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Research Article

Growth Response of (*Coffee Arabica)* to Plant Growth Promoting Rhizobia (PGPR) Inoculant Collected from Rhizosphere

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Abstract

Plant growth promoting rhizobia are beneficial bacteria that colonize plant roots and recover plant growth through wide variety of mechanisms. Productivity of coffee crop in Ethiopia is very low (0.7 ton ha-1 green coffee) as compared to other coffee producing countries. The low level of productivity of the crop stems from poor management of the plant during the initial stage of establishment in the field. The PGPR or Co- inoculant of PGPR and AMF can advance the nutrient use efficiency of plants. The aim of this study was to isolate, characterize (PGPR) and evaluate the Plant Growth Promoting Rhizobacteria inoculation effect on growth of coffee seedlings. Rhizosphere soil Sample was collected from Guwanguwa District Oromiya region. Samples were collected from growing coffee seedling rhizosphere. Random sampling was used to collect soil samples data to isolate PGPR using serial dilution methods. Isolates were allowed for d/f biochemical and physiological test. Each sample was designed in triplicate during laboratory experimentation to avoid variation. Most isolates were efficient in production of IAA, Phosphate solublization, Siderophore production, Hydrogen cyanide and Ammonia production. (PGPR) Isolates were also efficient to show significant effect on growth and growth related parameters of coffee seedling. PGPR inoculated coffee plant showed significance difference at ($P \leq 0.05$) compared to control group. (PGPR) isolates collected from coffee rhizosphere were effective on plant growth promotion. Inoculated plants showed Plant height of 11.71 cm^{-1} plant and un-inoculated plant recorded plant height of 8.87 cm-1 plant. Plant growth promoting bacteria inoculation has shown momentous effect on Coffee Arabica growth. Inoculation of PGPR on the Coffee Arabica Plant has shown substantial effect on increasing the growth & growth related parameters (vigor) of Coffee Arabica. Therefore, it is better to check the effect of PGPR isolates inoculation on yield and yield related parameters (Production) of coffee plant by inoculation on controlled and field trial.

Keywords

Coffee, Inoculation, Plant Growth Promoting Rhizobia, Productivity and Rhizosphere

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1. Introduction

Plant growth promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and recover plant growth through a wide variety of mechanisms [19, 29]. The PGPR or co-inoculants of PGPR and AMF can advance the nutrient use efficiency of plants [9]. Microbial communities in the rhizosphere are complex and have crucial functions that contributies to the development of sustainable agriculture [26]. Plant growth promoting rhizobacteria can affect plant growth via direct or indirect mechanisms. The direct promotions of plant growth by PGPR entails either providing the plant with compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment [10]. Besides IAA production, Ammonia production and HCN production ability most of the rhizobacteria (PGPR) also enhance plant growth by scavenging available iron (Fe3+), Plant growth promoting rhizobacteria can produce a certain amount of siderophore [26]. Coffee is the second most traded commodity in the world next to petroleum that grown in over 70 countries [17]. Coffee plays significant role in the Ethiopian economy [4]. Despite the existence of enormous genetic diversity of *Arabica Coffee* and its importance in the country's economy, productivity of the crop is very low (0.7 ton ha^{-1} green coffee) as compared to other coffee producing countries [9]. Such low level of productivity of the crop stems from erroneous (poor) management of the plant during the initial stage of establishment in the field and the use of weak and whippy seedlings with undesirable shoot and root growth for field planting and soil infertility were the major cause [18]. This comes mainly from lowly seed preparation and handling, use of declined and the application of synthetic fertilizer [19, 6]. To overcome these problems, different scholars carried out different researches. For instance, [5, 6] reported that good seeds preparation and handling gives better seedling than poorly prepared and handled seeds. Forest soil or a mixture of top soils (TS), compost and sand (S) in 3:1:0 or 2:1:1 ratio gives good seedling growth [15]. Plant growth promoting Rhizobacteria are beneficial bacteria that colonize plant roots and recover plant growth through a wide variety of mechanisms [19]. Therefore, this study was aimed to evaluate the plant growth promoting Rhizobacteria (PGPR) inoculation on the growth of coffee seedling.

2. Materials and Methods

2.1. Description of the Study Area

The study area was conducted in oromia Regional State, of Ethiopia, West Guji Zone, Abaya district. The district is located at 367 km away from Addis Ababa to Moyale road. Geographically it was found between 6°10'0"N to 6°30'0"N and 37°50'30"E to 38°18'0"E. bordered by Lake Abaya from the west and Galena district from the South, Sidama zone from the north and Gedeo zone from the East. The study area was located between the altitudinal ranges of 1566 to 1587 m above sea level. Its climate was bimodal rainfall with 800-1300 mm and the mean annual temperature of 16.5 to 28 ℃. The widely grown crops in the area were coffee maize, teff, Common bean, cabbage, banana and Enset. The total population of the study area was 139,914 of which 70,657 are male and 69,257 are female [9]

2.2. Collection and Isolation of PGPR Bacteria

The rhizosphere soil samples were collected according to [7]. The potential of Plant Growth Promoting Rhizobia bacterial isolates were isolated by using the procedure described by [27].

