Open Access

© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 14; Issue 02 (Mar-Apr 2023); pp 445–455

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Fusarium Wilt Biocontrol and Plant Growth Promotion in *Vigna radiata* L. (Mung bean) by a Drought Resistant Halo-alkali-tolerant *Pseudomonas aeruginosa* IRP169

Kankariya Raksha A[1], Chaudhari Ambalal B[2] and Dandi Navin D*[3]

[1-3] Department of Microbiology, School of Life Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon - 425 001, Maharashtra, India

Abstract

The soil-borne fungal phytopathogens of genus *Fusarium* are major threat to crops, particularly in non-disease suppressing soils and jeopardize agricultural productivity worldwide. The study examines rhizobacteria isolated from wheat rhizosphere for its effectiveness as plant growth promoter as well as biocontrol agent against phytopathogen *Fusarium oxysporum* infection of *Vigna radiata* L. (mung bean) by seed priming. A total of 39 rhizobacteria were screened for various plant growth promoting (PGP) traits and showed production of: (i) indole acetic acid (IAA; 94.87%), (ii) ammonia (87.17%), (iii) siderophore (79.48%), (iv) HCN (17.94%), and (v) P solubilization (79.48%). A drought and halotolerant bacterial strain IRP169 exhibited maximum fungal antibiosis against *F. oxysporum*, P solubilization along with pigment, catalase, oxidase, arginine dihydrolase, protease, amylase, IAA, ammonia, siderophore and HCN production. The 16S ribotyping revealed strain IRP169 to be *Pseudomonas aeruginosa* (99.8%). Further, mung bean seed biopriming with strain IRP169 and pot assay showed >90% germination. The seed treatments (i) sick pot (seeds primed with strain IRP169 + *F. oxysporum* mycelia), and (ii) IRP169 bioprimed seeds showed significant increase in all plant growth parameters vis-à-vis control. Thus, *P. aeruginosa* IRP169 emerged as a bespoke PGP rhizobacteria and an effective biocontrol agent for *Fusarium* wilt disease management.

***Key words*:** PGPR, Biopriming, Indole acetic acid, Siderophore, HCN

Globally, soil-borne fungal phytopathogen of Genus *Fusarium*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Verticillium*, and *Phytophthora* mediated diseases of major crop plants reported yield losses of 50-75% despite use of 2.66 and 200.57 million tonnes of synthetic pesticides and fertilizers, respectively [1]. A variety of diseases, such as root rot, stem rot, crown rot, damping-off, and vascular wilts frequently affects crops of economic importance. Crop productivity enhancement strategies such as crop rotation, plant breeding for disease resistance, nutrient inputs, and pesticide application are often limited and inefficient in current intensive agriculture practices [2]. Synthetic fungicides have undoubtedly prevented soil-borne fungal phytopathogen attack to some extent and contributed to increased crop yield, however, the recalcitrant and persistent nature of synthetic pesticides consequently led to biomagnification, residual leaching, soil-biota deterioration and eventual loss of soil fertility, major acute and chronic toxicity to non-target species, pesticide resistance and resurgence of pesticide resistant phytopathogens [3]. The use of synthetic agri-inputs significantly impacts agriculture budget leading to decreased yield and quality which in turn contributes to food shortage, inflation, hunger, and insecurity. As most favourable alternative, eco-friendly plant growth promoting rhizobacteria (PGPR) amendment has gained momentum to minimize the chemical inputs to achieve sustainable agriculture [4]. Rhizobacteria have appeared effective to (i) stimulate plant root growth, (ii) reduce incidence of crop diseases, (iii) provide the essential nutrition for plant growth, (iv) secrete plant root photosynthate exudates such as amino acids, sugars and proteins into the rhizosphere for colonization, (v) foster nutrient cycling, (vi) improve soil disease-resistance characteristic; and (vii) enhance crop productivity by 10-40% [5]. The microbial community in the rhizosphere milieu usually encompass about 10^{11} microbial cells g^{-1} root and more than $3x10^4$ prokaryotic species to promote plant growth either by direct or indirect mechanism [6]. The direct mechanism involves (i) release of indole acetic acid (IAA) and gibberellic acid (GA), (ii) delivery of nutrients through siderophores and phosphate solubilization, and (iii) diazotrophic nitrogen fixation [7]. On the contrary, indirect routes encompass production of (i) antibiotics, (ii)

Received: 28 Dec 2022; Revised accepted: 25 Feb 2023; Published online: 24 Mar 2023

Correspondence to: Dandi Navin D, Department of Microbiology, School of Life Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon - 425 001, Maharashtra, India, Tel: +91 9423951444; E-mail: navineo@gmail.com

Citation: Kankariya RA, Chaudhari AB, Dandi ND. 2023. *Fusarium* wilt biocontrol and plant growth promotion in *Vigna radiata* L. (mung bean) by a drought resistant halo-alkali-tolerant *Pseudomonas aeruginosa* IRP169. *Res. Jr. Agril Sci*. 14(2): 445-455.

hydrolytic enzymes, and (iii) hydrogen cyanide to control the plant pathogen challenge. Several bioactive compounds are secreted by rhizobacteria including 2,4-diacetylphloroglucinol (DAPG), phenazines, oomycin, pyrrolnitrin, pyoluteorin, kanosamine, zwittermycin-A, pantocin, aerugine, cepaciamide A, ecomycins, pseudomonic acid, azomycin, antitumor, cepafungins, antiviral karalicin, surface-active rhamnolipids, biocides like hydrogen cyanide (HCN), cell wall lytic enzymes, and ACC deaminase [8]. Overall, rhizobacteria of the holobiont contributes to biogeochemical nutrient cycling, and phytopathogen control, thereby contribute to improve the agricultural yield. Simultaneously, rhizobacteria reduce the occurrence of disease severity and impart induced systemic resistance (ISR) to the host plant and lowers pollutant toxicity [9]. Of the dominant representative rhizobacteria of genus *Acinetobacter, Agrobacterium, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Burkholderia, Enterobacter, Klebsiella, Rhizobium, Serratia, Thiobacillus, Pseudomonas* and *Bacillus* together with *Streptomyces* are often associated with the rhizosphere and demonstrated beneficial effects in several crop plants [10]. Predominantly, *Pseudomonas* spp. colonize root surface and suppress plant pathogens by producing antagonistic compounds. *Pseudomonas* spp. have demonstrated (i) widespread distribution in global soil, (ii) ability to colonize the host plant rhizosphere even under biotic as well as abiotic stress condition, and (iii) ability to produce a wide range of bioactive compounds inhibiting number of plant pathogens [11]. Members of the Pseudomonas genus, particularly *Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas stutzeri,* and *Pseudomonas fluorescens* are commonly found in the plant rhizosphere to improve plant growth [12] and confer protection to plants against root infection caused by *Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* [13]. The ability of Pseudomonads to suppress soil borne fungal pathogens is attributed to secretion of an array of bioactive metabolites, particularly secondary metabolites viz. pyoluteorin, pyrrolnitrin, phenazines and DAPG [14]. However, in the current scenario, climate-resilient and multi-stress tolerant plant growth promoting (PGP) traits are vital to address uncongenial soil conditions of pH and salinity. At present, the requirement of bespoke and robust rhizobacteria are essential for sustenance of crops under biotic and abiotic stress environment in the soil. Studies had shown that rhizobacterial priming to seeds activate defence responses when challenged by biotic and abiotic stress [15]. Few studies have demonstrated plant growth promotion and antagonistic activity against phytopathogens such as *Rhizoctonia solani*, *Fusarium oxysporum* by microbial biopriming with *Azotobacter*, *Azospirillum* and fluorescent *Pseudomonas* and reported higher grain yield [16]. Among the pulse crop, mung bean (*Vigna radiata* L.) is the major host of the fungal phytopathogen and responsible for 80% yield loss. The genus *Fusarium* is the widest host-range fungal phytopathogen affecting root, shoot and photosynthetic system [17]. The most widespread and frequent Fusarium-induced wilt is caused by saprophytic *F. oxysporum* and *F. solini* whose chlamydospores are capable of survival in wide range of habitats and persist for about a decade in infected soil as well as an endophyte to emerge between cropping seasons impacting subsequent crops. Phytopathogenic *Fusarium* is prevalent in environment under various abiotic and biotic stresses viz. physical (temperature, water, soil compaction, etc.), chemical (herbicide use) and biological (e.g., root lesion nematode) [18-19]. Therefore, the present study attempts to isolate and screen robust stress-resistant rhizospheric bacteria armed with PGP traits as well as *Fusarium* biocontrol by mung bean seed biopriming and as an effective substitute to toxic synthetic fungicide inputs.

