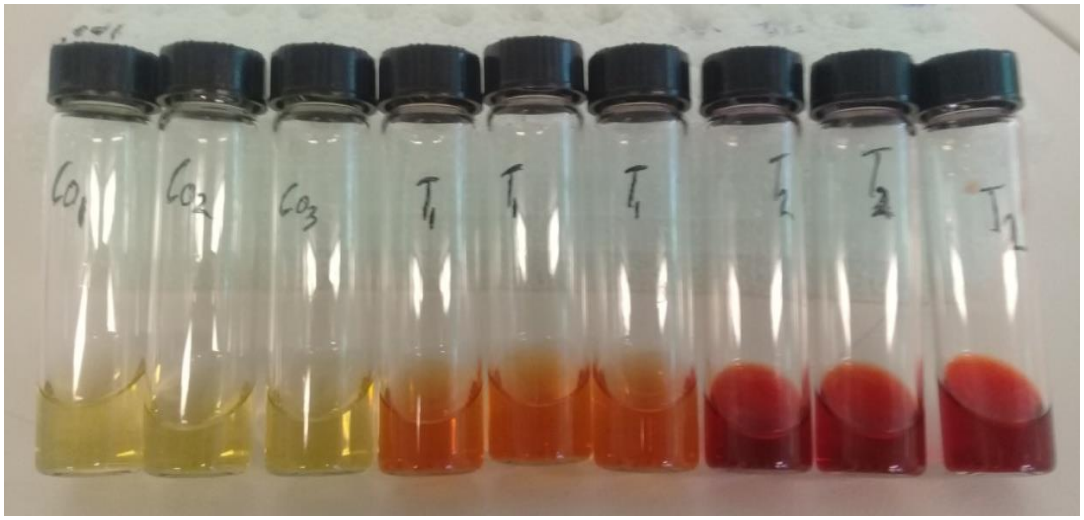

Figure 3. IAA production test at test tube

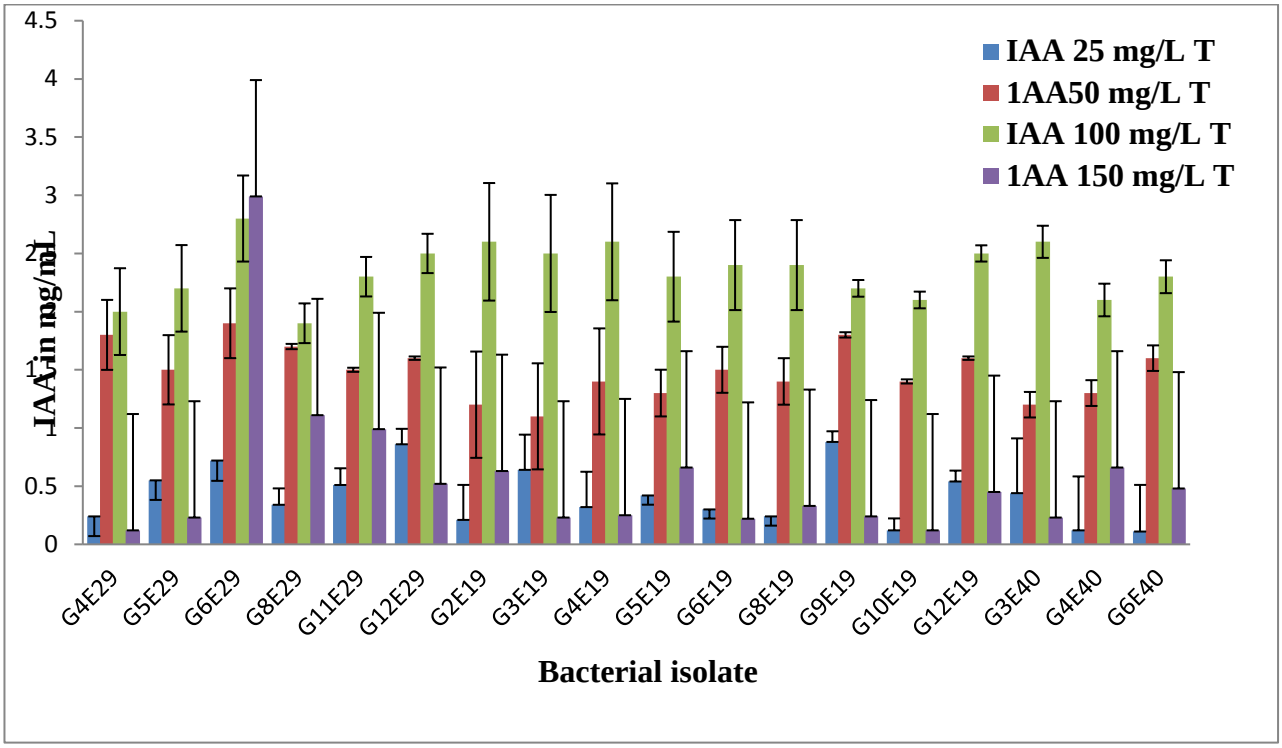

Figure 4. IAA production at 25 mg/L, 50 mg/L, 100 mg/L and 150 mg/L tryptophan

All 18 isolates produced IAA between the concentration ranges of 1.1 mg/ml to 1.9 mg/ml at 50 mg/l tryptophan (Figure 4). However, as the result indicated, those 18 selected isolates had a significant different IAA production potential at different concentration of tryptophan (25, 50, 100, 150 mg/L). At 50 mg/L tryptophan concentration, isolate G6E29 from Jigurti sorghum genotype and soil from Humera produced the highest amount of IAA 1.9 mg/ml. The lowest concentration was recorded from isolate G3E19 from ETWS 91242(Benishangul Region) isolated from the soil at Shoa Robit that produced 1.1 mg/ml. However, the concentration of tryptophan became lower to 25 mg/L of tryptophan IAA production became low for all 18 isolates. As indicated on (Figure 4)**,** isolates that produced IAA at 25 mg/ml tryptophan showed lower IAA production than from 50 mg/L tryptophan.

When the tryptophan concentration increased from 50 mg/L to 100 mg/L tryptophan, all the production of IAA increased for all the 18 isolates (Figure 4) such as G6E29 increased the production from 1.9 mg/ml to 2.8 mg/ml; G5E19 increased production from 1.3 mg/ml to 2.3 mg/ml, and G4E40 increased the production from 1.3 mg/ml to 2.1 mg/ml. However, at 150 mg/L tryptophan, all isolates produced low concentration of IAA, but one isolate (G6E29) significantly increased the production from 2.8 mg/ml at 100 mg/L tryptophan to 2.9 mg/ml at 150 mg/L tryptophan. Hence, at 100 mg/L of tryptophan concentration all 18 isolates produced higher amount of IAA. The tryptophan concentration affected each PGPR bacteria depending

on the isolates genetic makeup which are suitable used for instruction of the gene for the production of IAA. Sivasankari (2016) described that higher IAA was produced at 95 mg/L, whereas Indris *et al*. (2009) reported the highest IAA production at 2 mg/L of tryptophan concentration without the effect of genetic makeup of source sorghum and produce IAA with the given environmental conditions that the soil samples were together for the production of IAA. But Ahmad *et al*. (2008) reported the production of IAA increased when the concentration of tryptophan increased which is completely contradicted with the current study. According to the current study finding IAA production potential of each isolates show a discrepancy at different concentration of tryptophan depending on the sources of isolates. These might be due to the gene expression for IAA production of PGPR bacteria affected by the source of the isolate.

