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Phosphate solubilizing Rhizobacteria and Their Growth Promoting Ability from Sorghum Rhizosphere soil

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that can improve plant growth by different mechanisms such as phosphate solubilization. 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 Phosphate solubilizing bacteria from sorghum Rhizosphere soil and test for Phosphate solubilizing potential, evaluation of Phosphate solubilizing bacteria at greenhouse for sorghum growth performance potential. So that, in this study a total of 73sorghum rhizobacteria were isolated from the rhizosphere of 12 sorghum genotype by cultivating using 3 soil samples from the northern part of Ethiopia. Isolated PSB bacteria were screened for phosphate solubilization test. From the isolated bacteria 18 isolates were solubilized Phosphorous. Accordingly, eighteen PSB isolates 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 G_6E_{29} and G_4E_{19} had significantly increased all the parameter but two isolates ($G_{12}E_{19}$ and G_3E_{40}) were statistically non-significant for root fresh weight compared to the control..Thus, the use of sorghum 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 potentialof Rhizosphere bacteria in Ethiopia.

Keywords: Sorghum; Rhizosphere; Phosphors and Micro biome.

1. Introduction

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that can improve plant growth by different mechanisms such as phosphate solubilization, synthesis of plant growth hormones, biological nitrogen fixation, 1-Aminocyclopropane-1-carboxylate deaminase and siderophore productions, and antifungal activity (Gupta *et al*., 2018)

Phosphorus (P) is one of the major essential macronutrients for plants and is an indispensable component of nucleic acids (RNA and DNA), proteins, phospholipids, and cofactors (such as ATP). To provide plants with nutritional requirements, P generally relies on the application of chemical P fertilizers to the soil. Nevertheless, these chemical P fertilizers are easily fixed by Ca_2C, Fe_3C, and Al_3C in the soil, thereby resulting in low utilization efficiency (Wei *et al*., 2018; Xie*et al*., 2021). On the other hand, extensive usage of chemical P fertilizer may lead to numerous environmental issues, such as soil compaction and water pollution, and may increase economic burden. Considering the issue arising dueto low efficiency of chemical P fertilizers, it is crucial to improve P availability in soil, particularly for Ethiopian sorghum production area. (Sharma *et al*., 2013; Chakdar *et al*., 2018; Wang *et al*., 2019).

All the necessary biochemical reactions that occur within a plant are dependent upon the availability of phosphorous. It has been observed that majority of agricultural soils contain organic and inorganic forms of P extensively, however, quantity of accessible phosphorous to the plants is meager. Just 0.1 per cent of the overall soil P occurs in a soluble state for plant absorption due to soil fixation and poor solubility of phosphorous in the soil. It has also been

Observed that precipitation and immobilization of P inside the soilis usually related to pH of soils. Phosphorous immobilization in alkaline soils is brought about by calcium (Ca) whereas in acidic soils, fixation of P is brought about by aluminum (Al) and Iron (Fe) oxides. Application of P fertilizers is often practiced to maintain crop production. These when applied to soil get converted into lower solubility phosphorous containing compounds for use in small amounts by the plants. The efficiency of P fertilizers is increased through their repeated application to soil which in turn affects the diversity of microbes resulting in decreased soil fertility and loss in productivity(Gyaneshwar *et al*., 2002; Mahdi *et al*., 2011; Glick., 2012; Pereira and Castro., 2014).

In Ethiopia, sorghumisone of the staple food crops 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 sorghum 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).

Microorganisms play a pivotal role in the cycle of soil nutrients, wherein the phosphate-solubilizing bacteria (PSB) can convert insoluble phosphates into soluble forms that are available for plants. Recently, low-input agriculture has gained immense interest from researchers, with focus given to the development and use of commercial biological inoculants to the increase the

availability of key nutrients, particularly P, to crop plants (Xieet *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 and molecular 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 which can solublizing p (Atera and Itoh, 2011; Xie*et al*., 2015).

The mechanisms of PSB on phosphate solubilization are complex. The redox activity of microorganisms, production of CO2, secretion of siderophores, enzymes, and organic acid, and Nitrogen assimilation was considered to be PSB mechanisms that could transform insoluble P to soluble forms. In general, production of low molecular weight organic acids is the main phosphate-solubilizing mechanism of PSB (Sharma *et al*., 2013; Owen *et al*., 2015;Lelapalli *et al*., 2021).

Gluconic acid has immense importance and is mainly produced by the glucose dehydrogenase (GCD) which is encoded by gcd gene (Liang *et al*., 2020). Besides the solubilizing insoluble phosphates, most PSB can also produce plant growth-regulating substances, such as indole acetic acid (IAA) and ammonia, to promote plant growth (Ludueña *et al*., 2018; Wang *et al*., 2019).