MATERIALS AND METHODS

All the reagents, chemicals, solvents, and microbiological culture media used in this study were high purity analytical or research grade and procured from Hi-Media Pvt. Ltd. (Mumbai), Merck (Germany) or Sigma-Aldrich (USA). All the glassware were cleaned with 6N HCl and rinsed thrice with double distilled water before use. Microbial culture *Fusarium oxysporum* MTCC 9913 was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh (India) and maintained at 4˚C with regular subculturing on respective nutrient medium.

Soil collection and analysis

Soil sample was collected from rhizosphere of wheat cultivated in a farm at the depth of 15-20 cm by uprooting wheat. Samples were collected in a sterile plastic bag and processed within 6-12 hours. Further, composite soil sample was analysed for various physical and chemical parameters as per standard protocol [20].

Isolation of saline and drought tolerant rhizobacterial isolates

Wheat root was carefully brushed off to collect adhering soil. Samples (10 g) was added to conical flask containing 100 ml of 0.14 M sodium chloride supplemented with 0.01% Tween 20. Each flask was shaken for 30 min at 120 rpm followed by serial dilution up to 10^{-9}. An aliquot of 100 µl of the diluents were spread plated on non-selective nutrient agar medium in triplicates and incubated at 35ºC for 24-48h. The purified colonies of different morphotypes were separately inoculated in nutrient broth amended with PEG 8000 (150 and 200 gL^{-1}) and with NaCl (50 gL^{-1}) before incubation for 48-72 h at 35ºC, 120 rpm. Each culture was briefly centrifuged and those that showed absorbance >0.5 units were selected as halotolerant and drought resistant strains [21]. All the cultures were maintained on agar slopes and/or glycerol stocks at 4ºC for further use.

Preliminary identification of rhizobacteria

The selected isolates were primarily characterized based on (i) morphological traits such as cell morphology, Gram characteristics, motility, (ii) biochemical characteristics such as pigment production, catalase, cytochrome oxidase, OF (oxidation fermentation) test, arginine dihydrolase as per Bergey's Manual of Systematic Bacteriology. For hydrolytic enzymes, protease and amylase production, the bacterial strains were streaked on to casein hydrolysate medium and starch agar plates, respectively before incubation at 35 ±2°C for 24 h. Post-incubation, clear zone around each bacterial colony was recorded after addition of 1% iodine solution on the starch agar plate.

Assessment of plant growth promoting traits

a) *Indole acetic acid (IAA)*

For screening IAA production by the rhizobacteria, each pre-grown rhizobacterial culture (24 h old) was inoculated into sterile nutrient broth containing tryptophan (50 µg ml^{-1}) and incubated for 48h at 30ºC at 120 rpm. Each culture broth was centrifuged at 5000 x *g* for 30 min. The supernatant was mixed with two drops of o-phosphoric acid and Salkowski reagent in the ratio of 1:1. Development of pink colour was considered positive for IAA production [22]. Further, the absorbance of sample was measured at 530 nm using spectrophotometer (Mini UV 1240 Shimadzu). The amount of IAA produced by isolate

was evaluated from the standard curve of reference IAA in the range of 10–100 µg ml^{-1}.

b) *Ammonia production*

Bacterial isolates were grown separately in 20 ml peptone water broth at 28 ±2°C for 5 days. After incubation, the broth was centrifuged, and aliquots of 0.2 ml culture supernatant was mixed with 1 ml Nessler's reagent. Then, ammonia free distilled water was added to make final volume of 8.5 ml. Colour change (from brown to yellow) was indicative of ammonia production. Quantitative estimation of ammonia was performed using ammonium sulphate (0.1–1 µmol ml^{-1}) as reference compound and absorbance at 450 nm using spectrophotometer.

c) *Siderophore production*

Rhizospheric isolates secreting siderophores in the culture media were determined using the Chrome-Azurol S (CAS) assay [23]. Each pure culture was grown in an iron free succinate medium, incubated at 28 ±2 °C for 24-48 h with constant shaking at 120 rpm. The culture broth was centrifuged at 5000 rpm for 10 min and 50 µl of culture supernatant was mixed with equal amount of CAS reagent. Colour change from blue to orange after 20 min was noted as intensity of blue colour reduced due to the presence of siderophore that chelates with iron. For quantification, absorbance was recorded at 630 nm using succinate medium as blank on a spectrophotometer and siderophore units (%) were calculated using the following formula:

$$Siderophore\ unit\ (\%) = \frac{(Ar - As)}{Ar} \times 100$$

Where;
Ar = Absorbance of reference (succinate medium + CAS assay solution),
As = Absorbance of the sample at 630 nm

d) *Phosphate solubilization*

Phosphate (P) solubilization potential of each isolate was assayed by spot inoculation of pre-grown culture (24 h) on Pikovskaya agar plates containing 1% tricalcium phosphate and incubated for 3-5 days at 28ºC. The formation of transparent halos around each bacterial colony was suggestive of P solubilization activity and the diameter was used to calculate solubilization index (SI) as follows:

$$Solubilisation\ index\ (SI) = \frac{Colony\ diameter + Halo\ zone\ diameter}{Colony\ diameter}$$

e) *Hydrogen cyanide (HCN) and chitinase production*

Each bacterial isolate was spot inoculated on nutrient agar medium containing 4.4 g L^{-1} of glycine. A piece of sterile Whatman filter paper (1 cm^2) was dipped in a solution containing 2 g of picric acid and 8 g of sodium carbonate dissolved in 200 ml of sterile distilled water. The paper was air dried and stacked to the lid of each petri dish. Each plate was sealed with parafilm and incubated at 28 ±2ºC for 48 h. The change in colour of filter paper from yellow to orange-brown was indicative of HCN production [24]. Chitin degradation was monitored by spot inoculation of pre-grown rhizobacterial culture on colloidal chitin agar plates. Chitin hydrolysis was recorded as clear zones around colony after 14 days incubation at 30°C [25].

Antifungal activity of the soil isolates

The antifungal activity of isolates was examined by agar well diffusion method using phytopathogenic *Fusarium oxysporum* MTCC 9913. For this purpose, fungal spore suspension (0.1 ml in sterile saline; 10^6-10^7 spore ml^{-1}) was spread plated on potato dextrose agar (PDA). Wells (8 mm diameter) were prepared with sterile cork borer and separately filled with 100 µl of each rhizobacterial culture. The dual culture plates were observed for clear zone around the well after 3-5 days of incubation at 27 ±2ºC [26]. Sterile distilled water (100 µl) was used as a control.

16S ribosomal DNA sequencing

The genomic DNA of microbial cultures was isolated using single colony lysis method and its quality was evaluated on 1% agarose gel. The 16S rRNA gene was PCR amplified using 0.5 µM each of 27F and 1492R primers (5`-AGA GTT TGA TCC TGG CTC AG and 5`-TAC GGT TAC CTT GTT ACG ACT T, respectively) in 25 µl reaction mixture containing template (40 ng), Taq buffer (1X) with *Taq* polymerase, $MgCl_2$ (1.5 mM) and dNTPs (500 µM). The PCR conditions were 94ºC: 3 min followed by 35 cycles of 94ºC: 30 sec; 50ºC: 30 sec; 72ºC: 90 sec and final extension of 72ºC for 7 min. The PCR products were purified and visualized on 0.7% agarose gel [27]. Then, the forward and reverse DNA sequencing reaction of PCR amplicon was carried out using Big Dye Terminator ver. 3.1 cycle sequencing kit using POP7 polymer on ABI 3730XL Genetic Analyser as per manufacturer instructions. The basecalled nucleotide sequences were used for preparation of contig and comprised of 1489 bp non-ambiguous nucleotides. Phylogenetic analysis was performed through multiple alignments of homologous DNA sequences downloaded from GenBank database in MEGA11 [28] using UPGMA.

