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Indole-3-acetic acid (IAA) Production from Sorghum Rhizosphere Bacteria in Ethiopia

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Abstract: **Indole-3-acetic acid (IAA), a principal phytohormone, controls several crucial physiological processes of plants. Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that can improve plant growth through phytohormone (IAA) production. The application of herbicides and chemical fertilizers to overcome sorghum production constraints are markedly, but those chemicals have negative side effects. The current study was conducted with the objective of isolation of IAA produced sorghum rhizospheric bacteria and optimize for IAA production potential at different concentration of tryptophan and evaluation of IAA produced bacteria at greenhouse for sorghum growth performance potential. So that, in this study a total of 73 sorghum rhizobacteria were isolated from the rhizosphere of 12 sorghum genotype by cultivating using 3 soil samples from the northern part of Ethiopia. Isolated bacteria were tested for IAA production trait at different concentration of tryptophan. From the isolated 73 sorghum rhizospheric bacteria 18 isolates able to 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. Accordingly, the selected 18(eighteen) potential 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 G_6E_{29} and G_4E_{19} had highest IAA production potential and 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. The selected 18 potential isolates were grouped at Pseudomonas genera. Thus, the use of potential IAA produced and plant growth promoting sorghum rhizosphere bacteria to improve sorghum production and productivity in Ethiopia could be useful. However, further molecular identification and evaluation of the isolates exhibited multiple plant growth promoting traits on plant-microbe interaction for economic crop of Ethiopia is needed to uncover their efficacy as effective plant growth promoting rhizosphere bacteria. Therefore, this isolate could be a prospective candidate to be employed as biofertilizers.**

Keywords: **IAA; phytohormone; sorghum; and Micro biome.**

1. INTRODUCTION

In Ethiopia, sorghum is one 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 those 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).

Indole-3-acetic acid (IAA) is a heterocyclic compound containing carboxymethyl group (acetic acid) that belongs to the most studied phytohormone, and is involved innumerous mechanisms in plant physiology (Mike *et al*. 2018). IAA is a vital plant hormone that governs several facets of plant growth and development (Tan *et al*. 2007). It is the most common naturally available and physiologically crucial Phytohormone of the Auxin class, although there are various compounds like 4-chloroindole-3-acetic acid, phenylacetic acid, indole-3-butyric acid and indole-3-propionic acid with similar activity. The role of bacterial IAA is diverse in relation to the interaction between plants and microorganisms. Previously, bacterial Auxin production was believed to be mainly associated with pathogenesis, specifically with bacterial gall formation. However, it is obvious that several phytopathogenic bacteria including gall inducing as well as plant growth promoting bacteria are capable of synthesizing IAA. Besides being produced by plants, it is also synthesized by root associated bacteria like *Rhizobium* sp., *Pseudomonas* sp. And *Azospirillium* sp. (Spaepen *et al*. 2007).

To increase sorghum growth and grain yield by decreasing the impact of production costeraints 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 p^H (Ahemad and Kibret, 2014; Souza *et al*., 2015).

Beneficial bacteria which inhabit the soil of sorghum rhizosphere and manage the sorghum soil environment to achieve attainable sorghum yield. Bacteria use nutrients that are secreted by plant roots within the rhizosphere (Bloemberg and Lugtenberg, 2001). 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 sorghum yield increase (Cook, 2002).

The rhizosphere is the narrow region of soil which is directly influenced by root secretions and associated soil microorganisms. Many bacteria that feed on slough of plant cells, protein and sugars released by root are found in the rhizosphere (Mike *et al*., 2018).

