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Isolation, screening, and characterization of potassium-solubilizing bacteria from the rhizosphere of pearl millet (*Pennisetum glaucum*) and their effect on groundnut (Arachis Hypogaea)

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Abstract
Potassium is the most essential macro nutrient for the growth and development of plants. the current study was undertaken to isolate and characterize the potassium solubilizing bacteria (KSB) from the rhizosphere of *Pennisetum glaucum* grown in Surat. A total of 18 strains were isolated. Isolates were screened for their potassium solubilization efficacy using Aleksandrov agar and broth. Seven isolates were found to be good potassium solubilizers. The isolate PG10 showed the highest zone of solubilization (8.22±0.477cm) and soluble potassium production (125.3330.707 µg mL [1]). All the KSB isolates were characterized based on morphology, gram staining, and biochemical tests. The plant growth-promoting attributes of PG10 strains were assessed by a pot culture experiment conducted with *Arachis hypogaea*. Significant enhancement in the germination and growth of the inoculated seeds was observed. The results strongly indicate that the PG10 strain of *Bacillus spp*. is a potent potassium-solubilizing bacterial bio-inoculant.

Keywords: Potassium solubilizing bacteria, Aleksandrov media, *Pennisetum glaucum, Arachis hypogaea*

1. Introduction

Macro nutrient potassium (K+) is essential for the proper growth of plants [1]. intensive agriculture and high-yield crop varieties have accelerated potassium depletion in soils, leading to increased potassium deficiency [2]. Most of the earth's potassium reserves are in insoluble mineral forms, posing challenges for plant absorption. Soil microbes, especially potassium-solubilizing microorganisms, offer a promising solution to replenish potassium reserves and sustain crop production. Microbes secrete organic acids that help dissolve potassium from rock minerals or chelate silicon ions, improving their availability for plants. And they can release potassium from soil minerals [3]. Inoculating crops like *Cotton*, *rape*, *Pepper*, *Cucumber*, *Sorghum*, *Wheat,* and *Sudan grass* with potassium-solubilizing bacteria (KSB) has resulted in enhanced growth. As a result, the use of KSB as a bio fertilizer has become widespread, supporting sustainable agricultural practices [4-6].

The rhizospheric bacteria residing within the plant rhizosphere including strains of *Pseudomonas*, *Burkholderia*, *Acidothiobacillus, ferrooxidans*, *Bacillus mucilaginosus*, *Bacillus edaphicus*, *B. circulans*, and *Paenibacillus sp*. can convert insoluble soil potassium into soluble form enhancing plant access to this essential nutrient. Identifying efficient rhizobial strains can reduce reliance on chemical fertilizers, conserve resources, and mitigate environmental pollution risks [7-9].

Pearl millet (*Pennisetum glaucum*) is a vital cereal crop, renowned for its ability to thrive in adverse conditions and its importance as a primary food source across diverse global regions [10]. The current investigation aims to isolate bacteria from the rhizosphere of *Pennisetum glaucum* and screen their capacity for solubilizing potassium. Pot assays were conducted with ground nut seeds to evaluate these KSB's efficacy in promoting plant growth.

2. Materials and Methods

2.1 Collection of rhizospheric soil

The rhizospheric soil sample was collected from the rhizosphere of *Pennisetum glaucum* cultivated in the Surat region of South Gujarat. The sample was sealed in a UV-sterilized zip lock bag and carried to the laboratory for further study [11].

2.2 Isolation of the bacterial isolates
Bacterial strains were isolated from soil samples by the serial dilution technique. 0.1 ml of soil suspension was spread on Nutrient agar medium plates and incubated at 37 °C for 48 h. fast-growing and differential colonies were selected for further studies. Pure single-colony isolates were obtained by repeated streaking of bacterial cultures on a fresh nutrient agar medium [12].