Assessment of biopriming mung bean seeds

To assess the outcome of selected rhizobacterial isolate on mung bean (*Vigna radiata* L.) seed germination, biopriming procedure was employed [29]. The bacterial culture was grown in 500 ml flask containing 100 ml LB medium with incubation at 28ºC for 24 h and 120 rpm. Cell pellets were harvested using centrifugation, washed with sterile distilled water, and suspended in sterile saline. Initially, sodium hypochlorite solution at 10% (v/v) was used for surface sterilization of mung bean seeds and then washed thrice with sterile water. The surface sterilized seeds were soaked in the suspension containing 1.5×10^8 cells ml^{-1} for 30 min. About 15 untreated (non-bioprimed) and bacterized (bioprimed) seeds as a control and test, respectively were placed in a sterile petri plate containing moist filter paper and incubated (7 days, 28ºC). The experiment was conducted in duplicate to determine germination percentage and root length variation.

Biocontrol activity against Fusarium oxysporum on mung bean

The most potent rhizobacterial isolate IRP169 was evaluated for its disease suppression ability using pot assay at 30 ± 2°C. For this purpose, farm soil was sterilized thrice at 121°C for 20 min. Each pot was filled with the sterile soil (1.5 Kg) and used in duplicate Sick pots were prepared by amending mycelial fragments of *Fusarium oxysporum* (5%) as required. The experimental setup comprised of pots each containing 30 seeds designated as Treatment I: non-bioprimed (untreated) seeds sown in pots amended with mycelial fragments as control sick pots; Treatment II: non-bioprimed (untreated) seeds sown in pots; and Treatment III: *Pseudomonas aeruginosa* IRP169 bioprimed seeds sown in a sick pot amended with mycelial fragments. Pots were incubated in a greenhouse and periodically watered to maintain consistent soil moisture. After 15 days, plantlets were uprooted, and various parameters viz. root length, shoot length and germination percentage (GP),

Coefficient of seed germination, Seedling Vigour Index-I (SVI-I), Seedling Vigour Index -II (SVI-II), germination speed and typical disease symptoms were recorded [30].

Statistical methods

Data was analyzed to express mean ± standard deviation (SD) of three independent experiments unless otherwise mentioned and interpreted using one way analysis of variance (ANOVA) with $p \leq 0.05$ significance level using MS-Excel spreadsheet program.

RESULTS AND DISCUSSION

Soil characteristics

The detail characteristics of the composite soil sample of the wheat rhizosphere is summarized in (Table 1).

Table 1 Physico-chemical and particle size characterization of soil sample collected from rhizospheric area of wheat from Jalgaon used for isolation rhizobacteria

Properties	Unit	Findings	Max-Min limit	Inference
pH	--	8.0	6.5-7.5	Medium alkaline
Conductivity	mS cm^{-1}	0.61	0-1	Average
Organic carbon	%	1.02	0.40-0.60	More
Sulphur	Kg ha^{-1}	51.22	14-21	Abundant
Potassium	Kg ha^{-1}	873.6	150-200	Abundant
Free calcium	%	14.19	2.5-5.0	Saltier
Humidity	%	2.88	2.5-5.0	Humid
Sand	%	20.06	2.5-5.0	-
Clay	%	23.15	2.5-5.0	-
Poyta	%	15.43	2.5-5.0	-
Fine sand	%	33.08	2.5-5.0	-
Calcium	mEq	48.15	2.5-5.0	High
Magnesium	mEq	6.69	2.5-5.0	High
Sodium	mEq	63.40	5-15 ml	High
Water holding capacity	%	60.19	5-15	-
Unreal density	g cm^3	1.21	5-15	-
Specific density	g cm^3	2.03	5-15	-
Porous percentage	%	43.07	5-15	-
Increased density	%	27.71	5-15	-
Type	--	9.00	5-15	Dried clay

The soil was observed as humid and mildly alkaline (pH 8.0) and sodic with high Na (63.40 mEq), Ca (48.15 mEq), Mg (6.69 mEq), S (51.22 Kg ha^{-1}), K (873.6 Kg ha^{-1}) and a very high-water holding capacity (60.19%). The clay texture (23.15%) possibly promoted moisture, while the average salinity (0.61 mS cm^{-1}) appeared to be most conducive for microbial growth. The high organic C content (1.02%) was indicative of nutrient rich and diverse microbial eco-habitat [31].

Table 2 Morphological and biochemical characteristics of wheat rhizospheric isolates

Group	Strain	Gram character	Motility	Pigment	Catalase	Oxidase	Hugh-Leifson's test	Arginine dihydrolase	Protease	Amylase	Antifungal activity
1	IRP147	-	+	-	-	-	-	-	+	-	-
2	IRP165, IRP180, IRP181, IRP182, IRP183	-	+	-	-	-	-	+	-	-	-
3	IRP184	-	-	-	-	-	-	+	-	-	-
4	IRP163	-	+	-	-	-	-	+	-	+	+
5	IRP166	-	+	-	-	-	-	+	+	-	-
6	IRP164, IRP167	-	+	-	-	-	-	+	+	-	+
7	IRP146, IRP149	-	+	-	-	-	-	+	+	+	-
8	IRP151, IRP157, IRP171, IRP178, IRP179	-	+	-	-	-	-	+	+	+	+
9	IRP169	-	+	+	+	+	O	+	+	+	++
10	IRP168	+	-	+	+	+	-	-	+	-	-
11	IRP145, IRP150, IRP152, IRP158, IRP175	+	-	+	+	+	O	-	-	-	-
12	IRP148, IRP172	+	-	+	+	+	O	-	+	-	-
13	IRP153, IRP154, IRP155, IRP156, IRP159, IRP160, IRP161, IRP162, IRP170, IRP173, IRP174, IRP176	+	-	+	+	+	O	-	+	+	-

Keys: +, positive; -, negative; O, oxidative; IAA: Indole acetic acid

Isolation, identification, and biochemical characterization of potent PGPR

A total of 39 morphologically distinct colonies were obtained from the selected wheat rhizospheric soil. Each culture was designated as IRP145 to IRP184 and purified by successive sub-culturing on nutrient medium. (Table 2) summarizes preliminary culture characteristics of each strain.

Based on the results of biochemical and anti-fungal tests, the wheat rhizosphere microbiota could be distributed into 7 distinct groups, while 6 strains had unique metabolic characteristics. Nearly, half of the isolates were Gram negative and motile. Except IRP169, all Gram-negative strains tested negative for catalase, oxidase, Hugh Leifson's test and pigment production. Similarly, all Gram-negative isolates (except IRP147) used arginine as source of carbon and energy for growth and produced ammonia via arginine dihydrolase (including IRP169). The ammonia produced protects the cell from acidic environment. The starch and casein hydrolysing capability varied among the Gram negative and Gram-positive isolates, but nearly half of the isolates produced protease as well as amylase.