Synthesis of IAA by sorghum rhizosphere bacteria has been directly associated to the development of the sorghum root system and increased root growth and branching (Biswas *et al*. 2018). Therefore, auxins have been discovered in the culture media of *Bacillus pumilus* 8N-4 from rhizosphere of *Triticum aestivum*, *Zea mays* and *Oryza sativa* (Hafeez *et al*. 2006), *Pantoea agglomerans* from Legumes (Serdeva *et al*. 2007), *Rhizobium* sp. from root nodules of *Sesbania sesban*, *Bacillus* sp. from *Solanum tuberosum* (Ahmed and Hasnain, 2010), *Pseudomonas* sp. from apple rhizosphere (Sharma *et al*. 2014), *Pseudomonas fluorescens* from *Gossypium hirsutum* (Nehra *et al*. 2014), *Fluorescens pseudomonads* from *Oryza sativa* (Suresh *et al*. 2014) and *P. fluorescens* and *P. putida* from *Hordeum vulgaris* (Meliani *et al*. 2017).

There are limited research studies on auxin producing bacteria from sorghum rhizosphere in Ethiopia as well as in the world. However, isolated and evaluated the growth promoting capacity the IAA producing plant growth promoting rhizobacteria (PGPR) from sorghum rhizosphere. To mention some of the IAA producing sorghum rhizosphere bacteria and the diversity of plant growth promoting rhizobacteria was documented from rhizosphere of *Glycine max* (Adhikari *et al*. 2012) and *Oryza sativa* (Shrivastava, 2013), and their bio control activities also evaluated (Kumar *et al*. 2018)

Therefore, the objective of this study was to isolate IAA producing bacteria inhabiting sorghum rhizosphere, to estimate and optimize IAA production potential of sorghum rhizosphere bacteria at different concentration of tryptophan and to evaluate its efficiency for growth promoting ability of sorghum seedlings. As per the objectives, this study reports on the production of indole acetic acid by sorghum rhizobacteria and their growth promoting capabilities.

2. MATERIALS AND METHOD

2.1 Soil Sampling for Isolation of IAA producing rhizospheric bacteria

A total of 46 soil samples were collected randomly from the northern part of Ethiopia Tigray and Amhara regions in general and at Humera, Shoa Robit and Kemise specifically, in which sorghum is frequently cultivated for daily consumption of people which inhabited in the sampling area.

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2.2 Rhizosphere Soil Sampling

IAA producing rhizosphere bacteria were isolated from 12 sorghum genotypes (Table 1) using 3 soil samples, which are selected randomly from a total of 46 randomly collected soil samples. All the 12 sorghum genotypes were cultivated in greenhouse at NABRC, Holeta; using the selected 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 Whitman paper on a plate. Finally, the seedlings were transferred to pots in the greenhouse and allowed to grow for 40 days.

Table 1: 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 IAA producing bacteria

To isolate IAA produced sorghum rhizospheric 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 IAA produced sorghum rhizospheric 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. The diluted samples were poured on the selective media for *Pseudomona* genera 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 *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 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 IAA Production Test

IAA Production test was tested by following 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^oC 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% per chloric acid, and 1 ml per 1.5 M of $FeCl_3$ solution. The culture falcon tubes were incubated at 37°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 level of tryptophan high concentration IAA was produced.

2.5 Evaluation of Bacterial Isolates for Sorghum Growth Promotion

2.5.1. Inoculum Preparation

The isolates which have the potential to pass the IAA production 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°c. After 72 h of incubation, the standard concentration was adjusted at 1×10^{-9}

2.5.2. Greenhouse Evaluation

Growth promoting potential of the IAA produced sorghum rhizospheric 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 what man 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 IAA produced 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 in millimeter. 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 milligram. 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 milligram. The percent (%) of IAA produced bacterial performance for all agronomic parameters compared to the control was determined using the following formula.

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

2.6 Statistical Analysis

The significance effect of IAA produced sorghum rizospheric 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.

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3. RESULTS AND DISCUSSION

3.1 Isolation of IAA produced sorghum rhizosphere bacterial

Altogether 73 Rhizobacterial isolates were isolated from the sorghum rhizosphere. All isolates were selected as *Pseudomonas* as common genera using selective media and detection of plant growth promoting substances (IAA). 18 isolates were selected based on their ability to produce IAA in a preliminary screening (Blom *et al.* 2011). Those 18 isolates (Fig 1) had different potential in their IAA production 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 IAA produced 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}.