2.3 Screening of potassium solubilizing bacteria
2.3.1 Qualitative test
Potassium solubilizing rhizobacteria were isolated using solid Aleksandrov agar medium, which contained (per liter) 5 g glucose, 0.005 g $MgSO_4.7H_2O$, 0.1 g $FeCl_3$, 2.0 g $CaCO_3$, 3.0 g potassium aluminium silicate, 2.0 g calcium phosphate and 30 g agar. After that, the plates were incubated for seven days at 30ºC. Then each of the fast-growing colonies that showed a solubilization zone on the Aleksandrov agar plates was proved to be potassium solubilizing bacteria(KSB) in the primary screening. The K solubilization efficiency was calculated according to the solubilizing index [2].

$$\text{Solubilization Index (SI)} = \frac{\text{colony diameter} + \text{halo zone diameter}}{\text{colony diameter}}$$

2.3.2 Quantitative test
Quantitative estimation of the potassium solubilization was carried out using Alexandrov liquid media and a cobaltinitrite concentration of 12.5% was used to establish a standard curve for potassium. Each test tube containing varying potassium concentrations received five ml of sodium cobaltinitrite solution, with the volume adjusted to 10 ml using distilled water. The mixture underwent incubation at 37°C for 45 minutes to precipitate potassium, the mixture was centrifuged for 5 minutes at 13,000 rpm to settle the precipitate. The precipitate was collected and washed twice with distilled water and finally washed with absolute ethanol after decanting the supernatant. Next, 10 ml of concentrated hydrochloric acid was added to the precipitate and allowed to develop color for 15 to 20 minutes at 37°C. The absorbance was measured at 623 nm. 5 ml of culture supernatant was ascertained under the same conditions [13].

2.4 Morphological and Biochemical Characterization
The isolated bacterial strains were studied for microscopic, morphological, and biochemical characteristics. Morphological characterization was performed, such as size, shape margin, elevation, pigmentation, surface, constituency, and optic characteristics and the Gram staining reaction. For the genus identification biochemical tests like Methyl red (MR), Voges-Proskauer (VP), Catalase, Citrate, Nitrate, Indole, and Triple sugar iron agar (TSI) tests werecarried out [14, 15].

2.5 Effect of KSB on growing groundnut (*Arachis hypogaea*)
A pot assay was conducted using the most potent strain.It was tested on groundnut *(Arachis hypogaea*). Seeds were collected from Bilimora Nursery and Farm, Surat, Gujarat. The groundnut plant was authenticated by an expert [16-18].

2.5.1 Preparation of Bacterial inoculums
The strain showing maximum potassium solubilizing efficiency was grown in nutrient broth in a shaking incubator at 37±2 °C for 24 hours, with agitation at 180 rpm. The bacterial cell density in the suspension was adjusted to a final density of approximately 10^8 CFU ml [1.]

2.5.2 Inoculation of Groundnut seeds
The healthy groundnut seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes followed by repeated rinsing with sterile distilled water to remove any excess sterilizing agent. The pot study was conducted in plastic bags with 18.0 cm × 8.0 cm height and diameter. Groundnut seeds were inoculated with the bacterial suspension for 30 minutes. The seeds inoculated in a nutrient broth medium without bacterial suspension were used as a control. The plant from each pot was uprooted on the 60^{th} day to study the effect of potent strain on plant root length, shoot length fresh weight of root and shoot, number of nodules and number of roots, size of leaves, number of flowers, and individual plants [19].

2.6 Statistical analysis
Statistical analysis was evaluated by analysis of variance using the Statistical Analysis System software (WASP) Web Agri Stat Package. The experimental design comprised a complete randomized design (CRD) with a plot arrangement of treatment in seven replicates. Represent significant differences from respective control according to ANOVA.

3. Results and Discussion
3.1 Collection of rhizospheric soil sample
The rhizospheric soil sample of *Pennisetum glacum* (variety Ghb 526) was collected from Surat.

Fig 1: Rhizospheric soil sample of *Pennisetum glacum*

3.1 Isolation of the bacterial isolates
18 bacterial strains were isolated from the rhizospheric soil of *Pennisetum glaucum*. The strains were screened qualitatively for their ability to solubilize potassium and were further evaluated quantitatively. According to a previous report by Sharma, *et al*., 2014 [10] total 32 rhizospheric and non-rhizospheric bacterial strains were isolated from pearl millet rhizosphere and non rhizospheric area, Similar studies were carried out by Boobalan *et al*., 2021 [20] total 18 different isolates were isolated from the rhizosphere of minor millets.