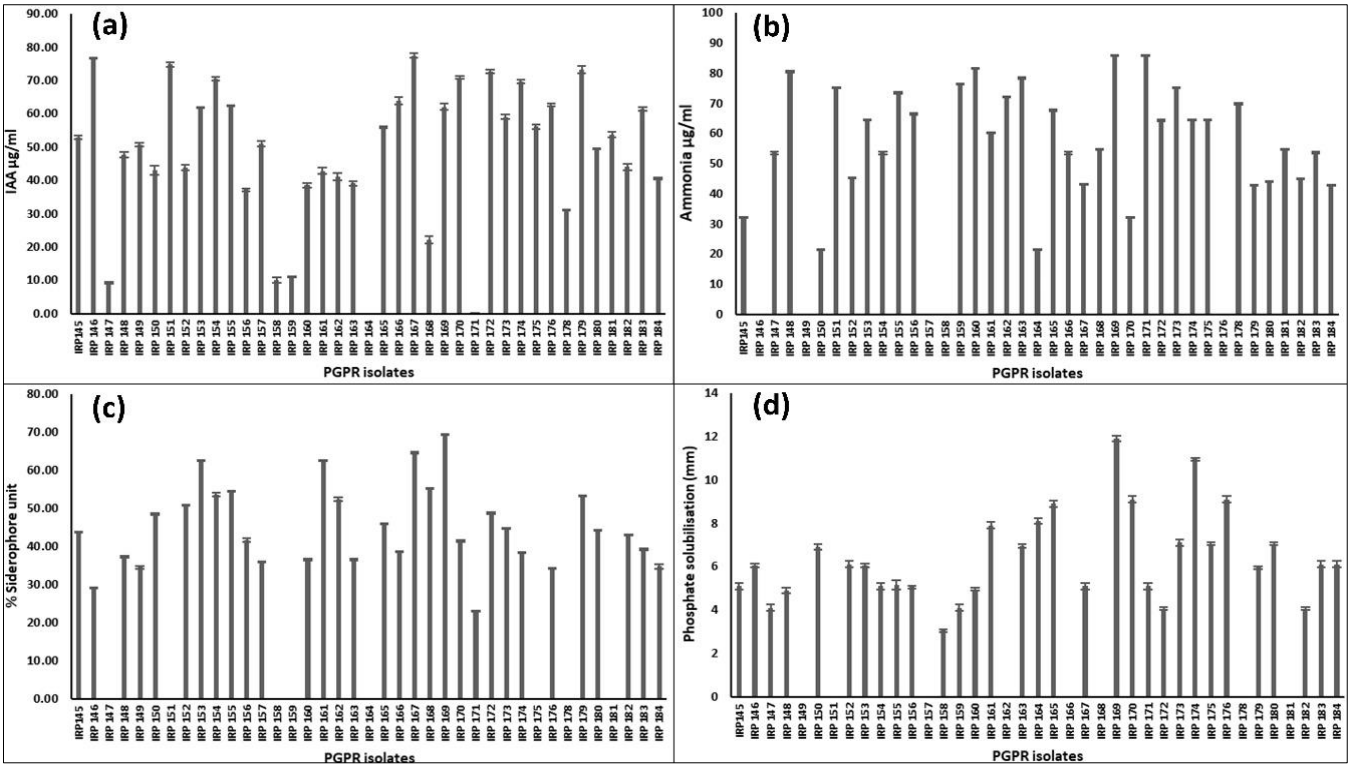

Fig 1(a) Quantitative estimation of IAA production by PGPR isolates in the nutrient medium amended with tryptophan (50 µg ml^{-1}); (b) ammonia production assayed in sterile peptone water and estimated using Nesslerization reaction; (c) estimation of siderophore using high-throughput 96-well CAS-Shuttle assay at A630; and (d) phosphate solubilization profile assayed in Pikovskaya's agar at 30^{o}C for 5 days

Evaluation of PGP traits of rhizospheric isolates

a) *Indole acetic acid production*

Indole 3-acetic acid (IAA), an indole derivative is most common naturally occurring phytohormone essential for plant growth and development and an emerging industrial commodity. Production of such auxins is a desirable PGP trait under drought stress as it stimulates root growth, modifies root system architecture, improves the water captivation and holding capacity [32]. A variety of soil-borne bacterial genera produce IAA which can be quantitated using nutrient broth amended with tryptophan (50 µg ml^{-1}). In the present study, nearly all isolates produced IAA, except isolates IRP164 and IRP171. The isolates IRP146, IRP151 and IRP167 showed IAA production of 76.77, 75.26 and 77.95 µg ml^{-1}, respectively. But isolate IRP147, IRP158 and IRP159 produced meagre viz. 9.46, 10.64 and 11.18 µg ml^{-1} IAA respectively (Fig 1a). The variable amount of IAA production among rhizobacterial isolates is probably dependent on the rate of carbon utilisation. In a recent study, IAA production was improved from 47 µg ml^{-1} to 148 µg ml^{-1} after statistically optimized conditions [33], hence the strains IRP164 and IRP171 hold promise for large-scale production.

b) *Ammonia production*

Nearly all the isolates showed ammonia production as indicated by yellow colouration after addition of Nessler's reagent. Out of 39 isolates, IRP169 and IRP171 produced 85.91 µg ml^{-1}, while isolate IRP172, IRP174 and IRP175 showed 64.51 µg ml^{-1} of ammonia, respectively. Only 5 isolates viz. IRP146, IRP149, IRP157, IRP158 and IRP175 failed to show ammonia in the medium (Fig 1b). As ammonia production is a vital PGP trait for supply of nitrogen to plant, it warrants root and shoot elongation thereby, plant biomass. Ammonia production is found only in selected groups of rhizobacteria. Its accumulation promotes plant growth and concurrently supress fungal pathogens and nitrobacteria in addition to improvising soil pH [34].

c) *Siderophore production*

Except IRP164, all rhizobacterial isolates showed siderophore production. Of these, isolate IRP167, IRP169 were able to secrete significant siderophore (65% units) but isolate IRP161and IRP 153 showed siderophore up to 62% unit (Fig 1c). Siderophores constitute low molecular weight iron-binding ligands secreted by rhizobacteria including, *Pseudomonas* Spp. that binds to ferric ion accessible to the plant [35]. Besides, siderophores secreted by rhizobacteria improves rhizosphere colonization, antagonistic activities and assist in iron availability to plant.

d) *Phosphate solubilization, HCN and chitinase production*

Phosphate solubilization capability of all the isolates was assessed on Pikovskaya's medium amended with tricalcium phosphate. As many as 79.48% of the isolates displayed considerable amount of solubilization of the inorganic tricalcium phosphate after 5 days of incubation. The isolates IRP169, IRP174, and IRP176 showed highest phosphate solubilization (12, 11 and 9 mm, respectively) corresponding to %SI of 3, 2.8 and 2.5%, respectively, however, the isolates IRP149, IRP151, IRP157, IRP162, IRP166, IRP178, and IRP180 could not solubilize phosphate (Fig 1d). Similarly, HCN production from isolate IRP165, IRP167, IRP169, IRP170, IRP171, IRP174, and IRP184 revealed significant change from yellow to brownish red (Fig 2). The phosphate solubilizing action of rhizobacteria provide soluble form of phosphate to plants, while HCN released by rhizospheric *Pseudomonas* facilitates phosphate availability to plant [36]. None of isolates showed chitinase activity.

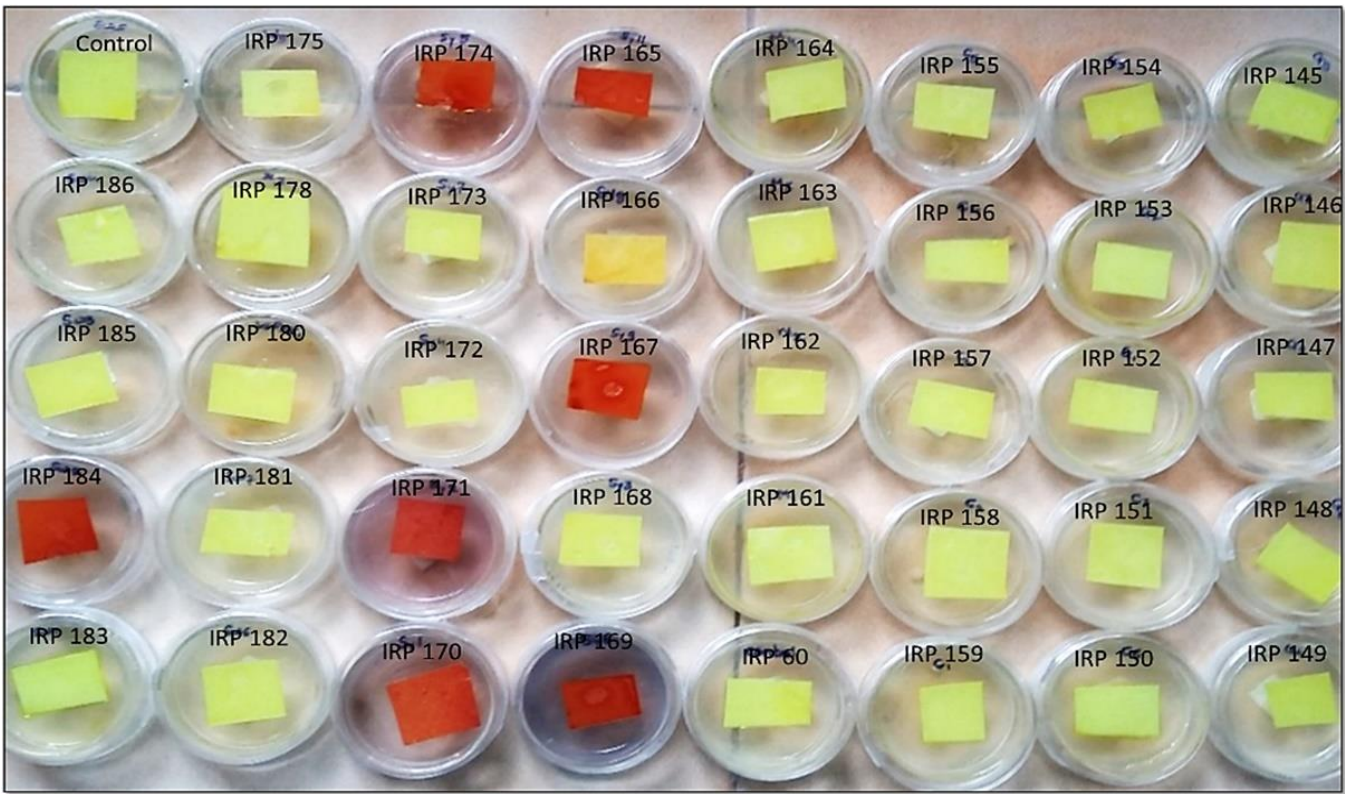

Fig 2 HCN production assay of the wheat rhizobacterial isolates depicting change in yellow to orange colour of filter paper of few isolates

