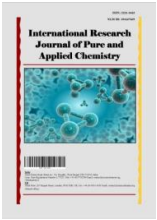

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Potential Screening of Indigenous Drought Stress Tolerant Bacteria for Plant Growth Promotion (PGP) Traits: An *In-vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author BPK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NT managed the analyses of the study. Author AVG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aims to formulate relevant microbial consortia against drought stress mitigation with potential drought stress tolerant bacterial isolates by polyethylene glycol 6000 (PEG 6000) different moisture stress levels to mitigate the drought stress which can finally helpful to increase plant and soil health under adverse stress conditions.
Study Design: Source of rhizosphere soil samples from groundnut drought prone areas of Andhra Pradesh.
Place and Duration of Study: Department of Agricultural Microbiology, Advanced Post Graduate Centre, Acharya N.G Ranga Agricultural University, Lam, Guntur, 522 034, between June 2017 and July 2020.
Methodology: Isolated strains were also tested for further drought stress screening by polyethylene glycol 6000 *In-vitro* screening was done for different plant growth promotion activities i.e. phosphate solubilization, IAA production, ammonia production, ACC deaminase activity, HCN

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production and catalase. HCN production, catalase positive, colony morphology, Gram staining and biochemical tests.

Results: Fifty-one efficient bacterial isolates were obtained from drought prone rhizosphere soils of groundnut. Isolated strains were also tested for further drought stress screening by polyethylene glycol 6000 at 0% (-0.05 MPa), 10% (-0.65 MPa), 20% (-1.57 MPa), 30% (-2.17 MPa) and 40% (-2.70 MPa). Thirty-seven bacterial isolates were further found to have an enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity which improved plant growth during stress conditions. The *In-vitro* screening was done for different plant growth promotion activities, twelve bacterial isolates were positive for phosphate solubilization. IAA production was shown by almost all the bacterial isolates. Three isolates were positive for ammonia production. Two isolates were positive for HCN production and all the isolates were found to be catalase positive. Seven isolates were showing maximum plant growth promotion activities and further identified based on colony morphology, Gram staining and biochemical tests.

Conclusion: This study suggests that 51 bacterial isolates exhibited the highest tolerance to moisture stress under *In-vitro*, these are screened and considered as potential isolates against plant growth promoting characteristics. Plant growth promoting bacteria that can modulate physiological response for water shortage, enhanced water or nutrient uptake and transpiration, induction of plant growth hormone signaling and increased antioxidase activity and photosynthetic rate thereby ensuring plant survival under such stressful conditions. In regard to isolates having PGP properties from the research work presented could be studied further under *In-vitro* and in vivo conditions from different soils with several crops for confirming their use as bio inoculants.

Keywords: Bacterial isolates; drought tolerance; groundnut; rhizosphere; PGPR; sustainable.

1. INTRODUCTION

Drought stress is one of the major agricultural problems reducing crop yield in arid and semiarid regions of the world. Changes in mean global air temperature and precipitation patterns are leading to longer drought periods and more extremely dry years, and more severe drought conditions will hinder food production in some countries [1]. At present, strategies to increase the ability of plants to tolerate drought stress involve the use of water-saving irrigation, traditional breeding, and genetic engineering of drought-tolerant transgenic plants. Unfortunately, these methods are also highly technical and labor-intensive, and thus difficult to practice. In addition to the above complications, one of the better way is to manage drought stress by using beneficial microorganisms which will promote plant growth under adverse conditions.

The Bacteria from a broad range of genus are an important component of the soil which will be involved in the various abiotic and biotic activities of the soil ecosystem for the exchange of nutrients and support soil health [2]. A group of bacteria that can be found in the rhizosphere, which is advantageous in improving the growth of plants, can be classified as plant growth-promoting rhizobacteria (PGPR). *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported as PGPR able to promote plant growth [3].