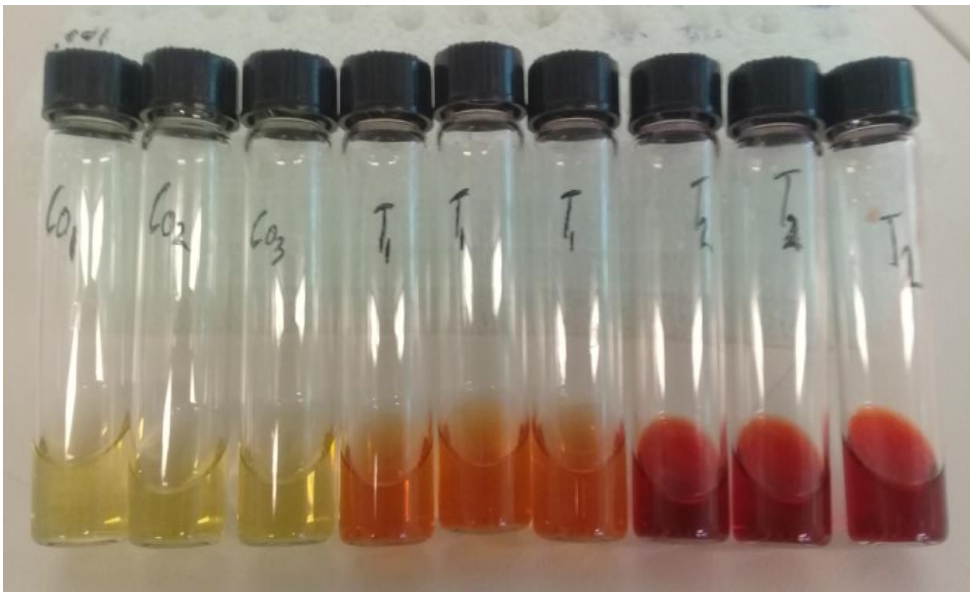

Figure 1: IAA production potential indication isolates

3.2 Optimization of IAA production by different level of tryptophan