Drought and saline tolerance of the rhizobacteria

All the strains were tolerant to PEG and NaCl induced drought and saline stress showed >0.5 units of absorbance in minor variation. The maximum tolerance was recorded for the isolate IRP169 which can be correlated to high IAA production [37]. The strain was also found to be superior in almost all selected PGP traits (IAA, ammonia and siderophore production, P solubilization) and inhibitory to the phytopathogenic *F. oxysporum*. The strain was biochemically distinct with Gram negative cell, motile, oxidative, and produced catalase, oxidase, protease, amylase, arginine dihydrolase and pigment production. Additionally, it showed drought and saline-stress tolerance.

Evaluation of biocontrol activity of the rhizospheric isolates

Fusarium wilt is a common soil-borne disease caused by members of genus *Fusarium* and *F. oxysporum* is responsible for vascular wilt and root infection in crops [38]. In earlier report, fungal phytopathogens mainly, *Colletotrichum dematium*, *Rhizoctonia solani* and *Sclerotium rolfsii* were inhibited *in vitro* by fluorescent Pseudomonads due to (i) siderophores production, and (ii) secretion of protease [39]. In the present study, all the strains showed varied antagonistic activity and only four groups (based on biochemical characteristics; (Table 2) showed significant zone of inhibition. These included biochemical group 4 strain IRP163 (Gram negative, motile, produce arginine dihydrolase and amylase), group 6 (Gram negative, motile, produce arginine dihydrolase and protease), group 8 (Gram negative, motile, produce arginine dihydrolase, protease and amylase) and group 9 strain IRP169 (Gram negative, motile, oxidative, produce arginine dihydrolase, protease and amylase). All the strains antagonistic to *F. oxysporum* were found to produce arginine dihydrolase. Among these groups, a strain designated as IRP169 exhibited maximum fungal antibiosis (16 mm) against the phytopathogen *F. oxysporum* (Fig 3).

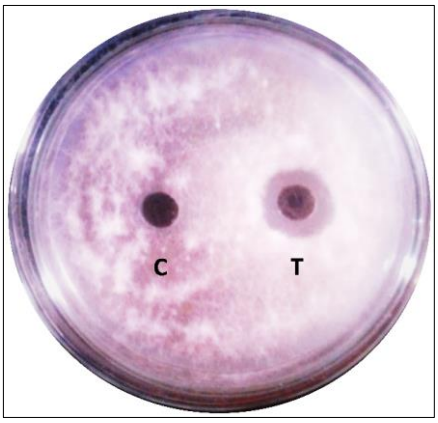

Fig 3 Antagonistic activity of IRP169 against fungal phytopathogen *Fusarium oxysporum* assayed using agar well diffusion method showing zone of inhibition after 3-5 days of incubation at 27 ± 2°C. Keys: C = Control (sterile distilled water), T = well inoculated with 100 µl of culture broth

These indices are comparable with antagonistic activity of *Bacillus altitudins* MS16 against *F. oxysporum* [40]. Hence, isolate IRP169 was selected and characterized by 16S ribotyping and examined for PGP and *Fusarium* biocontrol activity using mung bean seeds under plate and pot experiments.

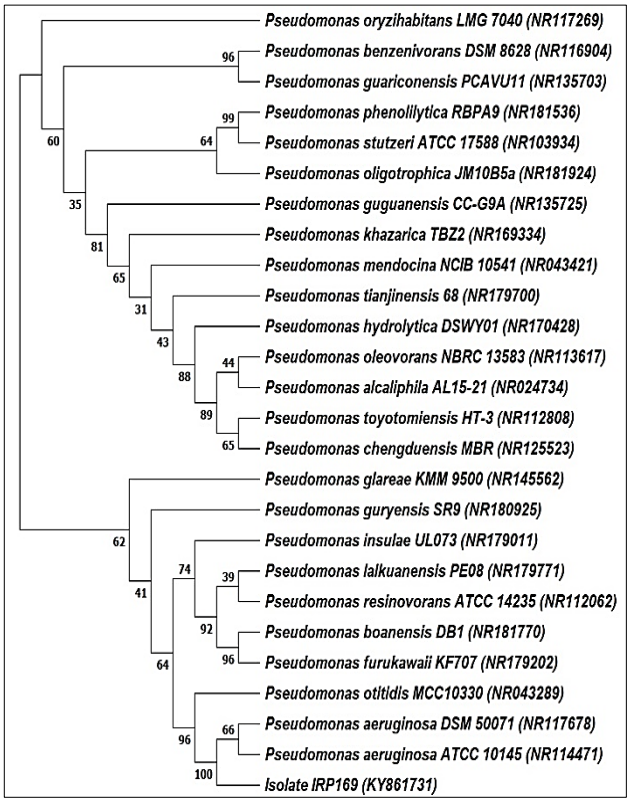

Fig 4 Phylogenetic analysis the isolate IRP169 inferred using the UPGMA method. The bootstrap consensus tree is inferred from 1000 replicates. The figures next to the branches represent percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. The evolutionary distances were computed using the Maximum Composite Likelihood method. The GenBank accession numbers are indicated in parenthesis. The analysis was performed in MEGA11

16S rRNA ribotyping

The genomic DNA extracted from isolate IRP169 showed a single band of high molecular weight DNA when resolved on 1% agarose gel electrophoresis. Further 16S ribosomal RNA gene amplification by PCR resulted in a DNA amplicon of approximately 1500 bp on agarose gel electrophoresis. The automated Sanger's dideoxynucleotide termination cycle sequencing, subsequent base calling, manual curation, and contig construction resulted in a non-ambiguous 1489 bp sequence. The NCBI BLAST analysis revealed close similarity of 99% to genus *Pseudomonas*. Further, UPGMA phylogenetic analysis suggested that the 16S rDNA gene of isolate IRP169 has close resemblance to *P. aeruginosa* with 100% bootstrap support (Fig 4). The DNA sequence data was submitted to NCBI GenBank under the accession number KY861731.

Effect of biopriming P. aeruginosa IRP169 on mung bean seed germination

Biopriming is seen as an emerging delivery method for inducing resistance against phytopathogens. Among these, seed biopriming presents many benefits including (i) activation of various cellular defence responses, (ii) alleviation of physiological and pathological stresses, (iii) plant resistance to phytopathogen challenge, (iv) completion of early germination phases, (v) rapid emergence of seedlings, (vi) facilitation of nutrient uptake, (vii) secretion of phytoalexins, and (vii) induces drought tolerance [41-43]. In the present study, seed germination capacity was primarily investigated using plate assay. The results are shown in (Fig 5, Table 3).

The seeds primed with *P. aeruginosa* IRP169 displayed 90% germination rate, while Vigour Index I and II were 5.2 and 0.7, respectively as compared to the non-bioprimed seeds. The germination (%) and the germination index was prominently higher in bioprimed seeds (90% and 5.7, respectively) as compared to the non-bioprimed seeds (73% and 4.8, respectively). Further, the root, shoot and plant length along with seedling weight were significantly improved due to biopriming. Thus, the improved growth indices can be attributed to the PGP activities of *P. aeruginosa* IRP169. Similar PGP effects were noticed for *P. aeruginosa* BHU B13-398 bioprimed mung bean [44].

Fusarium biocontrol activity of P. aeruginosa IRP169 under pot assay

In the present study, primed seeds were challenged with the test phytopathogen *F. oxysporum* using appropriate controls under pot experiment. The results are shown in (Fig 6, Table 4).