2.3. Characterization Plant Growth Promoting Properties of Bacterial Isolates

2.3.1. Production of in Dole - Acetic Acid (IAA)

IAA production was detected as described by [8, 16]. Freshly grown cultures in the Nutrient broth amended with tryptophan and incubated at 36±2°C for 48-72 h were centrifuged at 7500 rpm for 30 min. After which, 2.0 ml of the supernatant was mixed with two drops of 10 mM orthophosphoric acid and 4.0 ml of the Salkawaski reagent (50 ml, 35% of per chloric acid, 1.0 ml 0.5 M FeCl2 solution). The mixture was incubated at room temperature for 25 min.

2.3.2. Production of Hydrogen Cyanide (HCN)

Freshly grown isolates were screened for HCN production by adapting the method of [22, 16]. Isolates grown in nutrient broth amended with 4.4 g glycine/L were streaked on the modified nutrient agar plate. A sterile what man filter paper no.1 soaked in 2.5% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with Para film and incubated at 36±2 ℃ for four days. Color change was indication of the production of HCN according to [22].

2.3.3. Ammonia Production Test

Freshly grown cultures of the PGPR isolates were inoculated in 10 mL of peptone water in a tube and incubated for 48-72 hrs at 36±2°C. After incubation, 0.5 mL of Nessler's reagent was added in the tube. The development of brown to yellow color was detected for positive result of ammonia production [30, 1].

2.4. Inoculums Preparation and Evaluation of PGPR Isolates on Growth of Coffee Seedling

The isolates (PGPR), from Coffee Rhizosphere soil samples, which have the potential to pass the screening test, were selected as inoculums and evaluated their growth effect on

coffee seedling under agricultural shade by the following method described by [15]. Flasks, which have the capacity of 250 ml, were selected and filled with 150 ml of nutrient broth and was sterilized with steam sterilization method, and cooled down overnight by putting at the hood. Then, 200 µl of pure overnight suspension culture were added to the broth and incubated at incubator shaker for 72 hr by adjusting at 150 rpm and temperature 28 ℃. After 72 hr of incubation, the standard concentration were adjusted for inoculation at 1×10^{-9}.

2.5. Treatments and Experimental Procedure

The experiment was conducted at Dilla University Agricultural shade to evaluate rhizosphere bacterial isolates effectiveness on growth of (*Coffee Arabica)* varieties. Growth promoting potential of the isolated bacteria was evaluated with complete randomized block designs (CRBD) with three replications using *(Coffee Arabica)* seed. The seeds were surface sterilized by the following procedure, the seed washed by distilled water 3 times and then washed with 1.5% of 5% bleach by adding 2 drops [11]. Finally, the seeds were rinsed five times in sterile water and Pots (polyethylene tube) were filled with sterilized (steam sterilized top soil), compost and sand in the ratio of 2:1:1 respectively. Three seeds of (*Coffee Arabica*) varieties were placed in each plastic pots and thinned to one plants at emerging to prevent overcrowded of the plants. 2 ml of active growth of PGPR bacterial isolates were inoculated to per plot using syringe according to procedure of [32]. Complete randomized block designs (CRBD) with three replications were used as experimental design. There was Experimental group PGPR treated coffee seedling and Control group PGPR untreated Coffee seedling in the study.

2.6. Data Collection

Growth related parameters of coffee seedling were collected after treatment with PGPR bacterial isolates. Seedling height, leaf length, leaf width, number of paired leaves, number of nodes, leaf area, internode length, and stem diameter were taken to evaluate the effect of PGPR inoculation on Coffee Arabica Growth and related parameters.

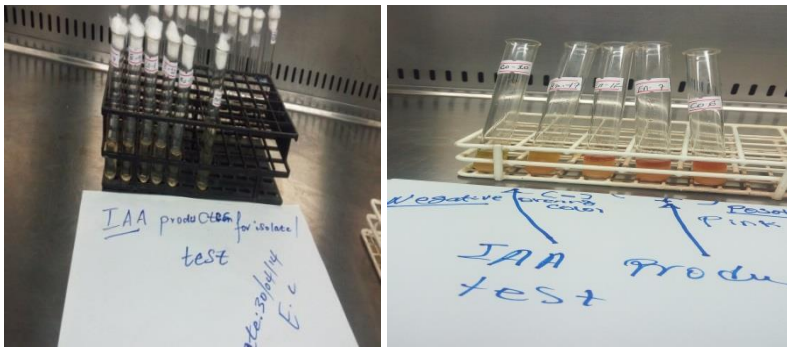
2.7. Statistical Analysis of the Data

The data obtained from the experiments were subjected to statistical analysis of variance (ANOVA) by using SAS software version 9.1. Means of all treatments was calculated and the differences tested for Significance using the Least significance difference (LSD) test at probability ($P<0.05$) level.