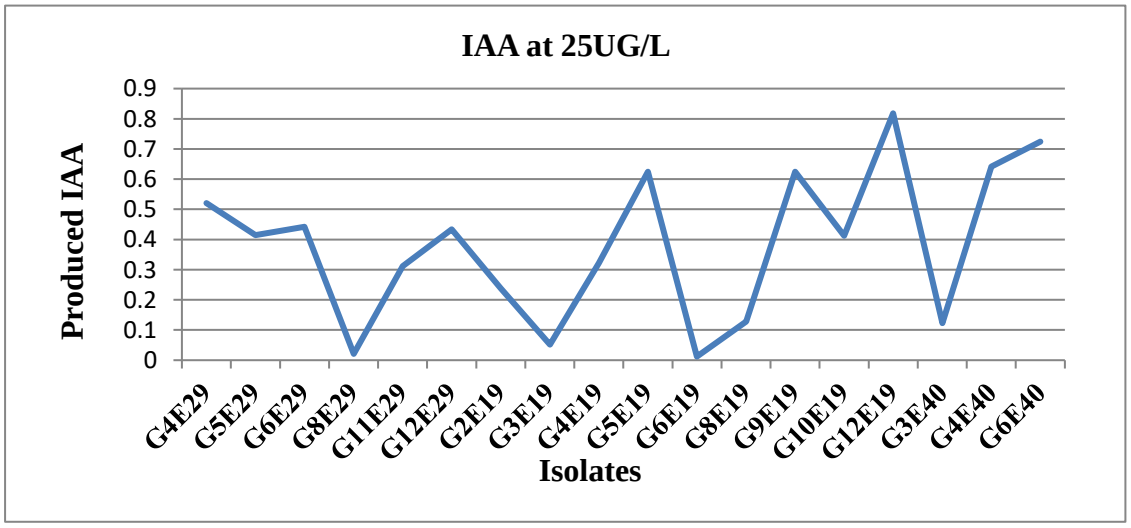

Figure 2: IAA production at 25 mg/L tryptophan

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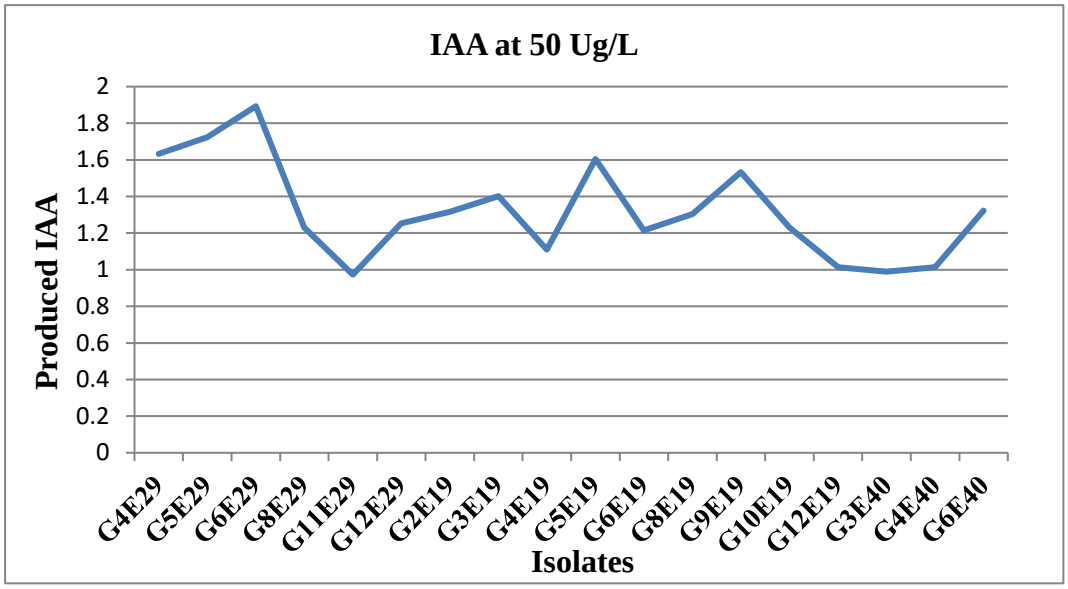