In general, isolates from Humera soil, with all 12 sorghum genotype rhizosphere, had the higher IAA production potential belongs to *Pseudomona and Bacillus* bacterial genera, whereas isolate from Shoa Robit and Kemise soil with 12 sorghum genotype rhizosphere had the lower IAA production potential in all tryptophan concentration which means plant genotype and soil type also affect the production of 1AA in addition to tryptophan concentration (Vejan *et al*., 2016).

4.2.3. Ammonia Production Test

Only 18 out of 26 isolates were able to produced Ammonia with the produced ammonia and 18 isolates had more potential for Ammonia production (Figure 5) and all 18 isolates had a significant different ammonia production potential at P=0.01(Figure 6).

Isolate G6E29, G6E40, G5E29, and G4E19 produced the highest amount of ammonia with 16.2 mg/ml, 14.3 mg/ml, 13.3 mg/ml and 13.2 mg/ml respectively which are isolated from the soil collected at Humera, Kemise and Shoa Robit with Jigurti(landrace sorghum genotype), Framida(Purdue University) and Hora-Doldy2(landrace) sorghum genotype. Isolates G4E29, G6E19, and G3E40 with 12.2 mg/ml, 12.6 mg/ml and 12.6 mg/ml, respectively, which are isolated from Framida (Purdue University), Jigurti (landrace sorghum genotype) and ETWS 91242 (Benishangul Region) sorghum genotype with the soil collected from Humera, Kemise and Shoa Robit. Isolate G3E19 produced the lowest amount 9.02 mg/ml ammonia which is isolated from the soil samples collected at Shoa Robit, and ETWS 91242(wailed) sorghum genotype.

In general isolate from the soils at Humera and Kemise with all sorghum genotypes had produced higher amount of ammonia compared to the isolate from the soil at Shoa Robit and

belongs to *Pseudomona and Bacillus* bacterial genera. These might be due to the soil type and sorghum genotype affect the production potential of ammonia produced PGPR isolate association with sorghum (Vejan *et al*., 2016). Mahbouba *et al*. (2013) reported that isolates from all genotype of wheat produced the same amount of Ammonia. Idris *et al*. (2008) and Sivasankari (2016) on the other hand reported ammonia production potential of rhizosphere bacteria depends on the soil nutrient availability and species of bacteria; which is contradicting to the current study. However, based on the current study, ammonia production of an isolate from different sorghum genotype and soil sample had different ammonia production potential; these might be due to the soil type and nutrient availability affect the ammonia production of PGPR bacteria.

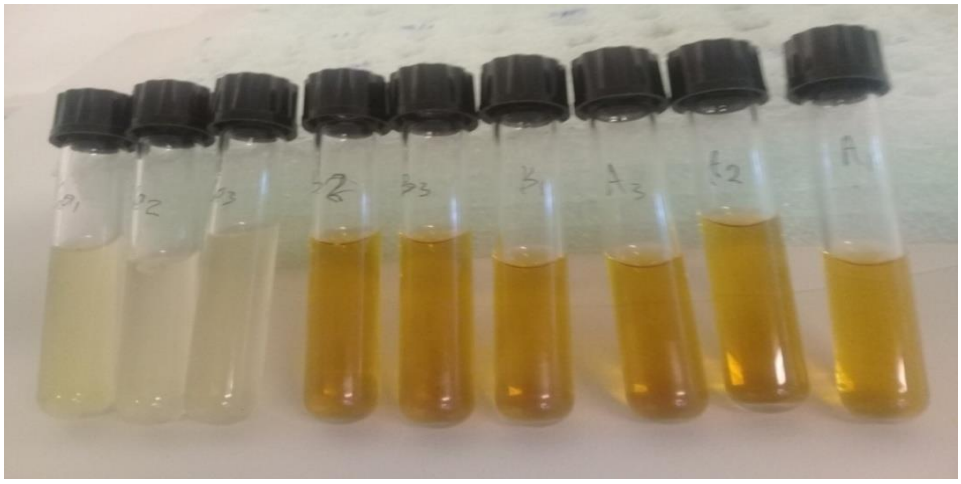

Figure 5. Ammonia production result in test tube.

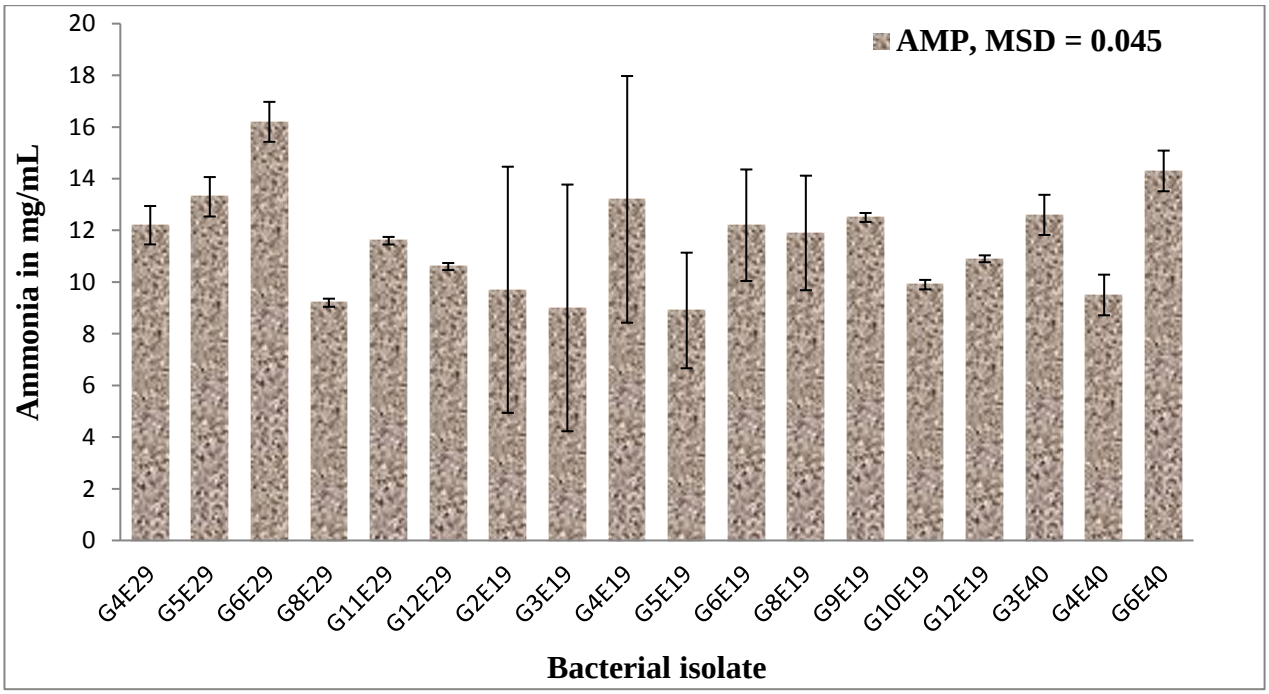

Figure 6. Ammonia production test for 18 potential PGPR

The analysis of variances of PGPR bacteria for sorghum growth related parameters such as Phosphate Solubilization, IAA production and Ammonia production tests were presented in (Table 5) below respectively. Mean squares were highly significant at (p = 0.01) for all parameters indicating that each isolate differed in the growth related trait cause variation which agreed with the finding of Indris *et al*. (2009).This might be due to the genetic makeup of the isolates and source genotype as well as the soil with the environmental condition Dinesh *et al.* (2015).