3. Result and Discussions

Plant Growth promoting rhizobia (PGPR) bacteria isolates from coffee rhizosphere were allowed for biochemical Characterization. Based on their biochemical characteristics of plant growth promotion efficiency 13(thirteen) isolates were identified with IAA production, HCN production, and Ammonia Production ability and checked inoculum effect on growth of (*Coffee Arabica*) seedling.

Study confirmed that PGPR isolates were capable to produce indole-3-acetic acid (IAA). IAA production was observed as the development of a pink-red color in the test tube. This result was in agreement to [31]. This confirmed that the isolates of rhizosphere PGPR were able to initiate plant growth through development of root to take nutrient from the soil [33]. IAA is one of the most important phytohormones, which may function as an important signal molecule in the regulation of plant growth [12]. This is an important mechanism of plant growth promotion because, IAA promotes root development and uptake of nutrients from the soil [33]. It has long been proposed that IAA act coordinate demand and acquisition of nitrogen and enhance crop yields [30]. The present result of our study was also in line to [30, 33].



(a) (b)

Figure 1. *IAA production of Isolates.*

Rhizosphere bacterial (PGPR) Isolates showed the characteristic of HCN production (Figure 1). Hydrogen cyanide production of PGPR isolates growth on the media changed the color of media from yellow to red orange indicating posi-

tive result for HCN production. HCN production is found to be common trait of PGPR, in the rhizosphere soil and is environmental pollutant and bio-control metabolite in PGPR species [2, 3]. Our result was in line to the result considered by [14]. This indicates that our isolates of PGPR have the activity of bio controlling agent around the rhizosphere of coffee seedling.

(a) (b)

Figure 2. *HCN production of isolates.*

Siderophore production of PGPR isolates was detected in the study. Rhizosphere isolates PGPR of coffee root were capable to show characteristics of siderophore production. It is one of the most abundant mineral deposits on earth. The unavailability of this element in it biological form for plant utilization creates perplexing circumstances for it growth. Siderophore, which literally means iron carrier or iron chelating is an important strategy developed to increase iron (Fe^{3+}) bio-availability as a Unique constituent of cytochrome, enzymes co-factor and heme or non-heme proteins [31, 33]. Suppression of soil borne plant pathogens by siderophore producing PGPR has been reported by [27, 23]. Our result agreed to [30]. Thus siderophore production potential of our PGPR isolates will play important role on the growth of coffee plant by suppressing soil born disease, inadvertently giving the plant to have good vigor index.

Ammonia production test of isolates was positive in the present study. According to [33] another important trait of PGPR is the production of Ammonia that indirectly influences the plant growth. The presence of ammonia producing PGPR Bacteria is an indicative for ammonification process were takes place in the rhizosphere than non -rhizosphere soil [20, 23] Plants can use ammonia as a nitrogen source which affects directly the growth of the plants [13]. Our result was supported by [20].

Table 1. *Soil Physico chemical property of study area.*

pH	Av. (P%)	TN (%)	Av. K (%)
5.13	0.0034	0.94	0.064

(a) (b)

Figure 3. *Ammonia Production potential of PGPR isolates.*

PGPR isolates of rhizosphere showed a significant effect on leaf length at ($p \leq 0.05$)**.** There was significant difference at ($P<0.05$) in plant leaf length b/n treated plant leaf length and control group (Table 2). Analysis of variance showed significant difference ($p \leq 0.05$) on leaf length due to interaction effect of seedling of coffee varieties and PGPR (Table 2). Besides IAA production, most of the soil microorganisms (PGPR) also enhance plant growth by scavenging available iron (Fe3+), Plant growth promoting rhizobacteria can produce a certain amount of siderophore [25, 21]. So the increase in plant height and leaf length might be due to the prominent bio control properties of (PGPR isolates) against pathogenic microbes in iron limiting condition [6, 21].

The highest leaf width was recorded by plants treated with CV1PGPRCS1 mean leaf width of 3.28 cm/p. my result showed that there was mean significant difference b/n coffee variety treated with PGPR isolate and the control group (Table 2). This variation in leaf width of plant treated with

PGPR and control group might be because of treated isolates might have difference Biochemical properties which will promote its growth by, phosphate solublization, IAA production HCN production or siderophore production properties of the isolates [30]. [32, 16] reported that Bacterial isolates of Rhizobia might have a potential of phytopathogen protection in advertently to promote growth of plant. However, there is insufficiency of information on the effect of PGPR effect on perennial plant this research is new and foot standing.