Figure 3: IAA production at 50 mg/L tryptophan

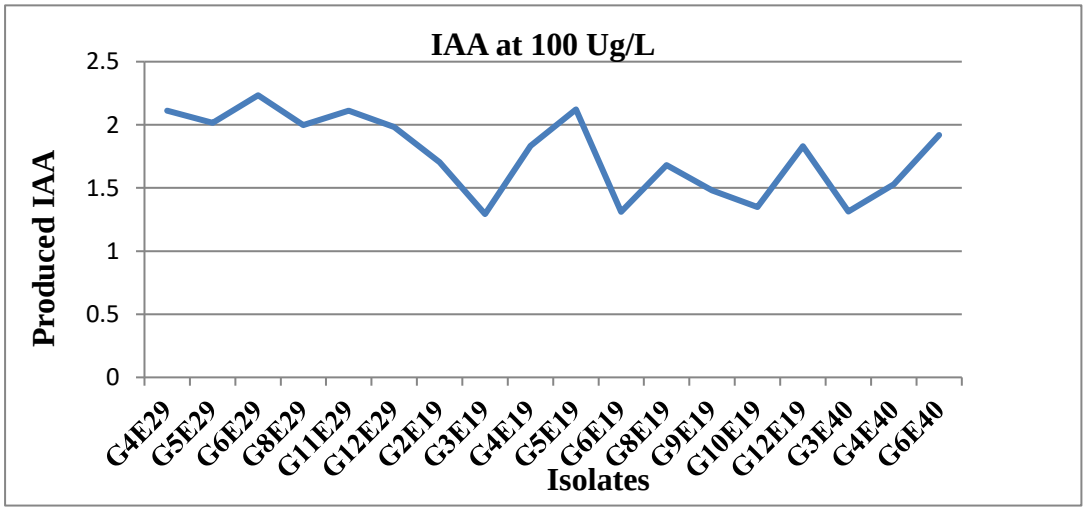

Figure 4: IAA production at 100 mg/L tryptophan

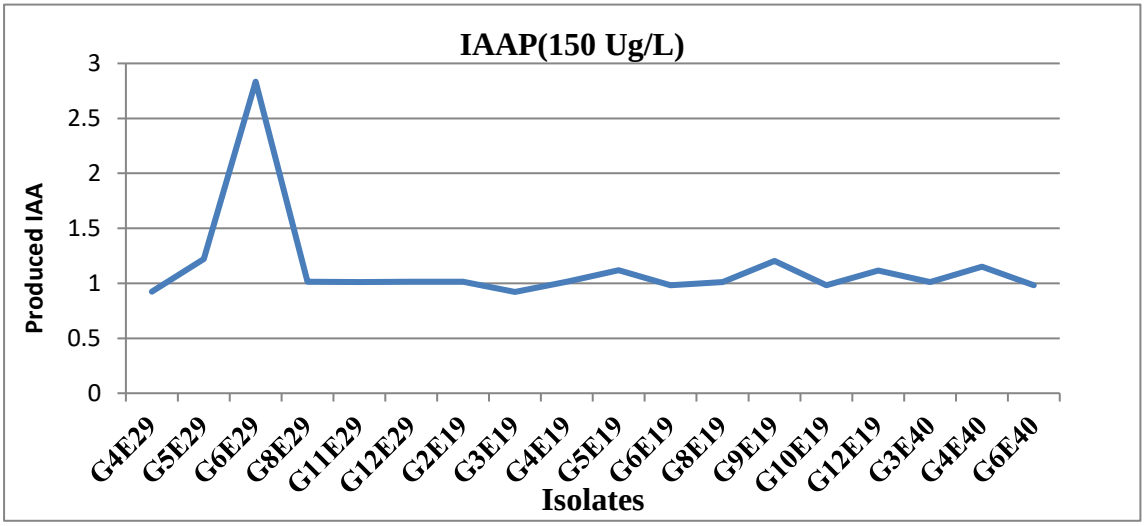

Figure 5: IAA production at 150 mg/L tryptophan

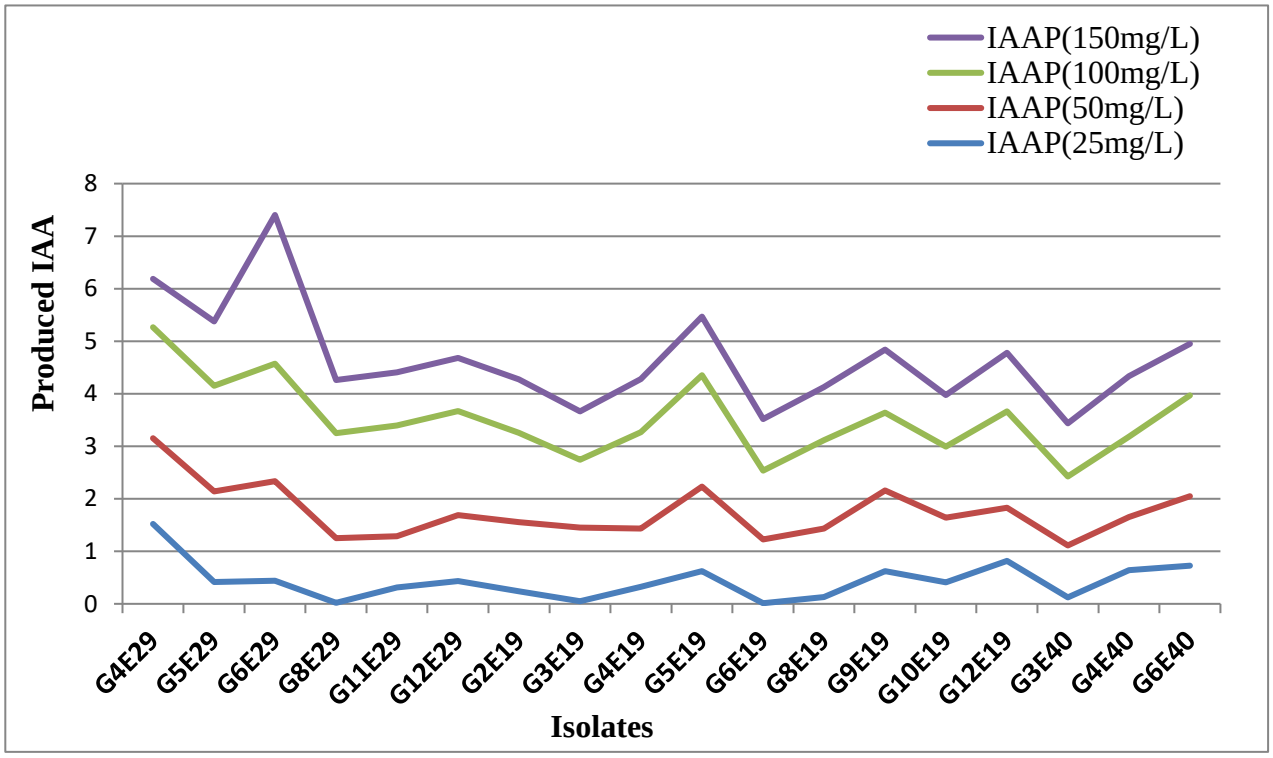

Figure 6: IAA production at 25 mg/L, 50 mg/L, 100 mg/L and 150 mg/L tryptophan

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 2,3,4,5 & 6). 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 (Figure 3), isolate G_6E_{29} 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 G_3E_{19} 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 2)**,** 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 G_6E_{29} increased the production from 1.9 mg/ml to 2.8 mg/ml; G_5E_{19} increased production from 1.3 mg/ml to 2.3 mg/ml, and G_4E_{40} 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 (Figure 5), but one isolate (G_6E_{29}) 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; 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).

3.3 Greenhouse evaluation of IAA produced isolates for Sorghum Growth Promotion performance

All the IAA produced potential 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 2**).

Table 2: 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 G_6E_{29} was isolated from Jigurti (landrace sorghum genotype) and soil from Humera and 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%. 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 2), 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 G_6E_{19} 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%. G_8E_{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 (Miransari and Smith, 2014). The above ground plant biomass growth promoting potential of PGPR also affected by environmental condition, soil type, level of IAA produced by plant and PGPR, 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 IAA produced 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.

Figure 7: Growth promoting ability of selected IAA produced isolates