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).

The majority of plant species are associated with PGPRwhich mainly belong to the following genera: Acinetobacter, Agrobacterium, Aeromonas, Alcaligenes, Arthrobacter,Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Bradyrhizobium, Caulobacter, Chromobacterium, Derxia,Enterobacter, Erwinia, Flavobacterium, Frankia, Herbaspirillum, Hyphomicrobium, Klebsiella, Micrococcus, Pseudomonas, Rhizobium, Serratia, Stenotrophomonas, Thiobacillus, Xanthomonas, and Zoogloea (Habibi *et al*., 2014; Lebrazi *et al*., 2020).

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; Hamdali *et al*., 2008 and Mayak *et al*., 2010).

To develop and restore the agricultural and ecological environment in Ethiopia to be suitable for sorghum production by reducing striga weed, Herbicide and chemical fertilizer impact, it is necessary to isolate and use PSB strains that are suitable for the native soil that also present an efficient phosphate-solubilizing ability and plant growth promoting effect in sorghum growing soil in Ethiopia. The present study aimed to isolate PSB strains from the sorghum Rhizosphere soil of

Ethiopia and to analyze the characteristics of phosphate solubilization and plant growth promotion potential. Thus, the research findings will provide a practical ground work for understanding the phosphate solubilization mechanism of rhizosphere bacteria and offer a basis for the ecofriendly application PSB for sorghum production improvement.

2. Materials and Methods

2.1. Soil Sampling for Isolation of Phosphorus solubilizing Bacteria(PSB)

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 were collected are presented in (**Table 1**).

Table 1. Soil sampling area with passport data

No	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

2.2 Rhizosphere Soil Sampling

Phosphorous solubilizing bacteria were isolated from 12 sorghum genotypes (**Table 2**) using 3 soil samples. All the 12 sorghum genotypes were cultivated in the NABRC greenhouse at Holeta; in the three 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 2. 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

2.3. Isolation of phosphorus solubilizing bacteria

To isolate PSB, 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 PSB.

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. The diluted samples were poured on the selective media for *Pseudomona*generafrom 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 *Pseudomona*genera selective medium contain per letter; 10 ml of glycerol, 10g sucrose, 1g casein hydrolysate, 5 g NH_4CL, 2.3g Na_2 HPO_4, 0.6g sodium dodecyl sulfate and 15g agar. The medium has the P^H of 6.8.

The plates were then incubated at 28^OC for 2 days. Individual bacterial colonies were selected and subculture on nutrient agar seven times for purification. Hence, 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^oc and another copy stored in 4^oC refrigerators for the active work.

2.4. Phosphate Solubilization Test

Phosphate solubilization activity of sorghum 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^oC 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.

$$\textbf{Phosphate solubilization index} = \frac{colonydiameter + clearhallozonediameter}{colonydiameter}$$

2.5. Evaluation of PSB Isolates for Sorghum Growth Promotion Efficiency

2.5.1. Inoculum Preparation

The isolates which have the potential to pass the screening test for Phosphate solubilization 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 Phosphate solubilized pure overnight suspension cultures were added to the broth and incubated at incubator shaker for 72 h by adjusting rpm 150 per minute and temperature 28^oc. After 72 h of incubation, the standard concentration was adjusted at 1×10^{-9}

2.5.2. Greenhouse Evaluation

Growth promoting potential of the isolated PSB 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°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 PSB bacterial suspension. The growth-promoting ability of PSB 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 in the unit of centimeter. 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°c for 4 hours and measured the weight using sensitive electronic balance in the unit of gram. The percent (%) of PSB bacterial performance for all agronomic parameters compared to the control was determined using the following formula.

$$\textbf{Increased \%} = \frac{Treatmentvalue - controlvalue}{controlvalue} \times 100$$

2.6. Statistical Analysis

The significance effect of PSB 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.

3. Results and Discussion

3.1. Isolation of phosphate solubilizing Bacteria

In the current study a total of 73 Sorghum Rhizosphere bacterial isolates were isolated. Out of the 73; 18 isolates were solubilized phosphate and selected as a potential PSB sorghum rhizosphere bacteria. However, those 18 isolates (Fig 2) had different potential in their phosphate solubilizing potential; these might be due to the potential of each isolate depending 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 (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),The eighteen potential phosphate solubilizing isolates are; G_4E_{29}; G_5E_{29}; G_5E_{29}; G_8E_{29}; $G_{11}E_{29}$; $G_{12}E_{29}$; G_2E_{19}; G_3E_{19}; G_4E_{19}; G_5E_{19}; G_6E_{19}; G_8E_{19}; G_9E_{19}; $G_{10}E_{19}$; $G_{12}E_{19}$; G_3E_{40}; G_4E_{40}; G_6E_{40}.