3.2 *In vitro* screening of the potassium solubilizing bacteria
3.2.1 Qualitative test
A total of seven potassium-solubilizing bacterial strains were obtained from the rhizosphere of *Pennisetum glaucum*. All the bacteria were capable of solubilizing potassium. The K solubilization efficiency of the isolates ranged from 2.75 to

8.22 cm (Table 1). Strain PG10 showed the highest pronounced solubilization ability in agar plates. A study conducted by Fatharani and Rahayu, 2018 [1] reported 22 isolates were potassium-solubilizing bacteria (KSB) from the rhizospheric soils of paddy (*Oryza sativa* L.). Among these isolates, seven demonstrated the capability to solubilize Feldspar when cultured on an Alexandrov agar medium. The solubilizing index (SI) ranges from 2.37 cm to 5.25 cm. Similarly Dhaked*et al.*, 2017 [5] documented those four bacterial isolates (KSB-1, KSB-2, KSB-3, and KSB-4) from various rhizosphere soils, demonstrated clear zones indicative of potassium solubilization. Among these, KSB-2 was from the cotton rhizosphere, KSB-1 and KSB-4 from maize, and KSB-3 from the rice rhizosphere.

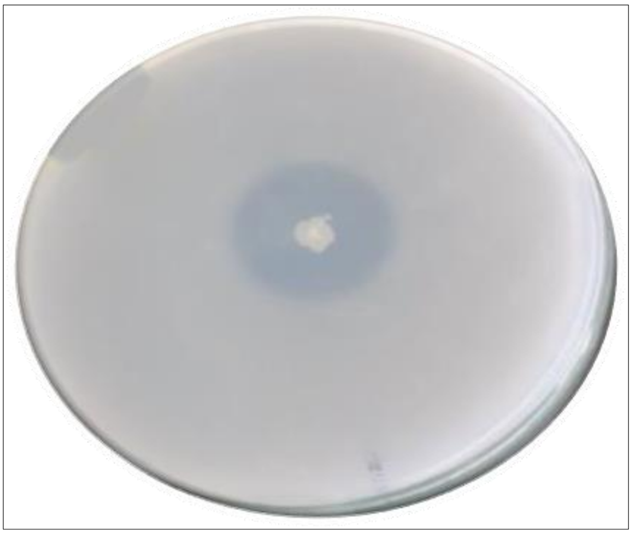

Fig 2: Isolate PG 10 on the solid Aleksandrov medium

Table 1: Screening of potassium solubilizing strains

Colony No	Solubilization index (SI) in cm
PG3	6.70±0.22
PG5	6.73±0.40
PG6	7.83±0.38
PG8	7.66±0.384
PG10	8.22 ±0.477
PG12	2.75 ±0.485
PG18	3.40±0.228

*The result indicates the mean of triplicates ±S.E

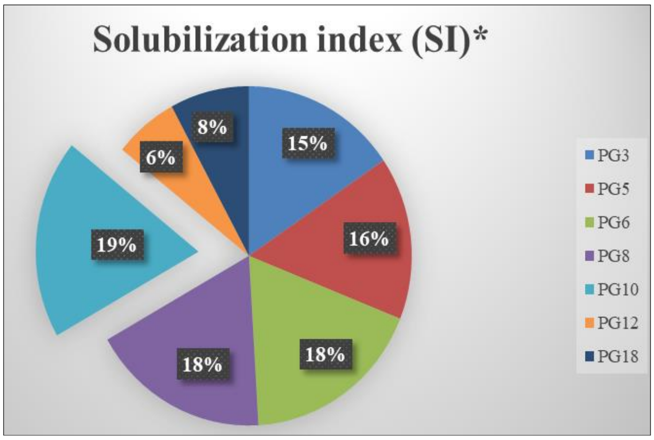

Fig 3: Potassium solubilizing efficiency of isolates

3.2.2 Quantitative test

The potassium solubilizing activities of all selected strains were determined quantitatively by the UV-VIS spectrophotometric method at 623 nm using KCl as standard ranging from 20-120µg/ml, (Figure 4). The range of concentration of soluble potassium varies from the concentration of 73.833±0.0833µg/ml observed in the isolate PG12 to maximum concentration 125.333±0.707 µg/ml detected in isolate PG10 (Figure: 05). Boubekri *et al.*, 2021 [20] demonstrated that the bacterial strains successfully solubilize potassium on Alexandrov medium.