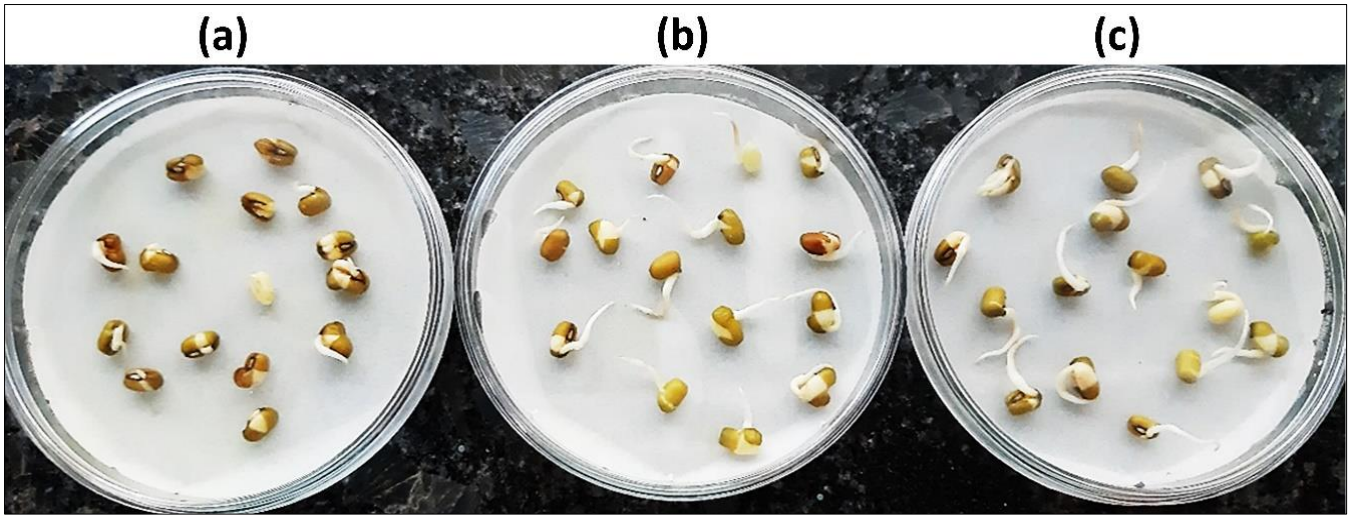

Fig 5 Mung bean seed germination plate assay illustrating (a) non-bioprimed seeds; and (b) and (c) seeds bioprimed with broth culture of IRP169 after 7 days of incubation at 28 °C

Table 3 Mung bean seed germination and growth indices observed in plate assay demonstrating *P. aeruginosa* IRP169 biopriming effect

Parameter	Non-bioprimed seeds	Seeds bioprimed with *P. aeruginosa* IRP169*
Total seeds germinated	11.00	13.50 ±0.71
Germination percent (%)	73.33	90.00 ±4.72
Shoot length (cm)	1.90	2.80 ±0.14
Root length (cm)	2.40	2.95 ±0.21
Plant length (cm)	4.30	5.75 ±0.35
Seedling fresh weight (g)	1.23	1.51 ±0.02
Seedling dry weight (g)	0.42	0.75 ±0.11
Seedling vigour index I	3.15	5.19 ±0.59
Seedling vigour index II	0.31	0.68 ±0.13
Germination speed (day/seed)	2.40	2.89 ±0.26
Germination index	4.80	5.75 ±0.21

*Values are represented as ± standard deviation of mean

Table 4 Evaluation of biocontrol activity of rhizobacterial isolate *P. aeruginosa* IRP169 on phytopathogenic *Fusarium oxysporum* by pot assay

Treatments	I	II	III
Pot amendments	Non-bioprimed seeds + *F. oxysporum* mycelia (Sick pot)	Non-bioprimed seeds (Healthy pot)	Bioprimed seeds + *F. oxysporum* mycelia (Biocontrol pot)
Germination percent (%)	77.78 ±1.15	94.44 ±0.57	97.78 ±0.57
Germination speed	2.87 ±1.23	2.89 ±0.61	2.97 ±0.35
Germination index	9.62 ±1.41	10.60 ±0.35	11.57 ±0.52
Coefficient of seed germination	1.56 ±0.04	1.89 ±0.07	1.96 ±0.05
Shoot length (cm)	21.83 ±0.5	24.20 ±1.05	25.90 ±1.52
Root length (cm)	8.97 ±0.61	12.70 ±1.34	13.40 ±0.72
Length of leaf (cm)	1.77 ±0.05	2.20 ±0.10	2.23 ±0.15
Plant height (cm)	30.80 ±1.11	36.90 ±2.39	39.30 ±2.24
Seedling Vigour Index - I	23.96 ±1.15	34.85 ±1.52	38.43 ±1.64
Seedling Vigour Index - II	0.10 ±0.01	1.66 ±0.03	1.78 ±0.03

*values are represented as ± standard deviation of mean

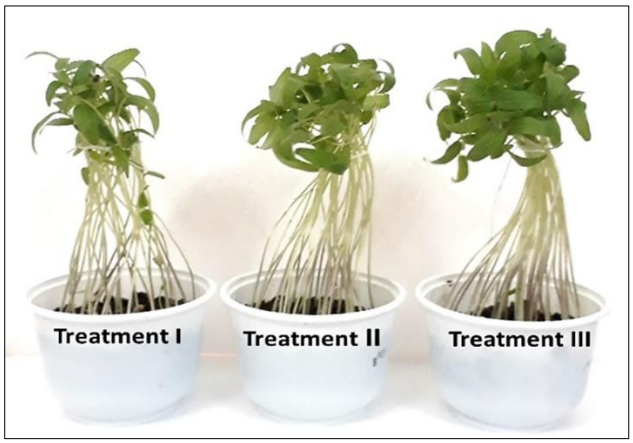

Fig 6 Biocontrol activity of *P. aeruginosa* IRP169 against *F. oxysporum* using mung bean growth assay conducted in pots at 30 ±2°C after 15 days. Treatment I: non-bioprimed seeds inoculated with *F. oxysporum* mycelia (sick pot); Treatment II: non-bioprimed seeds (healthy pot); Treatment III: bioprimed seeds with *F. oxysporum* mycelia (biocontrol pot)

A higher seed germination was noticed for bioprimed seeds challenged with fungi infested soil (Treatment III; 97.78%) as compared to the non-bioprimed seeds (Treatment I; 77.78%) and pots sown with non-primed seeds (Treatment II; 94.44%). The tolerance indices for (i) radicle growth was 8.97, 12.70 and 13.40 cm, (ii) plumule length of 21.83, 24.20 and 25.90 cm (iii) plant heights of 30.80, 36.90 and 39.30 cm, and (iv) germination indices of 9.62, 10.60 and 11.57 were recorded for Treatment I, II and III, respectively. The results prompted that *P. aeruginosa* IRP169 has pronounce biocontrol activity against *F. oxysporum* on seeds of mung bean. The length of leaf was not greatly influenced, but healthy leaves were observed in biocontrol and non-bioprimed pots (Treatment III and II) vis-à-vis sick pot (Treatment I). Overall, it was observed that *P. aeruginosa* IRP196 has a prominent incremental effect in root and shoot length even under *Fusarium*-stress over the non-bioprimed seeds. Based on percent germination, radicle height and plumule length, the order of effectiveness was biocontrol pot (Treatment III) > non-bioprimed pot (Treatment II) > sick pot (Treatment I). Throughout the experiment, the plantlets in the Treatment II and III consistently appeared healthy and fresh than Treatment I, indicating that *P. aeruginosa* IRP169 bioprimed seeds challenged with *Fusarium* phytopathogen (Treatment III) has a dual effect viz. multi-trait PGP activity as well as biocontrol. Previously, the PGP and biocontrol activity of a *P. aeruginosa* strains is reported against phytopathogenic *Rhizoctonia solani* causative to sheath disease of rice [45] and root rot in mung bean [46]. Similarly, *P. aeruginosa* is known to produce antifungal compound against *Macrophomina phaseolina* [47]. *P. aeruginosa* is also reported to control Fusarium wilt in pigeonpea and chickpea caused by *F. oxysporum* f.sp. *ciceris* and *F. udum* [48], *F. oxysporum*, *Trichoderma herizum* and *Alternaria alternata* [49]. The biocontrol potential of these strains was attributed to production of PGP traits (such as siderophore and HCN) and antagonistic compounds such as phenazine-1-carboxamide (PCN) and oxychlororaphin. A *P. aeruginosa* PGPR2 whole genome was mapped for biosynthetic genes of phenazine, paerucumarin, pyocyanin, pyoverdine, pyochelin, 3, 4-dihydroxy-N-methyl-4-(4-oxochroman-2-yl) butanamide and macrolide antibiotics