Leaf number and leaf length of coffee seedling were significantly influenced at ($P<0.05$) due to the inoculation of coffee rhizosphere PGPR isolates (Table 2). Leaf surface area of PGPR treated plant were significantly affected than the untreated. Having large surface area of leaf is important for the photosynthesis of plant [28]. The highest leaf surface area was recorded by coffee plant treated with CV1PGPRCS1 with mean (10.36 cm/p), followed by coffee plant treated with CV2PGPRCS1 with mean (10.06 cm/p). in general the leaf surface area of coffee plant treated with coffee rhizosphere was significantly affected at ($P<0.05$) (Table 2). The increase in leaf surface of plant might be because of the potential effect of plant growth promoting Rhizobia isolated from the rhizosphere of coffee [20] reported that rhizobial isolates with plant growth promoting potential might have effect on the growth of plant via different mechanism either directly or indirectly. On the other hand increased plant growth related parameters might be due to the PGPR having Indole- -3- acetic acid production ability of isolates [24]. Our result was in line to [24].

Table 2. *Effect of IAA, HCN and Ammonia producing Bacteria inoculation on growth & related parameters of coffee seedling.*

No	Treatment	PH (cm)	LL (cm)	LW (cm)	NL (cm)	NN	LA (cm)	INL (cm)	SD (cm)
1	CV1PGPRCS1	11.71^{a}	6.10^{a}	3.280^{a}	10.00^{a}	5.00^{a}	10.36^{a}	2.50^{a}	1.54^{a}
2	CV2PGPRCS1	11.12^{b}	6.01^{b}	2.810^{b}	9.00b^{a}	4.66^{ba}	10.06^{b}	2.31^{b}	1.44^{b}
3	CV3PGPRCS1	10.35^{c}	5.45^{c}	2.660^{c}	8.00b^{c}	4.00^{bac}	9.04^{c}	2.24^{c}	1.27^{c}
4	CV1PGPRCS0	9.05^{d}	5.21^{d}	2.136^{d}	7.00d^{c}	3.66^{bc}	5.40^{d}	2.22^{d}	1.06^{d}
5	CV2PGPRCS0	8.87^{e}	3.56^{e}	2.060^{e}	6.00^{d}	3.33^{c}	4.46^{e}	1.73^{e}	1.01^{e}
6	CV3PGPRCS0	7.91^{f}	3.22^{f}	1.656^{f}	5.33^{d}	3.33^{c}	4.26^{f}	1.03^{f}	0.86^{f}
7	*$LSD_{(0.05)}$*	0.018	*0.07*	0.038	1.67	1.32	0.063	0.04	0.01
8	*Mean value*	9.83	4.92	2.4338	7.55	4.00	*7.26*	2.00	1.20
9	CV	2.17	2.17	2.178	2.17	2.17	2.17	2.17	2.17

N.B: PH= plant height, LL=Leaf length, LW=Leaf width, NL=Number of leaf, NN=Number of Node, LA= Leaf area, INL= Internode length and SD= stem diameter, S0 = s not stands for control group. CV= Coffee Rhizosphere plant growth regulating with Variety 1, 2 & 3.

(a) (b)

Figure 4. *Growth of Coffee after inoculation by PGPR.*

4. Conclusion

Characterization of PGPR and evaluating its inoculation effect on Growth of coffee seedling was done successfully. Isolates of PGPR from coffee rhizosphere can improve plant growth and growth related parameters. From isolated PGPR, CV1PGPRCS1 shows maximum effect on different growth and related parameters. Furthermore, in addition to P solublization of PGPR isolates different biochemical characteristics like IAA, HCN and siderophore production of isolates was assessed and detected in the study. Therefore, it is better to check the effect of PGPR isolates inoculation on yield and yield related parameters of coffee seedling by inoculation on controlled and field trial.

Abbreviations

PH	Plant Height
LL	Leaf Length
NL	Nod Length
INL	Internode Length
LW	Leaf Width
LA	Leaf Area
IAA	Indole Acetic Acid
HCN	Hydrogen Cynide

Author Contributions

Andualem Arimo Turito: Formal Analysis, Methodology, Project administration, Resources, Writing – original draft

Adugna Abdissa Belew: Investigation, Validation, Visualization

Nigatu Ebissa Nemomsa: Data curation, Project administration, Resources, Software, Supervision

Muluye Asnakew Alemneh: Methodology, Software, Validation

Conflicts of Interest

The authors declare no conflicts of interest.

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