Table 5.The effect of selected PGPR inoculation variance on the PST, IAA and AMP. Mean ± S.D at P = 0.01

Isolate	**PST**	**AMP**	**IAA (25mg/L Trp)**	**IAA (50mg/L Trp)**	**IAA (100mg/L Trp)**	**IAA (150mg/L Trp)**
G4E29	22.6±0.010	12.2± 0.015	0.249±0.003	1.840±0.001	2.012±0.001	0.122±0.000
G5E29	20.11±0.005	13.32 ±0.010	0.559±0.005	1.559±0.004	2.214±0.001	0.233±0.000
G6E29	28.12±0.005	16.23±0.005	0.722±0.001	1.997±0.000	2.887±0.001	2.991±0.001
G8E29	22.55±0.007	9.26±0.041	0.341±0.000	1.740±0.001	1.997±0.000	1.112±0.000
G11E29	18.32 ± 0.015	11.63 ±0.026	0.516±0.005	1.559±0.004	2.312±0.001	0.996±0.002
G12E29	19.83 ± 0.020	10.68±0.005	0.865±0.038	1.651±0.000	2.523±0.001	0.521±0.001
G2E19	19.22 ± 0.011	9.77±0.017	0.214±0.001	1.240±0.001	2.641±000	0.631±000
G3E19	22.31 ± 0.011	9.02±0.010	0.643±0.001	1.159±0.004	2.541±000	0.232±0.016
G4E19	20.13±0.010	13.22±0.010	0.325±0.001	1.451±0.000	2.614±0.001	0.255±000
G5E19	18.65±0.010	8.98 ±0.010	0.425±0.001	1.340±0.001	2.332 ±0.001	0.662±0.001
G6E19	17.92±0.010	12.22±0.011	0.305±0.104	1.559±0.004	2.423±0.001	0.228±0.000
G8E19	23.56±0.005	11.92±0.015	0.247±0.012	1.401±0.000	2.462±0.001	0.334±0.000
G9E19	21.13±0.01	12.54±0.010	0.883±0.001	1.857±0.004	2.213±0.001	0.245±000
G10E19	20.33 ± 0.015	9.92±0.005	0.127±0.005	1.459±0.004	2.112±0.005	0.124±0.001
G12E19	19.50 ± 0.011	10.97±0.010	0.542±0.009	1.671±0.139	2.513±0.005	0.451±000
G3E40	22.82±0.152	12.64±0.010	0.443±0.002	1.240±0.001	2.641±000	0.235±000
G4E40	20.±0.005	9.51±0.005	0.124±0.001	1.359±0.004	2.111±000	0.662±0.001
G6E40	21.23±0.020	14.31±0.005	0.113±0.000	1.651±0.000	2.353±0.001	0.481±0.000
D.F	53	53	53	53	53	53
R^2	82.6%	99.9%	99.1%	98.5%	99.9%	99.9%
CV	6.452	0.1363	6.252	2.139	0.04	0.669
P	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001

*Where, **PST** = phosphate solubilization Test, **IAAPT** = Indole acetic acid production Test, **AMPT** = Ammonia production Test and **Trp** = Tryptophan*

4.3. Greenhouse Evaluation of PGPR for Sorghum Growth Promotion

All the 18 isolates have significantly increased all the agronomic parameters relative to the control. However, some of the isolates had highly significant compared to the others at p = 0.01(Table 6).

Table 6. Mean separation analysis result for each isolate in favor of agronomic data (PSH, PSFW, PSDW, RL, RFW and RDW) at P = 0.01

Isolate	PSH	PSFW	PSDW	RL	RFW	RDW
G4E29	35.2^{bc}	11.5^{ef}	8.2^{c}	36.2^{bc}	15.4^{bc}	9.5^{bc}
G5E29	33.2^{d}	11.4^{ef}	5.2^{l}	34.2^{de}	15.1^{cd}	8.8^{de}
G6E29	35.5^{a}	13.8^{bc}	8.8^{ab}	37.8^{a}	16.3^{ab}	9.7^{b}
G8E29	31.4^{f}	10.4^{h}	7.0^{fg}	34.1^{de}	14.9^{cd}	9.1^{cd}
G11E29	33.2^{d}	10.8^{gh}	7.8^{cd}	33.8^{e}	14.1^{de}	8.8^{e}
G12E29	30.2^{h}	9.8^{i}	5.5^{kl}	32.2^{f}	12.2^{fg}	7.2^{g}
G2E19	31.7^{f}	11.1^{fg}	6.3^{hi}	29.8^{g}	11.3^{gh}	5.1^{i}
G3E19	32.2^{e}	11.8^{de}	6.9^{fg}	35.2^{cd}	14.2^{de}	6.5^{h}
G4E19	35.2^{a}	14.3^{a}	9.2^{a}	37.2^{ab}	16.4^{ab}	9.7^{b}
G5E19	33.5^{d}	13.2^{c}	8.2^{c}	28.2^{h}	13.5^{e}	8.3^{f}
G6E19	33.1^{d}	13.8^{ab}	8.8^{ab}	31.3^{f}	12.2^{fg}	9.3^{c}
G8E19	34.6^{b}	14.1^{a}	9.1^{a}	35.6^{c}	12.3^{fg}	8.7^{e}
G9E19	30.7^{g}	9.7^{ij}	5.8^{jk}	25.1^{i}	10.2^{ij}	6.2^{h}
G10E19	34.2^{c}	11.7^{de}	7.4^{de}	32.0^{f}	13.2^{ef}	9.2^{cd}
G12E19	32.4^{e}	10.5^{h}	7.1^{ef}	28.2^{h}	9.1^{j}	6.5^{h}
G3E40	30.3^{h}	9.7^{ij}	6.7^{gh}	27.4^{h}	7.2^{k}	6.4^{h}
G4E40	34.2^{bc}	12.1^{d}	8.6^{b}	36.2^{bc}	17.1^{a}	12.1^{a}
G6E40	31.4^{f}	10.5^{h}	6.2^{ij}	24.4^{i}	10.2^{hi}	6.1^{h}
Control	20.3^{i}	9.3^{j}	4.2^{m}	21.2^{j}	9.8^{ji}	3.4^{j}
CV	0.428	1.388	1.804	1.305	2.732	1.727
R^2	99.8%	99.3%	99.4%	99.5%	98.9%	99.7%
MSD	0.426	0.495	0.404	1.275	1.089	0.425

*Where, **PSH** = Plant Shoot Height; **PSFW** = Plant Shoot Fresh Weight; **PSDW** = Plant Shoot Dry Weight; **RL** = Root Length; **RFW** = Root Fresh Weight and **RDW** = Root Dry Weight; **CV** = Coefficient of Variation; **MSD** = Minimum Significance Difference.*

Isolate G6E29 was isolated from Jigurti (landrace sorghum genotype) and soil from Humera; it was significantly increased plant shoot height by 75%. Whereas isolate G4E19 was isolated from Framida sorghum genotype and the soil from Shoa Robit; it was significantly increased plant shoot by 74%. Next to G6E29 and G4E19, three isolates (G4E29, G8E19 and G4E40) showed a significant increase in plant shoot height, and isolated from the rhizosphere of Framida and S35 sorghum genotypes along with the soil collected at Humera, Shoa Robit and Kemise and significantly increased plant shoot height by 73%, 70% and 68% respectively. As described in (Table 6), the rest isolates also significantly increased the plant shoot height compared to the control. But compared to each other, they had lower potential relative to the above one; these might be due to the tested sorghum genetic makeup and environments are comfortable for PGPR to increase the plant shoot height. Ahmad *et al*. (2008), Noumavo *et al*. (2013) and Andreote *et al*. (2010) reported that all the tested isolates did not significantly increase the plant shoot height compared to the control which is contradicting to the current study. However, in the current study, all the isolates were increased the plant shoot height compared to the control with different plant shoot height increasing potential. The report by Indris *et al*. (2009) is analogous with the current study which reported that all selected potential isolates increased plant shoot height compared to the control.