besides rhamnolipid biosurfactant and quorum sensing N-acyl-homoserine lactone production [50]. In a recent study, *Fusarium* biocontrol and insecticidal activity by a 2,4-diacetylphloroglucinol (DAPG) producing moderately haloalkalitolerant *P. guariconensis* VDA8 in chickpea (*Cicer arietinum* L.) was established [51]. The strain VDA8 was statistically optimized for large-scale DAPG production. The present study points to a similar mechanism of action against the phytopathogenic *F. oxysporum* as the strain IRP169 produce siderophore and HCN. Several rhizobacteria including *Azotobacter chroococcum, Azospirillum spp., Bacillus megaterium, B. azotofixans, B. subtilis, P. putida, P. fluorescens, Serratia ficaria* have been reported for mung bean growth promotion effects with antagonistic activity [52]. A *P. aeruginosa* strain was found to alleviate drought stress in mung bean [53] but rhizobacteria showing tolerance towards saline as well as drought conditions is not reported. In the present study, biopriming of mung bean seeds with *P. aeruginosa* IRP169 has potential to provide protection during the initial phase of plant growth under saline soil environment and draught condition. Additionally, *P. aeruginosa* IRP169 is also demonstrated to produce IAA (61.99 µg ml^{-1}), ammonia (85.91 µg ml^{-1}), siderophore (69.21 units), HCN, phosphate solubilisation and secretion of hydrolytic enzymes (protease, amylase). Thus, the PGP activity of the isolate IRP169 warrants an effective tool to promote high seed germination rate as well as control yield loss due to *Fusarium* wilt incidence, thereby contribute to transform the soil to become disease suppressant soil in a most sustainable manner as compared to conventional synthetic fungicide-based cultivation.

CONCLUSION

The present study proves the saline and drought tolerant rhizobacterial isolate *Pseudomonas aeruginosa* IRP169 to have most prominent PGP traits viz. IAA, ammonia, HCN, siderophore and P solubilization along with effective antifungal activity. Biopriming application of the strain proved supportive to mung bean seed germination and a potential biocontrol tool for prevention of *Fusarium* induced wilt. The multi-trait *Pseudomonas aeruginosa* IRP169 is an effective alternative to chemical fungicides and can be integrated into cost-effective synthetic fungicide-free farming. Further, the strain either alone or with other integrated approaches could lead to improved economic cultivation of mung bean and develop disease-resistant soils. Further study on characterization of the anti-microbial compound produced by *P. aeruginosa* IRP169 and evaluation of its efficacy against different phytopathogens can benefit to develop a commercial scale PGP-biocontrol agent.

Acknowledgement

The work is supported by Rajiv Gandhi Science and Technology Commission, Mumbai (KBCNMU / RGSTC / Sanction Order / 30 (LSR-13) to Dr. Navin Dandi and Ms. Raksha Kankariya by Department of Science and Technology (DST), New Delhi (SR/FST/LS-II/2017/101(C)). Departmental assistance by DST FIST program (SR/WOS-A/LS-1209/2014(G)) and University Grants Commission (UGC), New Delhi under SAP (F.4-23/2015/DRS-III(SAP-II)) is also acknowledged.

LITERATURE CITED

1. FAO. 2022. World food and agriculture—statistical yearbook 2022. FAO, Rome, Italy. https://doi.org/10.4060/cc2211en
2. Panth M, Hassler SC, Baysal-Gurel F. 2020. Methods for management of soilborne diseases in crop production. *Agriculture*,10(1): 16. https://doi.org/10.3390/agriculture10010016
3. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GP, Handa N, Kohli SK, Yadav P, Bali AS, Parihar RD, Dar OI. 2019. Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences* 1(11): 1-6. https://doi.org/10.1007/s42452-019-1485-1
4. Lahlali R, Ezrari S, Radouane N, Kenfaoui J, Esmaeel Q, El Hamss H, Belabess Z, Barka EA. 2022. Biological control of plant pathogens: A global perspective. *Microorganisms* 10(3): 596. https://doi.org/10.3390/microorganisms10030596
5. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology* 18(11): 607-621. https://doi.org/10.1038/s41579-020-0412-1
6. Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PA, Raaijmakers JM. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332(6033): 1097-1100. https://doi.org/10.1016/j.envres.2022.114498
7. Vyas P, Gulati A. 2009. Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiology* 9(1):174. https://doi.org/10.1186/1471-2180-9-174
8. Haas D, Défago G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology* 3(4): 307-319. https://doi.org/10.1038/nrmicro1129
9. Kumar P, Dubey RC, Maheshwari DK. 2012. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiological Research* 167(8): 493-499. https://doi.org/10.1016/j.micres.2012.05.002
10. Tailor AJ, Joshi BH. 2014. Harnessing plant growth promoting rhizobacteria beyond nature: a review. *Journal of Plant Nutrition* 37(9): 1534-1571. https://doi.org/10.1080/01904167.2014.911319
11. Kankariya RA, Chaudhari AB, Gavit PM, Dandi ND. 2019. 2, 4-Diacetylphloroglucinol: a novel biotech bioactive compound for agriculture. *In*: Microbial Interventions in Agriculture and Environment. (Eds) Singh D, Gupta V, and Prabha R. Springer, Singapore. pp. 419-452. https://doi.org/10.1007/978-981-13-8391-5_16
12. Kumudini BS, Jayamohan NS, Patil SV. 2017. Integrated mechanisms of plant disease containment by rhizospheric bacteria: Unraveling the signal cross talk between plant and fluorescent *Pseudomonas*. In: Agriculturally Important Microbes for Sustainable Agriculture. (Eds) Meena V, Mishra P, Bisht J, Pattanayak A. pp 263-291. https://doi.org/10.1007/978-981-10-5343-6_9
13. Noreen R, Ali SA, Hasan KA, Sultana V, Ara J, Ehteshamul-Haque S. 2015. Evaluation of biocontrol potential of fluorescent *Pseudomonas* associated with root nodules of mungbean. *Crop Protection* 75: 18-24. https://doi.org/10.1016/j.cropro.2015.04.018