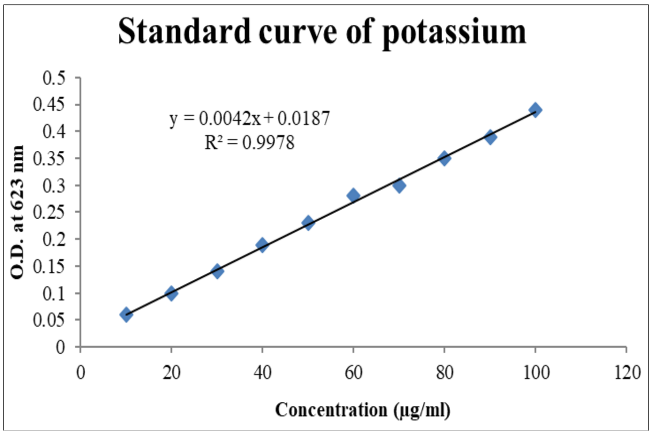

Fig 4: Standard for potassium solubilization

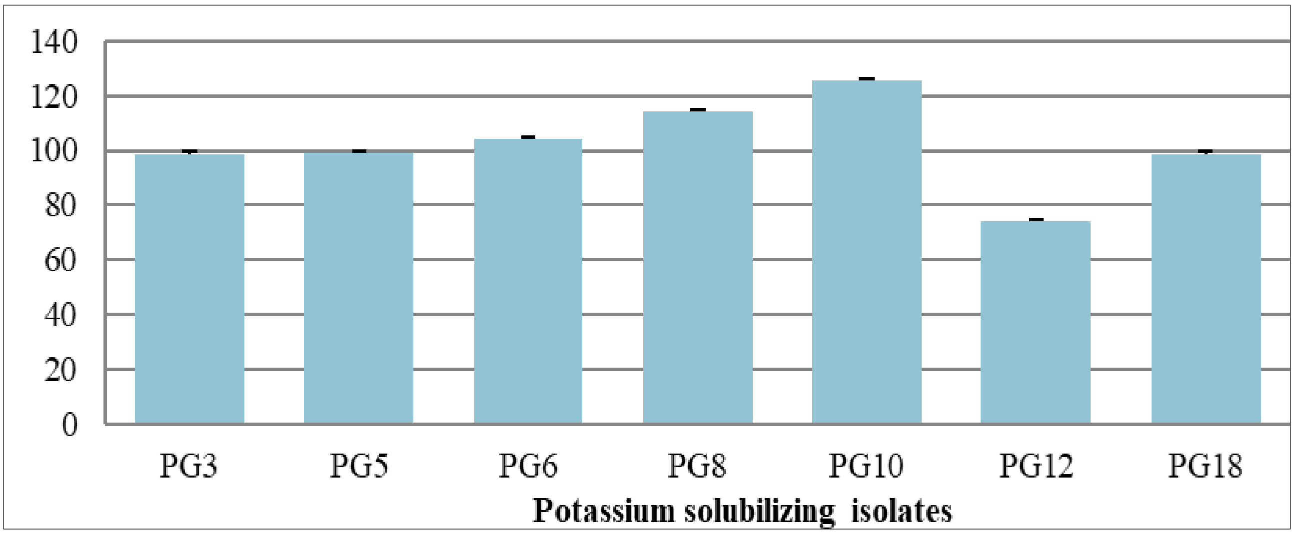

Fig 5: Potassium solubilization ability of KSB isolates in liquid medium.

Table 2: Quantitative estimation of available potassium

Isolates	Soluble potassium concentration (µg/ml)
PG3	98.75±0.520
PG5	99.083±0.440
PG6	103.917±0.330
PG8	113.917±0.363
PG10	125.333±0.707
PG12	73.8333±0.083
PG18	98.667±0.546

*The result indicates the mean of triplicates ±S.E

3.4 Morphological characterization

The preliminary observations on the colony morphology including size, shape, margin, elevation, pigmentation, surface, constituency, and optic characteristics, as well as the Gram staining of seven isolated bacteria, are presented in Table 3. The morphological analysis of these potassium-solubilizing bacterial isolates showcased a wide range of characteristics. Five were classified as gram-positive, while the remaining two were categorized as gram-negative.

Table 3: Morphological characteristics of KSB isolates

	PG3	PG5	PG6	PG8	PG10	PG12	PG18
Colony size	Pinpoint	Intermediate	Small	Pinpoint	Intermediate	Pinpoint	Small
Colony shape	Round	Round	Round	Round	Round	Round	Round
Colony margin	Erose	Wavy	Entire	Wavy	Wavy	Wavy	Repand
Colony elevation	Thin	Flat	Effused	Raised	Raised	Convex	Smooth
Colony pigmentation	White	Creamy yellow	creamy	Off white	Creamy	Light yellow	orange
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Constituency	Dewdrop	Butyrous	Viscous	Dry	Dry	Most	Viscous
Optical characteristic	Transparent	Opaque	Opaque	Opaque	Opaque	Transparent	Opaque
Gram reaction	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve	+Ve