Figure 1. Phosphate solubilizing test on the pleat

As the result showed, isolate G_6E_{29} 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 G_5E_{29}, G_4E_{19}, G_9E_{19}, $G_{10}E_{19}$, G_4E_{40} and G_6E_{40} resulted 20.1 mm, 20.1 mm, 21.1 mm, 20.3 mm, 20 mm and 21.23 mm diameter clear hallo zone and Isolates were 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 $G_{11}E_{29}$ which was isolated from SRN39(Purdue University) genotype and Haftay Humera soil; G_5E_{29} which was isolated from Hora-Doldy2 (landrace) genotype and Haftay Humera soil; G_6E_{19} 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 Gottumukkala*et al*. (2016). 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 (Fig 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.

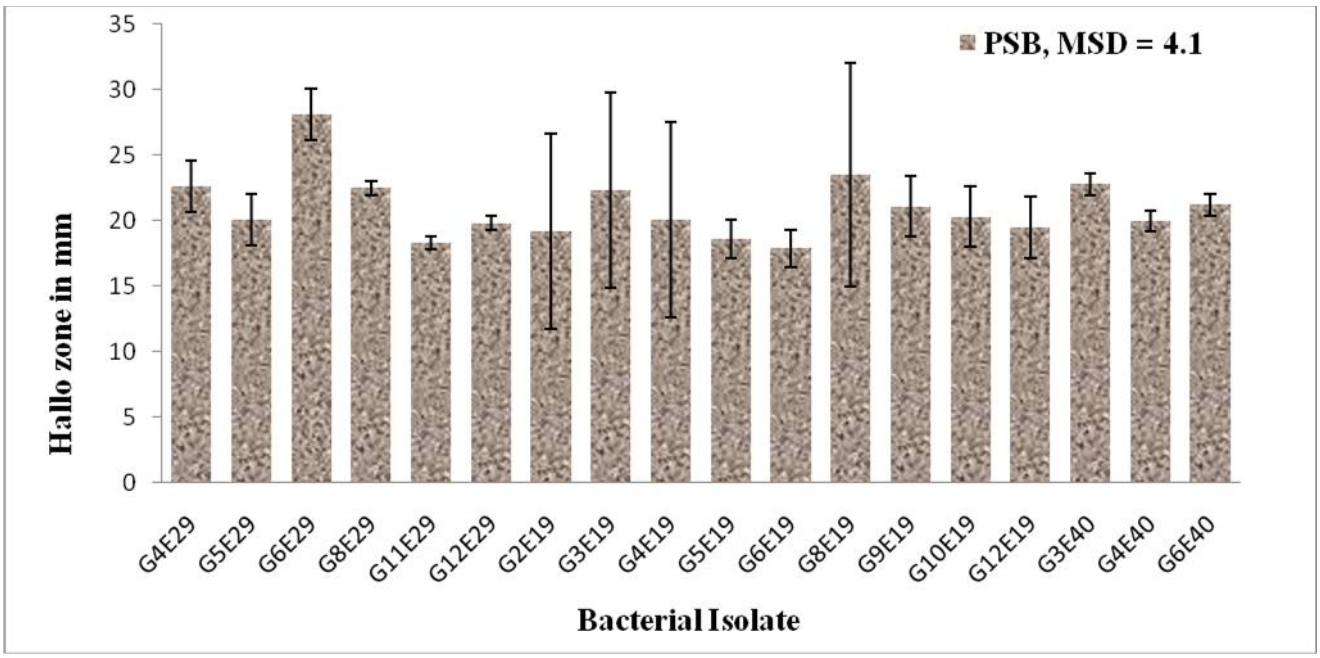

Figure 2. Diameter of phosphate solubilization test clear hallo zone

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 sample. However, the isolate from Kemise had the medium phosphate solubilizing bacteria associated with all sorghum genotype. Isolate from the Soil at Shoa Robit and all sorghum genotypes had low phosphate solubilizing PGPR association; 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.