14. Keswani C, Singh HB, García-Estrada C, Caradus J, He YW, Mezaache-Aichour S, Glare TR, Borriss R. Sansinenea E. 2020. Antimicrobial secondary metabolites from agriculturally important bacteria as next-generation pesticides. *Applied Microbiology and Biotechnology* 104(3):1013-1034. https://doi.org/10.1016/j.cropro.2015.04.018
15. Mitra D, Mondal R, Khoshru B, Shadangi S, Mohapatra PK, Panneerselvam P. 2021. Rhizobacteria mediated seed bio-priming triggers the resistance and plant growth for sustainable crop production. *Current Research in Microbial Sciences* 2: 100071. https://doi.org/10.1016/j.crmicr.2021.100071
16. Rajput RS, Singh P, Singh J, Ray S, Vaishnav A, Singh HB. 2019. Seed biopriming through beneficial rhizobacteria for mitigating soil-borne and seed-borne diseases. *In*: *Plant Growth Promoting Rhizobacteria for Sustainable Stress Management. Microorganisms for Sustainability* (Eds) Sayyed R, Vol 13. Springer, Singapore. https://doi.org/10.1007/978-981-13-6986-5_7.
17. Chang Y, Sun F, Sun S, Wang L, Wu J, Zhu Z. 2021. Transcriptome analysis of resistance to Fusarium wilt in mung bean (*Vigna radiata* L.). *Frontiers in Plant Science* 12: 1213. https://doi.org/10.3389/fpls.2021.679629
18. Pandey AK, Burlakoti RR, Kenyon L, Nair RM. 2018. Perspectives and challenges for sustainable management of fungal diseases of mungbean [*Vigna radiata* (L.) R. *Wilczek* var. *radiata*]: a review. *Frontiers in Environmental Science* 6: 53. https://doi.org/10.3389/fenvs.2018.00053
19. Batzer JC, Singh A, Rairdin A, Chiteri K, Mueller DS. 2022. Mungbean: A preview of disease management challenges for an alternative US cash crop. *Journal of Integrated Pest Management* 13(1): 4. https://doi.org/10.1093/jipm/pmab044
20. Daji JA, Kadam JR, Patil ND. 1996. *A Text Book of Soil Science*. Media Promoters and Publishers Pvt. Ltd., Bombay.
21. Busse MD, Bottomley PJ. 1989. Growth and nodulation responses of *Rhizobium meliloti* to water stress induced by permeating and nonpermeating solutes. *Applied and Environmental Microbiology* 55(10): 2431-2436. https://doi.org/10.1128/aem.55.10.2431-2436.1989
22. Gordon SA, Weber RP. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiology* 26(1): 192. https://doi.org/10.1104%2Fpp.26.1.192
23. Schwyn B, Neilands JB. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* 160(1): 47-56. https://doi.org/10.1016/0003-2697(87)90612-9
24. Kesaulya H, Zakaria B, Syaiful SA. 2015. Isolation and physiological characterization of PGPR from potato plant rhizosphere in medium land of Buru Island. *Procedia Food Science* 3: 190-199. https://doi.org/10.1016/j.profoo.2015.01.021
25. Nawani NN, Kapadnis BP. 2003. Chitin degrading potential of bacteria from extreme and moderate environment. *Indian Journal of Experimental Biology* 41(3): 248-254. http://nopr.niscpr.res.in/handle/123456789/17062
26. Kuzin AI, Tagaev AA, Ovchinnikova TV, Kuznetsova NI, Nikolaenko MA, Morozova OA, Azizbekyan RR. 2019. Study of the strain *Bacillus pumilus* B-13176 and its metabolites with fungicidal and antibacterial activities against *Aspergillus niger* and *Staphylococcus aureus* (MRSA). *Applied Biochemistry and Microbiology* 55(7): 748-755. https://doi.org/10.1134/S0003683819070056
27. Dandi ND, Dandi BN, Chaudhari AB. 2013. Bioprospecting of thermo-and osmo-tolerant fungi from mango pulp–peel compost for bioethanol production. *Antonie Van Leeuwenhoek* 103(4): 723-736. https://doi.org/10.1007/s10482-012-9854-4
28. Tamura K, Stecher G, Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38(7): 3022-3027. https://doi.org/10.1093/molbev/msab120
29. Mahmood A, Turgay OC, Farooq M, Hayat R. 2016. Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS Microbiology Ecology* 92(8): 1-14. https://doi.org/10.1093/femsec/fiw112
30. Dandi B, Dandi N, Chaudhari A, Chincholkar S. 2015. Biodegradation of high gravity distillery effluents using microbes from different ecological habitats. *Environmental Engineering and Management Journal* 14(12): 2871-2880.
31. White RE. 1987. *Introduction to the Principles and Practice of Soil Science*. Blackwell Scientific Publications.
32. Korver RA, Koevoets IT, Testerink C. 2018. Out of shape during stress: A key role for auxin. *Trends in Plant Science* 23: 783-793. https://doi.org/10.1016/j.tplants.2018.05.011
33. Benadjila A, Zamoum M, Aouar L, Zitouni A, Goudjal Y. 2022. Optimization of cultural conditions using response surface methodology and modeling of indole-3-acetic acid production by *Saccharothrix texasensis* MB15. *Biocatalysis and Agricultural Biotechnology* 39: 102271. https://doi.org/10.1016/j.bcab.2021.102271
34. Bhattacharyya C, Banerjee S, Acharya U, Mitra A, Mallick I, Haldar A, Haldar S, Ghosh A, Ghosh A. 2020. Evaluation of plant growth promotion properties and induction of antioxidative defense mechanism by tea rhizobacteria of Darjeeling, India. *Scientific Reports* 10: 15536. https://doi.org/10.1038/s41598-020-72439-z
35. Dane PR, Pawar SP, Kankariya RA, Chaudhari BL. 2016. Pyoverdin mediated sunlight induced green synthesis of silver nanoparticles. *RSC Advances* 6(10): 8503-8510. https://doi.org/10.1039/C5RA20856D
36. Sehrawat A, Sindhu SS, Glick BR. 2022. Hydrogen cyanide production by soil bacteria: Biological control of pests and promotion of plant growth in sustainable agriculture. *Pedosphere* 32(1): 15-38. https://doi.org/10.1016/S1002-0160(21)60058-9
37. Uzma M, Iqbal A, Hasnain S. 2022. Drought tolerance induction and growth promotion by indole acetic acid producing *Pseudomonas aeruginosa* in *Vigna radiata*. *PloS One* 17(2): e0262932. https://doi.org/10.1371/journal.pone.0262932
38. Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD. 2012. The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* 13(4): 414-430. https://doi.org/10.1111/j.1364-3703.2011.00783.x
39. Dunne C, Crowley JJ, Moënne-Loccoz Y, Dowling DN, O'Gara F. 1997. Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. *Microbiology* 143(12): 3921-3931. https://doi.org/10.1099/00221287-143-12-3921
40. Goswami M, Deka S. 2020. Isolation of a novel rhizobacteria having multiple plant growth promoting traits and antifungal activity against certain phytopathogens. *Microbiological Research* 240: 126516. https://doi.org/10.1016/j.micres.2020.126516

41. Liu X, Quan W, Bartels D. 2022. Stress memory responses and seed priming correlate with drought tolerance in plants: An overview. *Planta* 255(2): 1-14. https://doi.org/10.1007/s00425-022-03828-z
42. Aswathi KP, Kalaji HM, Puthur JT. 2022. Seed priming of plants aiding in drought stress tolerance and faster recovery: a review. *Plant Growth Regulation* 97(2): 235-253. https://doi.org/10.1007/s10725-021-00755-z
43. Saha D, Choyal P, Mishra UN, Dey P, Bose B, Prathibha MD, Gupta NK, Mehta BK, Kumar P, Pandey S, Chauhan J. 2022. Drought stress responses and inducing tolerance by seed priming approach in plants. *Plant Stress* 4: 100066. https://doi.org/10.1016/j.stress.2022.100066
44. Kumari P, Meena M, Upadhyay RS. 2018. Characterization of plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere of *Vigna radiata* (mung bean). *Biocatalysis and Agricultural Biotechnology* 16: 155-162. https://doi.org/10.1016/j.bcab.2018.07.029
45. Aye SS, Matsumoto M. 2012. Characterisation of antagonistic soil microbes against *Rhizoctonia* spp. and *Sclerotium hydrophilum*. *Archives of Phytopathology and Plant Protection* 45(20): 2465-2473. https://doi.org/10.1080/03235408.2012.729421
46. Sun F, Sun S, Zhu L, Duan C, Zhu Z. 2019. Confirmation of *Fusarium oxysporum* as a causal agent of mung bean wilt in China. *Crop Protection* 117: 77-85. https://doi.org/10.1016/j.cropro.2018.11.017
47. Shahid S, Khan MR. 2019. Evaluation of biocontrol agents for the management of root-rot of mung bean caused by *Macrophomina phaseolina*. *Indian Phytopathology* 72(1): 89-98. https://doi.org/10.1007/s42360-018-0098-8
48. Anjaiah V, Cornelis P, Koedam N. 2003. Effect of genotype and root colonization in biological control of fusarium wilts in pigeonpea and chickpea by *Pseudomonas aeruginosa* PNA1. *Canadian Journal of Microbiology* 49(2): 85-91. https://doi.org/10.1139/w03-011
49. Bano N, Musarrat J. 2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Current Microbiology* 46(5): 0324-0328. https://doi.org/10.1007/s00284-002-3857-8
50. Illakkiam D, Shankar M, Ponraj P, Rajendhran J, Gunasekaran P. 2014. Genome sequencing of a mung bean plant growth promoting strain of *P. aeruginosa* with biocontrol ability. *International Journal of Genomics* 2014: 123058. https://doi.org/10.1155/2014/123058
51. Kankariya RA, Chaudhari AB, Dandi ND. 2022. Statistical production optimisation, *Fusarium* biocontrol and insecticidal activity of 2, 4-diacetylphloroglucinol produced by a newly discovered moderately haloalkalitolerant *Pseudomonas guariconensis* VDA8. *Zemdirbyste-Agriculture* 109(2): 139-148. https://doi.org/10.13080/z-a.2022.109.018
52. Paul D, Lade H. 2014. Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. *Agronomy for Sustainable Development* 34(4): 737-752. https://doi.org/10.1007/s13593-014-0233-6
53. Sarma RK, Saikia R. 2014. Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. *Plant and Soil* 377(1): 111-126. https://doi.org/10.1007/s11104-013-1981-9.