Three isolates (G4E19, G8E19 and G6E19) significantly increased the plant shoot fresh weight. G4E19 was isolated from the rhizosphere of Framida sorghum genotype, and the soil at Shoa Robit; it was significantly increased the plant shoot fresh weight by 54%. G8E19 was isolated from the rhizosphere of S35 sorghum genotype, and the soil collected from Shoa Robit; it was significantly increased the plant shoot fresh weight by 52%, and G6E19 was isolated from Jigurti landrace sorghum genotype, and Shoa Robit soil; it was significantly increased plant shoot fresh weight by 48%. G5E19 was isolated from Hora-Doldy2 Ethiopian landrace sorghum genotype and the soil at Shoa Robit; it was significantly increased the plant shoot fresh weight by 48%. The remaining isolates also significantly increased the plant shoot fresh weight compared to the control. However, compared to each other, they had lower potential relative to the above, may be due to sorghum genetic makeup of the tested genotype and favorable environmental conditions required by PGPR. Each isolate might have also different potential based on their Genome. Indris *et al*. (2009) reported that the isolates increased the plant shoot height but not the plant shoot fresh weight which is contradicted to the current study. But here, all 18 isolates increased plant shoot height and plant shoot fresh weight compared to the control. Zinniel *et al*. (2002) reported that isolates that increase the plant shoot height also increase plant shoot fresh weight which is related to the current study.

Three isolates; such as G4E19, G8E19 and G6E29 are significantly increased the plant shoot dry weight. G4E19 was isolated from the rhizosphere of Framida sorghum genotype, and the soil at Shoa Robit; it was significantly increased the plant shoot dry weight by 119%. G8E19 was isolated from the rhizosphere of S35 sorghum genotype, and the soil at Shoa Robit, it was significantly increased plant shoot dry weight by 116%. G6E29 was isolated from rhizosphere of Jigurti landrace sorghum, and soil at Humera; it was significantly increased plant shoot dry weight by 109%. Such statistically significance difference might be due to the tested sorghum genetic makeup and conducive environment for PGPR isolates for plant soot dry weight(Andreote *et al*., 2010). PGPR bacterial genera might have different potential based on their genome to increase the plant shoot dry weight (Miransari and Smith, 2014). The above ground plant biomass growth promoting potential of PGPR also affected by environmental condition, soil type and green house condition (Glick, 2012; Vejan *et al*., 2016). Giongo (2010) and Ahmad *et al*. (2008) reported that all tested PGPR increased in shoot dry weight by 80% compared to the control which but in the current study all tested PGPR increased in different amount. Indris *et al*. (2009) reported that isolates increase plant shoot dry weight in different amount which is comparable to the current study.

The two isolates (G6E29 and G4E19) significantly increased root length. G6E29 was isolated from the rhizosphere of Jigurti landrace sorghum genotype, and from the soil at Humera; it significantly increased root length by 78%, whereas G4E19 was isolated from the rhizosphere of Framida sorghum genotype, and the soil at Shoa Robit; it was significantly increased the root length by 75%. The three isolates such as G4E29, G4E19 and G4E40 have significantly increased the root length next to G6E29 and G4E19. G4E29 was isolated from the rhizosphere of Framida sorghum genotype, and the soil at Humera, it was significantly increased the root length by 71%. G4E19 was isolated from the combination of Framida sorghum genotype, and the soil at Shoa Robit, it was significantly increased the root length by 75%. G4E40 was isolated from Framida sorghum genotype and the soil collected at Kemise, it was significantly increased the root length by 71%. The other isolates also had significant increasing effect in the root length compared to the control. But compared to each other, they had lower potential relative to the above one, these difference might be due to the tested sorghum genetic makeup and environmental condition is comfortable for PGPR, as well as each isolate might have different potential based on their genome to increase the root length or the sorghum genotype that have more carbon root exudates which are used for PGPR to colonize the root and increase the root length (Bloemberg and Lugtenberg, 2000). Giongo (2010) and Ahmad *et al*. (2008)

reported that most of the isolates increased the root length in the same amount 16 cm compared to the control, which contradict the current study. Indris *et al*. (2009) reported that isolates were significantly increased the root length in different potential which is similar to the current study reported that all the isolates increased the root length significantly with different manner depending on source genotype and soil sample.

The three isolates such as G4E40, G6E29 and G4E29 have significantly increased the root fresh weight. G4E40 was isolated from the rhizosphere of Framida sorghum genotype, and the soil at Kemise; it was increased the root fresh weight by 74%, G6E29 was isolated from the rhizosphere of Jigurti landrace sorghum genotype, and the soil collected at Humera; it was significantly increased root fresh weight by 66% and G4E29 was isolated from the rhizosphere of Framida sorghum genotype, and the soil collected at Humera; it was significantly increased the root fresh weight by 56%. The two isolates (G5E29 and G8E29) were isolated from the rhizosphere Hora-Doldy2 and S35 sorghum genotype with the combination of soil from Humera. Compared to the control, both isolates were increased the root fresh weight by 54% and 52% respectively. The rest isolates also had significantly increased in the root fresh weight compared to the control. But compared to each other, they had a lower potential relative to the above one. But two isolates (G12E19 and G3E40) no significant for root fresh weight. Compared to the control, the root fresh weight decreased by 7% and 26% respectively from the control; but they had a significant increasing effect for the rest agronomic parameter. These might be due to the isolate was not contented association to the tested genotype or affect the environmental condition for root fresh weight (Andreote *et al*., 2010). Indris *et al*. (2009) and Ahmad *et al*., (2008) reported that all the isolates increased the root length also increased the root fresh weight which is contradict to the current study. However, the current study reports that all the isolates significantly increased the root fresh weight with different amount, these might be due to the tested sorghum genotype genetic makeup and environmental condition is comfortable for PGPR, as well as each isolate might have different potential based on their genome and colonize the root to increase the root fresh weight or the sorghum genotype that more carbon root exudates which is used for PGPR to colonize the root (Vejan *et al*., 2016).

Intended for root dry weight, isolate G4E40 which was isolated from the rhizosphere of Framida sorghum genotype, and soil at Kemise; it was significantly increased the dry weight of root by 256%. The three isolates (G4E29, G6E29 and G4E19) were isolated from the rhizosphere of Framida and Jigurti sorghum genotype with a combination of soil collected from Humera and Shoa Robit; they have significantly increased the root dry weight by 256%,

185% and 185% respectively. The other isolate also significantly increased the root dry weight compared to the control, these might be due to the tested sorghum genetic makeup and environmental condition is contented for PGPR function, as well as each isolate might have different potential based on their genome to increase the root dry weight (Table 6) compared to each other (Cakmakci *et al*., 2006). Anjum *et al*. (2011); Abedinzadeh *et al*. (2019) and Khalid *et al*. (2004) reported that isolates were isolated from different crop rhizosphere and genotype increased root dry weight differently which is similar to the current study. To the contradict Indris *et al*. (2009) and Khalid *et al*. (2004) reported that all the isolates did not significantly increase all the agronomic parameter which is isolated from single soil sample and sorghum genotype. However, in the current study, all the isolates were significantly increased all the parameter in a significance variation, except two *Bacillus* and *Azotobacter* bacterial genera *(*G12E19 and G3E40).