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. 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%. G_4E_{40} 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 G_4E_{29} 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 2) 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 also belongs to *Pseudomona* bacterial genera and had the highest amount of IAA production potential; which indicates the IAA production potential of PGPR have the potential of growth promotion. 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 along with the IAA production potential affects the growth promoting potential of PGPR.

Table 3: 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 IAA producing sorghum 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 3). 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 sorghum rhizosphere bacteria have different growth promoting potential depending their source and IAA production potential.

3.4. Correlation analysis for agronomic parameter

Table 4: 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 4). 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 such as IAA and affected all agronomic parameter in 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. CONCLUSION

The production of IAA by PGPR isolated from different sorghum genotype rhizosphere and three soil samples is confirmed. This is the very impressing kind of research carried out regarding the production of IAA by PGPR isolated from sorghum rhizosphere soil. This study extends the knowledge on auxin producing PGPR inhabiting the sorghum rhizosphere. *Pseudomona* bacterial genera produces maximum IAA in LB medium with 25mg/L, 50mg/L, 100mg/L and 150mg/L concentration of tryptophan at 28°C after 72 hour of incubation. It demands similar kind of research in rhizosphere of other sorghum genotypes distributed across the country. Bacteria associated with sorghum rhizosphere may be a good source for development of biofertilizers for organic production. Therefore, we conclude that genus *Pseudomona* can be considered as a potential source for the commercial production of IAA which is environment friendly to mitigate chemical pollution of soil in Ethiopia. However, an intensive and extensive study in the field condition is expected particularly for the economically important crops with different types of soil in the field at varying climatic condition

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