3.2. Greenhouse Evaluation of PSB isolates 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 3).

Table 3. 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
G_4E_{29}	35.2^{bc}	11.5^{ef}	8.2^{c}	36.2^{bc}	15.4^{bc}	9.5^{bc}
G_5E_{29}	33.2^{d}	11.4^{ef}	5.2^{l}	34.2^{de}	15.1^{cd}	8.8^{de}
G_6E_{29}	35.5^{a}	13.8^{bc}	8.8^{ab}	37.8^{a}	16.3^{ab}	9.7^{b}
G_8E_{29}	31.4^{f}	10.4^{h}	7.0^{fg}	34.1^{de}	14.9^{cd}	9.1^{cd}
$G_{11}E_{29}$	33.2^{d}	10.8^{gh}	7.8^{cd}	33.8^{e}	14.1^{de}	8.8^{e}
$G_{12}E_{29}$	30.2^{h}	9.8^{i}	5.5^{kl}	32.2^{f}	12.2^{fg}	7.2^{g}
G_2E_{19}	31.7^{f}	11.1^{fg}	6.3^{hi}	29.8^{g}	11.3^{gh}	5.1^{i}
G_3E_{19}	32.2^{e}	11.8^{de}	6.9^{fg}	35.2^{cd}	14.2^{de}	6.5^{h}
G_4E_{19}	35.2^{a}	14.3^{a}	9.2^{a}	37.2^{ab}	16.4^{ab}	9.7^{b}
G_5E_{19}	33.5^{d}	13.2^{c}	8.2^{c}	28.2^{h}	13.5^{e}	8.3^{f}
G_6E_{19}	33.1^{d}	13.8^{ab}	8.8^{ab}	31.3^{f}	12.2^{fg}	9.3^{c}
G_8E_{19}	34.6^{b}	14.1^{a}	9.1^{a}	35.6^{c}	12.3^{fg}	8.7^{e}
G_9E_{19}	30.7^{g}	9.7^{ij}	5.8^{jk}	25.1^{i}	10.2^{ij}	6.2^{h}
$G_{10}E_{19}$	34.2^{c}	11.7^{de}	7.4^{de}	32.0^{f}	13.2^{ef}	9.2^{cd}
$G_{12}E_{19}$	32.4^{e}	10.5^{h}	7.1^{ef}	28.2^{h}	9.1^{j}	6.5^{h}
G_3E_{40}	30.3^{h}	9.7^{ij}	6.7^{gh}	27.4^{h}	7.2^{k}	6.4^{h}
G_4E_{40}	34.2^{bc}	12.1^{d}	8.6^{b}	36.2^{bc}	17.1^{a}	12.1^{a}
G_6E_{40}	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 G_6E_{29} was isolated from Jigurti (landrace sorghum genotype) and soil from Humera; it was significantly increased plant shoot height by 75%. Whereas isolate G_4E_{19} was isolated from Framida sorghum genotype and the soil from Shoa Robit; it was significantly increased plant shoot by 74%. Next to G_6E_{29} and G_4E_{19}, three isolates (G_4E_{29}, G_8E_{19} and G_4E_{40}) 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 3), 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 (G_4E_{19}, G_8E_{19} and G_6E_{19}) significantly increased the plant shoot fresh weight. G_4E_{19} 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%. G_8E_{19} 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%. G_5E_{19} 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 G_4E_{19}, G_8E_{19} and G_6E_{29} are significantly increased the plant shoot dry weight. G_4E_{19} 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%. $G8E_{19}$ 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%. G_6E_{29} 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 (Hamdali *et al*., 2018). 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 (G_6E_{29} and G_4E_{19}) significantly increased root length. G_6E_{29} was isolated from the rhizosphere of Jigurti landrace sorghum genotype, and from the soil at Humera; it significantly increased root length by 78%, whereas G_4E_{19} 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 G_4E_{29}, G_4E_{19} and G_4E_{40} have significantly increased the root length next to G_6E_{29} and G_4E_{19}. G_4E_{29} was isolated from the rhizosphere of Framida sorghum genotype, and the soil at Humera, it was significantly increased the root length by 71%. G_4E_{19} 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, 2001). 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 G_4E_{40}, G_6E_{29} and G_4E_{29} have significantly increased the root fresh weight. G_4E_{40} was isolated from the rhizosphere of Framida sorghum genotype, and the soil at Kemise; it was increased the root fresh weight by 74%, G_6E_{29} 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 (G_5E_{29} and G_8E_{29}) 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 ($G_{12}E_{19}$ and G_3E_{40}) 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 G_4E_{40} 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 (G_4E_{29}, G_6E_{29} and G_4E_{19}) 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 3) 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.

The two isolates such as G_6E_{29} and G_4E_{19} 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. PSB rhizosphere bacteria isolated from the soil collected at Humera and Shoa Robit increased all the parameter compared to each other. PSB bacteria which are isolated from the Humera soil had the higher growth promoting potential compared to the soil collected from Shoa Robit, whereas PSB bacteria which are isolated from the soil at Kemise had the growth promoting potential but low growth promoting potential compared to PSB 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 (Ahmad *et al*. 2008).