The two isolates such as G6E29 and G4E19 have increased all the sex parameters isolated from the rhizosphere of Jigurti and Framida sorghum genotype, and the soil collected from Humera and Shoa Robit also belongs to *Pseudomona* bacterial genera. Bacteria isolated from the soil collected at Humera and Shoa Robit increased all the parameter compared to each other. PGPR bacteria which are isolated from the Humera soil had the higher growth promoting potential compared to the soil collected from Shoa Robit, whereas PGPR bacteria which are isolated from the soil at Kemise had the growth promoting potential but low growth promoting potential compared to the bacteria which are isolated from soil at Humera and Shoa Robit, these might be the soil and environmental condition effect the growth promoting potential PGPR bacteria (Giongo 2010 and Ahmad *et al*. 2008).

All the isolates had the growth promoting potential compared to the control but had different growth promoting potential depending on the source genotype. So, bacteria isolated from Framida and Jigurti sorghum genotype significantly increased all the parameter followed by bacteria isolated from the landrace's sorghum genotype having growth promoting potential compared to the bacteria isolated from the other sorghum genotype, these might be due to the genetic makeup of source sorghum genotypes are affect the type and potential of PGPR. Bacteria isolated from sorghum Framida, Jigurti and landrace sorghum genotype with the combination soil collected at Humera and Shoa Robit significantly increased the six parameters such as: plant shoot height, plant shoot fresh weight, plant shoot dry weight, root length, root fresh weight and root dry weight compared to bacteria isolated from the rest of sorghum genotype and soil collected at Humera, these might be due to plant genotype and soil type together with environmental condition affect the potential of PGPR.

Table 7.The effect of PGPR inoculation variance on sorghum agronomic data (PSH, PSFW, PSDW, RL, RFW and RDW). Mean ± SD at P =0.01.

Isolate	PSH	PSFW	PSDW	RL	RFW	RDW
G4E29	34.3 ± 0.10	11.5 ± 0.03	8.2 ± 0.03	36.2 ± 0.06	15.4 ± 0.05	9.5 ± 0.05
G5E29	33.2 ± 0.08	11.4 ± 0.15	5.2 ± 0.06	34.2 ± 0.03	15.1± 0	8.8 ± 0.03
G6E29	35.5 ± 0.05	13.4 ± 0.11	8.8 ± 0.03	37.8 ± 0.01	16.3 ± 0.05	9.7 ± 0.03
G8E29	31.4 ± 0.05	10.4 ± 0.03	7.0 ± 0.06	34.1 ± 0.03	14.9 ± 0	9.1 ± 0.03
G11E29	33.2 ±0.08	10.8 ± 0.03	7.8± 0.06	33.8±0.03	14.1 ±0.03	8.8 ± 0.03
G12E29	30.2 ±0.12	9.8±0.03	5.5±0.05	32.2±0.08	12.2±0.05	7.2 ± 0.03
G2E19	31.7±0.08	11.1±0.06	6.3± 0.01	29.8± 0.03	11.3± 0	5.1± 0.03
G3E19	32.2 ± 0.06	11.8±0.03	6.9 ± 0	35.2 ± 0.13	14.2±0.03	6.5 ± 0.03
G4E19	35.2 ± 0.03	14.3 ± 0.11	9.2 ± 0.05	37.2 ± 0.03	16.4 ± 0.10	9.7 ± 0.08
G5E19	33.5 ± 0.05	13.2 ± 0.08	8.2 ± 0.05	28.2 ± 0.08	13.5 ± 0.86	8.3 ± 0.05
G6E19	33.1 ± 0.03	13.8 ± 0.03	8.8 ± 0.03	31.3 ± 0.11	12.2 ± 0.08	9.3 ± 0.05
G8E19	34.6± 0.08	14.1 ± 0.03	9.1 ± 0.03	35.6 ± 0.03	12.3 ± 0.11	8.7 ± 0.08
G9E19	30.7 ± 0.08	9.7 ± 0.05	5.8 ± 0.03	25.1 ± 0.03	10.2 ± 0.05	6.2 ± 0.08
G10E19	34.2 ± 0	11.7 ± 0.10	7.4 ± 0.089	32.0 ± 0.03	13.2 ± 0.089	9.2 ± 0.03
G12E19	32.4 ± 0.12	10.5±0.05	7.1±0.03	28.2± 0.08	9.1 ± 0.03	6.5 ± 0.02
G3E40	30.3 ± 0.05	9.7 ± 0.11	6.7 ± 0.15	27.4 ± 0.05	7.2 ± 0.05	6.4 ± 0.15
G4E40	34.2 ± 0.12	12.1 ± 0.06	8.6 ± 0.12	36.2 ± 0.08	17.1 ± 0.03	12.1 ± 0.06
G6E40	31.4 ± 0.05	10.5 ± 0.20	6.2 ± 0.05	24.4 ± 0.06	10.2 ± 0.06	6.1 ± 0.06
Control	20.3 ± 0.12	9.3 ± 0.12	4.2 ± 0.12	21.2 ± 0.10	9.8 ± 0.03	3.4 ± 0.05
DF	56	56	56	56	56	56
MSD	0.426	0.495	0.404	1.275	1.089	0.425
P	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001

*Where, **DF** = Degree of Freedom**; M.S.D *** = Minimum Significance Difference **PH** = Plant Height; **PFW** = Plant Fresh Weight; **PDW** = Plant Dry Weight; **RL** = Root Length; **RFW** = Root Fresh Weight and **RDW =** Root Dry Weight*

The analysis of variances of plant growth promoting rhizosphere bacteria for sorghum growth and growth-related parameter; such as plant shoot height, plant shoot fresh weight, plant shoot dry weight, root length, root fresh and dry weight related traits were presented in (Table 7). Significant differences were detected between each isolate for all of the studied parameters which indicates that each isolate differed in the growth promoting potential for Teshale sorghum genotype cause variation which goes with the finding of Indris *et al*., (2009). Entry mean squares were significant (p<0.01) for all agronomic parameter; these might be due to all the tested PGPR rhizosphere bacteria have different growth promoting potential depending their source.

4.4. Correlation analysis for agronomic parameter

Table 8.Correlation relationship for PSH, PSFW, PSDW, RL, RFW and RDW at P = 0.01

	PH	PFW	PPDW	RL	RFW	RDW
PH						
PFW	0.674**					
PDW	0.769***	0.832 ***				
RL	0.747***	0.611**	0.655 **			
RFW	0.559**	0.564**	0.509 **	0.819***		
RDW	0.768***	0.616**	0.746***	0.793***	0.783 ***	

*Where ** moderate (significance), *** strong (highly significance), **PSH** = Plant Shoot Height; **PSFW** = Plant Shoot Fresh Weight; **PSDW**= Plant Shoot Dry Weight; **RL** = Root Length; **RFW** = Root Fresh Weight and **RDW** = Root Dry Weight*

Plant height, plant fresh and dry weight, root length, root fresh, and dry weight positively correlated among each other (Table 8). Ratner (2009) categorized the Pearson correlation coefficient as weak, moderate and strong for values ranging from 0 to ± 0.29, ± 0.3 to ± 0.69 and ± 0.7 to ±1.0, respectively. So all the agronomic parameters (Plant height, plant fresh and dry weight, root length, root fresh, and dry weight) exhibited a positive correlation with strong

and moderate relation, these might be due to growth promoting rhizobacteria can produced appropriately all growth related trait and affected all agronomic parameter in an the same manner. The current study results were following the finding of Indris *et al*. (2009) and Khalid *et al*. (2004). In contrast, Abedinzadeh *et al*. (2019), Ahmad *et al*. (2008) and Anjum *et al*. (2011) reported plant height was negatively correlated with root length and fresh weight, but in the current study all the agronomic parameters were positively correlated.