3.5 Biochemical characterization

The biochemical tests are an important method for the initial identification of the isolates. The results of the biochemical test are shown in Table 4. Differential biochemical tests such as Methyl-red, Vogus-Proskauer, Citrate utilization, Nitrate reduction, Gelatin hydrolysis, and TSI test results are observed after their respective incubation times. Two isolates were tested positive for the Methyl-red test, one isolate was for the Indole test, five isolates were for the Nitrate test, two isolates were for Gelatinase, two isolates were for the Triple sugar iron test, and four isolates were for the Citrate test. These results provide valuable insights into the metabolic capabilities and potential identification of the bacterial strains [7]. Based on morphological and biochemical tests the strains were identified using the guidelines of Bergey's manual of systematic bacteriology Bergey, 1923 [21].

Based on the morphological characterization and biochemical reaction, the potassium solubilizing isolates were identified with the help of Bergey's manual of systematic bacteriology as PG3 and PG6 *Proteus* spp, PG5 and PG12 *Staphylococcus spp*, and PG8 and PG10 is *Bacillus* spp, PG18 is *Rhodococcus*. Verma *et al*., 2020 [15] reported that the rhizospheric bacteria *Bacillus spp*, and *Pseudomonas spp*, *Paenibacillus* strains were able to solubilized potassium.

Table 4: Biochemical characteristics of the KSB isolates

	PG3	PG5	PG6	PG8	PG10	PG12	PG18
M-R	+	-	+	-	-	-	-
V-P	-	-	-	-	-	-	-
Indole	-	+	-	-	-	-	-
Nitrate	+	-	-	+	+	+	+
Gelatine	-	-	-	-	+	+	-
TSI	+	-	+	-	-	-	-
Citrate	+	-	+	-	-	+	+

*Here; + indicates presence, -Indicates absence

3.6 Pot assay

Based on the solubilizing ability of KSB isolates, the effect of *Bacillus spp*. PG10 was tested on groundnut (*Arachis hypogaea*) growth. There is increasing evidence suggesting that the addition of *Bacillus* spp. PG10 culture to the groundnut plant resulted in significant improvement in plant growth. (Root length, shoot length fresh weight of root and shoot, number of nodules, and number of flowers).

Fig 6: Growth promotion activity: with and without treatment of KSB strain (*Bacillusspp.)*

Table 5: Effect of PG10 isolates on groundnut (*Arachis hypogaea*) growth

Sr. No	RL (cm)	SL (cm)	LS (cm)	FWR (gm)	FWS (gm)	NN	NR	NL	NF
Control	3.606 ^{b}	11.930 ^{b}	2.271 ^{b}	0.574 ^{b}	1.613 ^{b}	1.857 ^{b}	55.571 ^{b}	20.143 ^{b}	2.00 ^{b}
PG10	16.419 ^{a}	33.261 ^{a}	3.514 ^{a}	2.268 ^{a}	9.945 ^{a}	14.857 ^{a}	156.286 ^{a}	54.714 ^{a}	5.41 ^{a}
S. Em ±	0.151	0.293	0.04	0.02	0.063	0.094	0.764	0.272	0.1
CD at 5%	0.469	0.908	0.118	0.069	0.210	0.44	2.357	0.843	0.311
CV%	4.019	3.452	3.498	4.151	3.126	4.523	1.919	1.934	7.483

Here; Means with the same letters are not significantly different at 5%, $p<0.05$ which indicates a 95% confidence level *Each value represents the mean of seven replications according to a degree of freedom

Growth was measured at 60 days after seed germination. RL root length, SL shoot length, LS leaves size, FWR fresh weight of roots, FWS fresh weight of shoot, NN no of nodules, NR no of roots, NL no of leaves number, NF no of flowers. The values are mean and S. Em±. Biostatical analysis was performed using the WASP Web Agri Stat Package. Represent significant difference from the respective control according to ANOVA. The P value was obtained $p<0.05$ which indicates 95% confidence level.