All the PSB 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 PSB 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 growth promoting potential of phosphate solubilizing PGPR.

Table 4.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
G_4E_{29}	34.3 ± 0.10	11.5 ± 0.03	8.2 ± 0.03	36.2 ± 0.06	15.4 ± 0.05	9.5 ± 0.05
G_5E_{29}	33.2 ± 0.08	11.4 ± 0.15	5.2 ± 0.06	34.2 ± 0.03	15.1± 0	8.8 ± 0.03
G_6E_{29}	35.5 ± 0.05	13.4 ± 0.11	8.8 ± 0.03	37.8 ± 0.01	16.3 ± 0.05	9.7 ± 0.03
G_8E_{29}	31.4 ± 0.05	10.4 ± 0.03	7.0 ± 0.06	34.1 ± 0.03	14.9 ± 0	9.1 ± 0.03
$G_{11}E_{29}$	33.2 ±0.08	10.8 ± 0.03	7.8± 0.06	33.8±0.03	14.1 ±0.03	8.8 ± 0.03
$G_{12}E_{29}$	30.2 ±0.12	9.8±0.03	5.5±0.05	32.2±0.08	12.2±0.05	7.2 ± 0.03
G_2E_{19}	31.7±0.08	11.1±0.06	6.3± 0.01	29.8± 0.03	11.3± 0	5.1± 0.03
G_3E_{19}	32.2 ± 0.06	11.8±0.03	6.9 ± 0	35.2 ± 0.13	14.2±0.03	6.5 ± 0.03
G_4E_{19}	35.2 ± 0.03	14.3 ± 0.11	9.2 ± 0.05	37.2 ± 0.03	16.4 ± 0.10	9.7 ± 0.08
G_5E_{19}	33.5 ± 0.05	13.2 ± 0.08	8.2 ± 0.05	28.2 ± 0.08	13.5 ± 0.86	8.3 ± 0.05
G_6E_{19}	33.1 ± 0.03	13.8 ± 0.03	8.8 ± 0.03	31.3 ± 0.11	12.2 ± 0.08	9.3 ± 0.05
G_8E_{19}	34.6± 0.08	14.1 ± 0.03	9.1 ± 0.03	35.6 ± 0.03	12.3 ± 0.11	8.7 ± 0.08
G_9E_{19}	30.7 ± 0.08	9.7 ± 0.05	5.8 ± 0.03	25.1 ± 0.03	10.2 ± 0.05	6.2 ± 0.08
G_{10}E19	34.2 ± 0	11.7 ± 0.10	7.4 ± 0.089	32.0 ± 0.03	13.2 ± 0.089	9.2 ± 0.03
$G_{12}E_{19}$	32.4 ± 0.12	10.5±0.05	7.1±0.03	28.2± 0.08	9.1 ± 0.03	6.5 ± 0.02
G_3E_{40}	30.3 ± 0.05	9.7 ± 0.11	6.7 ± 0.15	27.4 ± 0.05	7.2 ± 0.05	6.4 ± 0.15
G_4E_{40}	34.2 ± 0.12	12.1 ± 0.06	8.6 ± 0.12	36.2 ± 0.08	17.1 ± 0.03	12.1 ± 0.06
G_6E_{40}	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 PSB for their plant growth promoting potential 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 4). Significant differences were detected between each PSB isolate for all of the studied parameters which indicates that each PSB isolate contrasted 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 PSB sorghum rhizosphere bacteria have different growth promoting potential depending their source.

4. Conclusion

This is the report to reveal the potency of *Pseudomona* Genera, a sorghum rhizobacterium for phosphate solubilization and plant growth promotion potential. In general, phosphate solubilizing PGPR are known for its significant role in improving plant growth, health and crop yield. Hence, based on the finding suggest the usage of this PSB isolates in the preparation of biofertilizers which can be considered as a replacement to chemical fertilizers for sorghum production in Ethiopia.

The two isolates G_6E_{29} and G_4E_{19}showing its sorghum growth promoting potential compared to other PSB isolates and the capability to promote all the parameter growth in broad range of soil sample and sorghum genotype indicates its environment and different genotype adaptability and PSB isolates possessing various enzyme activities for phosphate solubilization and plant growth promoting activity, which are known for its biotechnological and industrial applications in agricultural crop production and productivity, recommends this promising isolate for subsequent research. Strongly conclude that this potential bacterium, *Pseudomona* Genera, has eventual commercial applications after further molecular research.

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