4.5. Biochemical and Morphological characterization

In the current study, a total of 18 potential isolates were obtained from sorghum genotype based on the fact that they fulfilled all growth promoting characteristics. As described in (Table 9)**.** All the tested isolates were rood shaped and utilized carbon source. Isolate G5E29, G12E19, G6E40 were gram - positive, whereas the rest isolates were gram-negative. Isolate G4E29, G5E29, G6E29, G4E19, G6E19, G8E19, G9E19, G10E19 and G12E19 were catalase-negative, whereas G8E29, G11E29, G12E29, G2E19, G3E19, G5E19, G3E40, G4E40 and G6E40 were catalase-positive. All the eighteen isolates were groped in two colony morphology such as button and serrated margins shaped.

Eight isolates (G4E29, G5E29, G6E29, G4E19, G6E19, G8E19, G9E19 and G10E19) were classified under the taxonomic genera of *pseudomonas*. Six isolates (G8E29, G11E29, G12E29, G2E19, G3E19 and G3E40) were classified under the taxonomic genera of *Azotobacter* and four isolates (G5E19, G12E19, G4E40 and G6E40) were classified under the taxonomic genera of *Bacillus*. Indris *et al*., (2009) reported that Pseudomonas, Azotobacter and Bacillus were associated with the rhizosphere of sorghum. Ahmad *et al*., (2008) reported that *Actinomycetes* were also associated in addition to *Pseudomonas, Azotobacter* and *Bacillus* genera. So, in the current study, the majority of the isolates from Landrace sorghum genotype and all the 3 soil samples were classified under *Pseudomonas*. *Azotobacter* PGPR bacteria were associated with the developed variety of sorghum genotype with all soil samples; *Bacillus* PGPR bacteria were associated with Striga susceptible sorghum genotype. So, sorghum genotype affected the association of PGPR bacteria at rhizosphere of sorghum, might be depending on the sorghum genotype and soil sample, taxonomic classification and the Carbon source utilization of growth promoting bacteria is diverse. Based on the current study the *Pseudomonas* PGPR genera are the beast performance for both plant growth related screening test and sorghum growth promoting performance in greenhouse. However, the growth promoting potential of *Pseudomonas* genera had a significance difference depending on the source sorghum genotype and soil type. Based on the current study the *Pseudomonas* genera have the greatest potential for both growth related trait such as phosphate solubilization test, IAA production test and ammonia production test along with the potential of in all agronomic parameter for greenhouse evaluation and followed by *Bacillus* genera in all growth related trait and growth parameter.

Table 9. Biochemical and morphological characterization of 18 selected potential isolates

Isolate	Glucose	Lactose	Sucrose	Gram stain	shape	Catalase test	Colony morphology	Genera
G4E29	+	+	+	-	rods	-	Button shaped	*Pseudomona*
G5E29	+	+	+	-	rods	-	Button shaped	*Pseudomona*
G6E29	+	+	+	-	rods	-	Button shaped	*Pseudomona*
G8E29	+	+	+	-	rods	+	Serrated margins	*Azotobacter*
G11E29	+	+	+	-	rods	+	Serrated margins	*Azotobacter*
G12E29	+	+	+	-	rods	+	Serrated margins	*Azotobacter*
G2E19	+	+	+	-	rods	+	Serrated margins	*Azotobacter*
G3E19	+	+	+	-	rods	+	Serrated margins	*Azotobacter*
G4E19	+	+	+	-	rods	-	Button shaped	*Pseudomona*
G5E19	+	+	+	+	rods	+	Serrated margins	*Bacillus*
G6E19	+	+	+	-	rods	-	Button shaped	*Pseudomona*
G8E19	+	+	+	-	rods	-	Button shaped	*Pseudomona*
G9E19	+	+	+	-	rods	-	Button shaped	*Pseudomona*
G10E19	+	+	+	-	rods	-	Button shaped	*Pseudomona*
G12E19	+	+	+	+	rods	+	Serrated margins	*Bacillus*
G3E40	+	+	+	-	rods	+	Serrated margins	*Azotobacter*
G4E40	+	+	+	+	rods	+	Serrated margins	*Bacillus*
G6E40	+	+	+	+	rods	+	Serrated margins	*Bacillus*

Were, + = can utilize the tested Carbone, the gram positive isolate and can produced catalase enzyme; - = gram negative isolate or can't produce catalase enzyme

5. SUMMARY AND CONCLUSION

Nowadays, it is very important to improve sorghum production and productivity using plant growth promoting rhizosphere bacteria in Ethiopian agriculture. The result of the current study revealed that the objective such as to isolate and screen isolated PGPR for growth promoting trait and evaluate their growth promoting potential in the greenhouse and identify potential growth promoting PGPR using biochemical and morphological characterization which are isolated from 12 sorghum genotype by cultivating on 3 collected soil samples from Northern part of Ethiopia. A total of 117 rhizosphere bacteria were isolated from 12 sorghum genotype rhizosphere sample.

All the 117 isolates were subjected to growth promoting test; such as phosphate solubilization test using PVK culturing media and 33 isolates solubilized phosphate, in addition to phosphate solubilization, all 33 isolates were subjected for IAA production test using different concentration of tryptophan; such as 25 mg/L, 50 mg/L, 100 mg/L, and 150 mg/L of tryptophan. Twenty-six isolates produced IAA from the total 33 isolate PGPR. The production potential of each IAA produced 26 isolates increased from 25 mg/L to 100mg/L of tryptophan concentration but decreased using 150 mg/L of tryptophan concentration, except one isolate, G6E29, which increased IAA production at 150 mg/L of tryptophan. Based on the current study, the higher IAA production scored at tryptophan concentration of 100 mg/L. In addition to phosphate solubilization and IAA production test, all the 26 isolates were subjected to ammonia production test using Nessler's reagent. Eighteen isolates produced ammonia from all 26 tested PGPR based on the screening test. Eighteen isolates (G4E29, G5E29, G6E29, G8E29, G11E29, G12E29, G2E19, G3E19, G4E19, G5E19, G6E19, G8E19, G9E19, G10E19, G12E19, G3E40, G4E40 and G6E40) were selected based on those isolates which solubilize phosphate, produce IAA and produce ammonia. Those potential selected 18 isolates were subjected to further greenhouse evaluation and biochemical characterization.

Eighteen of the most potential isolates were evaluated in a greenhouse by adding 1×10^{-19} standard concentrations on Teshale sorghum genotype at Holeta National Agricultural Biotechnology Research Center. Plant shoot height, plant shoot fresh and dry weight, root length, root fresh, and dry weight were collected after 35 days of inoculation. Analysis of variance revealed the presence of significant variation among isolates for all studied traits. Mean square of all isolates for all parameter was significant indicating that all the isolates significantly promote sorghum growth.