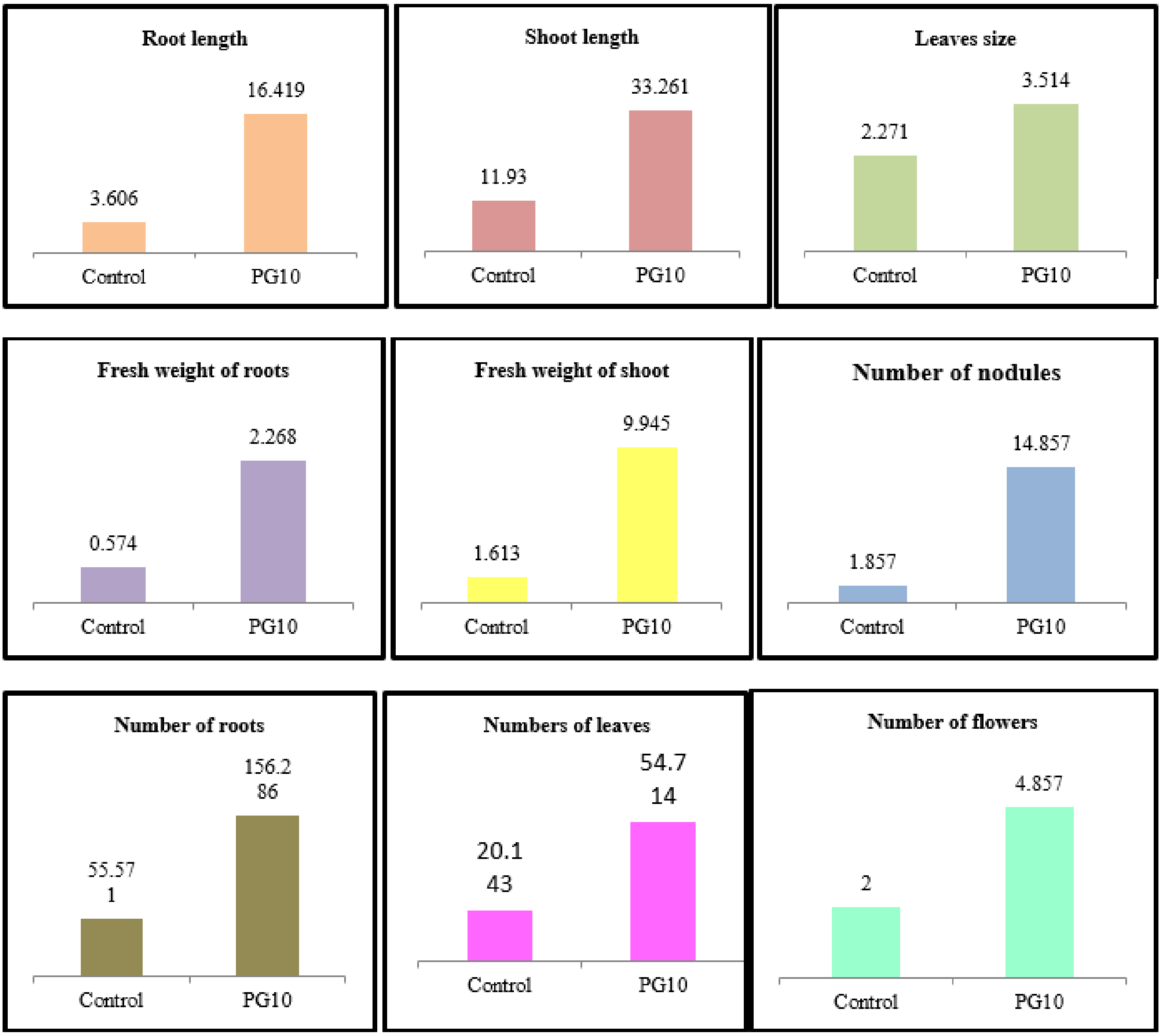

Fig 7: Effect of PG10 on Plant Growth

The average root and shoot length and fresh weight are considerably higher in treated with the KSB pot and comparatively lower in the control pot. Similarly, result of Pérez-Pérez *et al*., 2021 [4] indicated that the root and shoot length and mass were notably higher in treated with KSB compared to control. Gohil *et al*., 2022 [22] studied the effects of PG-8 on *Arachis hypogea* growth and the outcomes were noted with control. Their results showed the higher growth with *Bacillus spp.*

4. Conclusion

The results of our investigation demonstrate the capacity of seven bacterial isolates to solubilize potassium, the *Bacillus species* PG10 has proven to be the most potent strain. Application of this strain improved *Arachis hypogea* growth as compared to control. It showed considerable improvements in the shoot length, root length, root hair number, leaf number, and leaf area, nodules, flowering in both plants. This suggests its potential application as an environmentally friendly alternative to toxic agro chemicals and makes it a potential candidate for application as a bio-inoculant.

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6. Conflict of interest
The authors declare that no conflicts of interest exist.

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