For plant shoot height, all the 18 isolates significantly increased plant height when compared to the control. But when compared to each other, two isolates G6E29 and G4E19 significantly increased the plant shoot height better than the other. For plant shoot fresh and dry weight, all the isolates significantly increased plant shoot fresh and dry weight compared to the control but G6E29, G4E19 and G8E19 the potential one compared to each other. For root length, all the isolates significantly increased root length when compared to the control, but when compared to each other, they have different potential for root length growth, and G6E29 and G4E19 were the most important ones in this respect. For root fresh and dry weight, all the isolates significantly increased the root fresh and dry weight compared to the control, except two isolate, G12E19 and G3E40, which are non significance for the root fresh weight compared to the control. The isolates G6E29, G4E19 and G4E40 significantly increased the root fresh and dry weight.

Pearson correlation coefficient analysis revealed that plant soot height, plant shoot fresh and dry weight, root length, root fresh and dry weight growth and growth-related traits had a highly significant ($p<0.01$) positive correlations with each other. Based on the findings of the current study, the following recommendations and feature line of work have been suggested.

- Isolates with good sorghum growth promoting potentialities were characterized and the best 2 efficient isolates (G6E29 and G4E19) were identified. The results are promising for the design of potentially active sorghum growth promoting PGPR strain which would be beneficial for improvement of sorghum production and productivity for sustainable agriculture.
- The experiment was conducted using soil collected from the Northern part of Ethiopia; it is realistic to conduct similar experiments for other parts of Ethiopia across wider ranges of agro ecology to get other potential PGPR strain.
- The experiment was conducted at in vivo level for sorghum only; it is realistic to carry out a similar experiment for other crops across wider ranges of agro ecology.
- Furthermore, assessing different types of effective and compatible PGPR strains along with different sources of crop and environment to increase crop production efficiency and grain yield of sorghum and other cereal crop should require further investigation in the future.

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7. APPENDICES

Appendices 1. Step by step practical laboratory work

1.1. Experimental Design: Total number of observations (experimental and control samples)

- 12 sorghum genotypes * 3 soil sample * 4 replications = 144 total experimental samples were used.
- Rhizosphere soils from the 144 total samples were used to isolate plant growth promoting rhizosphere bacteria (PGPRB) from the respective sorghum roots.
- A total of 9 control samples from the three soil samples and three replications (3 soil samples * 3 replications) = 9 control samples were used to compare the change in the functional PGPR relative to the experimental observations.
- Hence, this project had a total of 153 experimental and control observations (144 experimental or treatment and 9 control bulk soils of the three sample types).
- From 153 total observations of this study used only one replication or 39 samples, the rest 114 were lived for the project worker.

1.2. Research Activities and Methods

Activity.1.2.1. Soil sample and pot preparation

- 156 plastic pots with the capacity of 700 g were prepared to cultivate the 12 sorghum genotypes on the three selected soil samples.
- The pots were fulfilled with 500 g collected soil sample of both treatment and control one.
- The fulfilled soils were moistened with sterile distilled water by adding 250 ml.

Activity.3.2.Surface sterilization and germination of sorghum seeds

- 25 Sorghum seeds per genotype having same or nearly the same morphological appearance (shape, color and texture) were selected for surface sterilization.
- The seeds were transferred to autoclaved 50 mL falcon tubes containing sterile 30 mL distilled water with two drops of tween 20.
- The seeds were first thoroughly washed for 15 minutes on a shaker at 120 rpm.

- Surface sterilization was conducted by incubating the seeds in falcon tubes containing 30 mL of bleach (1.5% concentrated) for 5 min with shaking at 120 rpm and followed by washing in distilled water for 5 minutes for 3 consecutive times.
- The sterilized seeds were transferred to autoclaved whatman paper placed in 90 mm Petri dished moistened by 2 ml distilled water.
- The plates were sealed using Para film and incubated at 28°C.
- The seeds were monitored for their spatial arrangement and in case they overlap manual adjustment was made.
- The Petri dishes containing the soaked seeds were sealed with Para film and left to germinate in growth chamber for 2 days at 28°C.

Activity.3.3. Transferring sorghum seedlings to greenhouse pots

- The greenhouse condition was adjusted to 28°C and 16 hours of light and 9 hours of darkness.
- The germinated seeds were planted on the greenhouse pots and watered with 250 mL of distilled water every three days gap.

Activity.3.4. Collecting rhizosphere soil

- After setting the third leaf the roots were harvested by removing the plants from the pots.
- The contents of the pots were discarded carefully on to 70% alcohol cleaned large greenhouse box and the plants were pulled out of the bulk soil.
- The root section grown in the top fill portion was cut off from the rest of the plant.
- The roots were shaken gently to remove the loose attached soil and cut using sterile surgical blade for each plant.
- The root systems were put in a sterile 50 ml falcon tube containing sterilized phosphate buffered solution (30 ml in volume per falcon tube).
- The plant roots were shaked for 30 minutes at 120 rpm and centrifuged at 10000rpm.
- The root portion were removed and transferred to another set of buffer containing tubes, shaken at 120 rpm for 10 minutes, centrifuged at 10000rpm.
- The pellets of the two preparations were mixed after discarding the supernatant.
- The pellets were weighed and kept in a freezer till needed.

Activity.3.5. Rhizosphere soil bacteria isolation

- The pellets in activity 3.4. Were diluted in 1:9 manners in sterile normal saline (0.85 % NaCl) solution.

- Dilutions up to 10^{-8} was made and 100 µl of the solution were spread on nutrient agar plates in three replication for each dilution.
- The plates were incubated for 24 hours at 28°C aerobically and distinct colonies (up to 12 per plates) were picked and transferred to nutrient broth solution and incubated at the same temperature overnight.
- Purification of isolated bacteria was done by streaking the isolates on nutrient agar till consistent morphology is achieved.
- Broth culture of the pure isolates were prepared in two copies of glycerol stocks and preserved at -80°C till needed.

1.4. Screening of the isolated microbes for sorghum PGP traits

Activity.4.1. Screening for ammonia production potential

- Prepare nutrient broth like the above one and full fill 1000 µl to the eppendorf tube.
- Take long term preserved isolates and vortex it very well.
- Take 100 µl of bacterial isolate and put in to the eppendorf broth.
- Vortex in very well to mixes with broth.
- Incubate overnight at the temperature of 28^{o}c.
- Prepare peptone water and full fill 30 ml of peptone to appropriate test tube.
- Take 50 ml from each overnight activated cell suspension to 30 ml of peptone broth then Incubate for 72 h at 25^{o}C.
- After incubation 1ml of Nessler reagent were added to previous broth culture bacteria.
- The formations of brown color are indicator of ammonia production.
- Select only isolates that can produce ammonia and use for farther test.
- Isolates that can't produce ammonia are removed.

Activity.4.2. Screening for IAA production potential

- Take only isolates that can produce ammonia for IAA production as follow.
- Activate selected isolates overnight as like the above one.
- Peptone yeast extract broth were prepare as follow for 1 litter.
- 5ml of prepared peptone yeast extract broth were full filled to appropriate test tube.

- Take 50 µl of bacterial cell suspension of overnight activated isolate and added to 5 ml test tube peptone yeast extract broth.
- Incubate for 72h at 28^{o}C in the dark place.
- After incubation for 72h take 1.5 ml of incubated isolate and put to eppendorf tube.
- Then centrifuge by 10300 rpm for 10 minutes.
- After centrifuge take 1ml of supernatant and add to eppendorf tube.
- 1ml of Salkkawaski reagent (50 ml of35% of perchloric acid and 1mlof 0.5 M $FeCl_3$ solution) was added to 1ml of supernatant.
- Then the culture tubes were incubated at 37^{o}C for 1h at dark place.
- Then formation of red color is indicator of IAA.
- Select isolates that can form red color but isolates that can't for red color were removed.

Activity.4.3. Screening for phosphate solubilization potential

- Take only isolates which have the potential to produce both ammonia and IAA.
- Phosphate solubilization was done by using Pikovskaya agar medium.
- Pikovskaya agar medium was prepared for inorganic phosphate and Autoclave by 121^{o}C for 15 minute.
- Then pour to plate and wait tell solidify the media.
- Then cool down overnight to streak activated isolate.
- Overnight activated bacterial isolates were streaked on the medium.
- The culture mediums were incubated for 48 h at 30^{o}C.
- Then formation of clear halo zone around the media was indicator of phosphate solubilization capacity.
- Measure the halo zone and the isolate area by ruler.

Activity 1.5 Greenhouse Evaluation of bacteria isolates for sorghum growth promotion

Activity.5.1. Sorghum seed selection, sterilization and germination

Seed selection and sterilization were performed according the methods discussed above

Activity.5.2. Pot preparation for sorghum cultivation at greenhouse

- Depending on number of potential isolates identified a subset of sorghum genotypes were used to screen the effectiveness of the isolates in PGP.

- 18 potential isolates were evaluated at greenhouse.
- One susceptible sorghum Genotype Teshale was used.

Activity.5.3. Cultivation of seedling at the greenhouse

- Each pot was planted with 3 seedlings with 3 replication sorghum.
- 100ml of bacterial suspension with the concentration of 10^{-9} will be applied after germination seedling for the test one but the control was used only 100ml distill water instead of isolate.
- The temperature of greenhouse was maintained at 28^{o}C after planting of sorghum.
- Watering (500ml) is done in three days gap regularly at evening time.
- The plants were harvest 5 weeks after the first inoculation.
- Growth promoting ability of isolates were determined based on the data collect plant height, plant dry and fresh weight, root length, root dry and fresh weight with relative to control one.
- Greenhouse arrangement for test treatments were used completely randomized design and for randomization, table number randomization method was used for all treatment.

Appendices 2. Chemical composition of the media

Appendices 2.1. Pikovskaya agar media component grams in litter

Component	Grams/Litter
Yeast extract	0.050g/L
Dextrose	10 g/L
Calcium phosphate	5 g/L
Ammonium sulphate	0.5 g/L
Potassium chloride	0.2 g/L
Magnesium sulphate	0.1 g/L
Manganese sulphate	0.0001 g/L
Ferrous sulphate	0.0001 g/L
Agar	15 g/L

Appendices 2.2. Component of Nessler reagent mL per litter

Component	mL or g/Litter
Distilled water	25mL
Potassium iodide	50g
Mercuric chloride	35mL
Potassium hydroxide (40%)	400mL

Appendices 2.3. Component of Salk waski reagent mL per litter

Component	mL /Litter
35% perchloric acid	50mL
0.5M ferrous chloride solution	1m

Appendices 2.4. Media component for IAA production grams in litter

Component	mL /Litter
Peptone/ ……..	10g
Biff extract…….	3g
Sodium chloride (NaCl)……	5g
L-tryptophan……..	0.204g
Distilled water ……....1litter	
P^H…….	7

Appendices 3. Different ANOVA tables

4.1. ANOVA Table for Plant height

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	583.77	29.18	534	<.0001
Error	36	0.68	0.019		
Corrected Total	56	584.4			

4.2. ANOVA Table for plant fresh weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	135	6.7	262	<.0001
Error	36	0.927	0.025		
Corrected Total	56	135			

4.3. ANOVA Table for plant dry weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	112	5.6	328	<.0001
Error	36	0.61	0.017		
Corrected Total	56	112			

4.4. ANOVA Table for Root length

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	1225	61	359	<.0001
Error	36	6.1	0.17		
Corrected Total	56	1231			

4.5. ANOVA Table for Root fresh weight

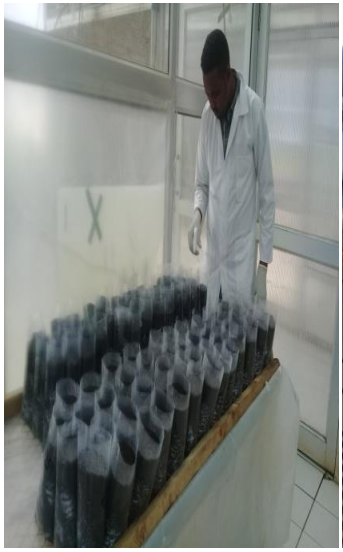
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	405	20	163	<.0001
Error	36	4.4	0.124		
Corrected Total	56	410			

4.6. ANOVA Table for Root dry weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	227	11	600	<.0001
Error	36	0.68	0.018		
Corrected Total	56	227			

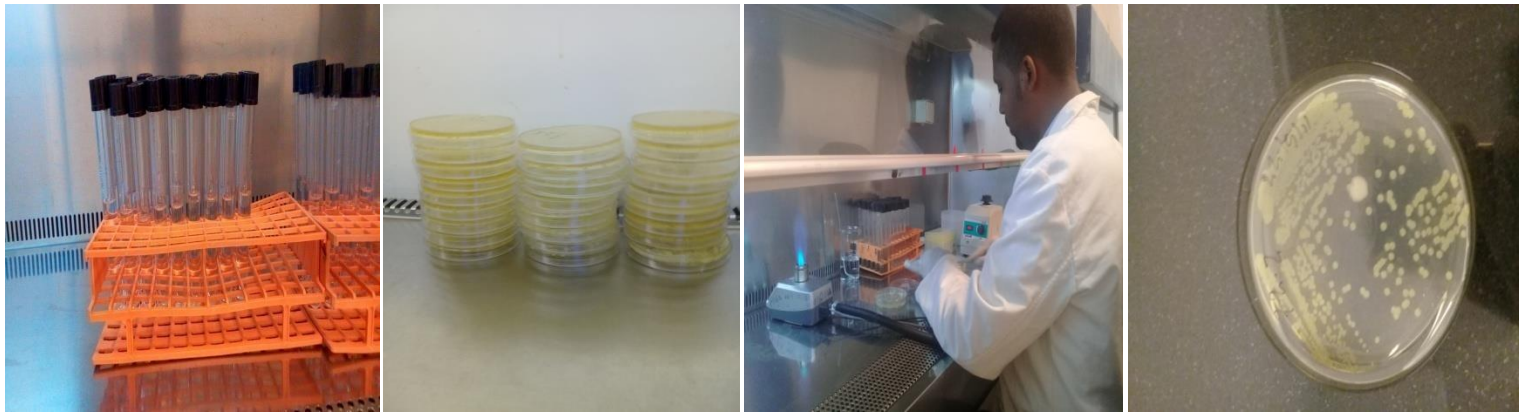
Appendix 4. Photo during the work

Appendix 5.1.Photo during Soil sample preparation, seed sterilization, transfer seedling to pot and cultivate for 40 day.




Appendix 5.2.Photo during rhizosphere Soil sample preparation after 40 days cultivation and isolation of PGPR.

Appendix 5.3.Photo during isolation and purification of PGPR after collecting Rhizosphere soil.



Appendix 5.4. Photo during screening of isolated pure PGPR for PST, IAA and Ammonia.

Appendix 5.5. Photo during Greenhouse evaluation of selected PGPR isolate