One alternative for growing plants under dry conditions is the use of plant growth promoting rhizobacteria (PGPR). PGPR is a group of bacteria that can be associated with plant root systems, both at the root surface and in endophytic associations, and which can either directly or indirectly facilitate plant growth in optimal, biotic, or abiotic stress conditions [4-5]. PGPR has been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores, and synthesis of plant growth hormones i.e. Indole-3-acetic acid (IAA), gibberellic acid, cytokinins, and ethylene [6]. Indirect mechanisms involve the biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzymes, hydrogen cyanide, catalase and siderophore or through competition for nutrients and space can improve significantly plant health and promote growth, as evidenced by increases in seedling emergence, vigor, and yield [7]. In addition, PGPR is linked to the catabolism of molecules related to stress signaling, such as bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase [8]. Many PGPR has been shown to alleviate drought stress effects in plants

by reducing plant ethylene levels that are usually increased by unfavorable conditions by increasing the level of reactive oxygen species (ROS) in subcellular compartments [9]. Antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), though, can preserve cellular stability and play an essential role in scavenging ROS and preventing oxidative damage. Bacteria can withstand stress conditions because of the production of exopolysaccharides, during water stress conditions [10]. Field application of microorganisms having drought-tolerant ACC deaminase may influence plant growth.

Drought stress tolerant rhizobacteria have been established for systemic resistance and stress tolerance inducers as well as a plant growth promoter in previous studies where they were inoculated solely. But scarce information is available for the combined use of drought stress tolerant bacteria as consortia inoculation for improving drought stress tolerance in groundnut crops. For this reason, the present study was planned for the isolation of drought-tolerant plant growth-promoting rhizobacteria and evaluation of their PGP activities under drought stress. The inoculation with consortia of drought stress tolerant bacteria would further improve the growth of groundnut under drought. The results may be useful for explaining the mechanisms and the screened PGP bacteria have great potential for biotechnological applications in drought-stressed agricultural systems under drought prone rhizospheric soils of Andhra Pradesh.

2. MATERIALS AND METHODS

2.1 Soil Sampling

Rhizosphere soil had been drawn from different areas of Anantapur and Prakasam districts such as soils from different drought prone areas. In the Anantapur District (Mandals of Anantapur, Kambadur, Gummagatta and Vidapanikal) and Prakasam District (Mandals of Chirala, Chinaganjam, Kothapatnam and Vetapalem) along with particular GPS Coordinates for each sampling area was fixed Fig 1. Forty-eight soil samples were collected from different villages of four mandals in the Anantapur (Upland) and Prakasam (Coastal) districts for the isolation of drought stress tolerant bacterial strains. The soil samples were mainly collected from groundnut rhizosphere fields along with their GPS Coordinates. Crop plants were selected randomly in the field and the intact root system was dug out, carefully taken in plastic bags, labeled well and stored at 4^{o}C. The chemicals and media components used in the present investigation were of analytical grade (AR) obtained from Hi-media, Merck limited, Fischer and Sigma chemicals, India.

2.2 Isolation of Bacteria from Rhizosphere Soils

For isolation of Rhizobacteria, the method proposed by Vlassak et al. [11] was followed. In this procedure, 10 g of soil from each soil sample was taken in a conical flask of 90 ml saline. The sample was agitated for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. 0.1 ml was spread on sterilized petri plates containing Nutrient agar media the petri plates were incubated at room temperatures (28^{o}C ± 2^{o}C) for 24-72 h. Two replicates were maintained for each dilution. The plates were examined daily for up to 3 days for bacterial colonies.

2.3 Cultural Characterization

The plates incubated for a day at 30 ± 1°C were observed for the growth of colonies on Nutrient agar media colonies were screened by enumeration. All the bacterial isolates were studied for their colony morphology, cell morphology (Gram reaction), pigmentation, spore production and biochemical characteristics according to the standard methods described in Bergey's Manual of Determinative Bacteriology [12-13].

2.4 Maintenance of Isolates

All the isolates were maintained at 4 °C in equal volumes of nutrient broth and 30% glycerol can be preserved as long as 12-15 months [14].

2.5 Screening of Bacterial Isolates for Plant Growth Promoting Traits Under In Vitro

2.5.1 Drought tolerance

Trypticase soy broth (Tryptone (Pancreatic Digest of Casein) 17.0 g/L, Soytone (Peptic Digest of Soybean) 3.0 g/L, Glucose (Dextrose) 2.5 g/L, Sodium Chloride 5.0 g/L, Dipotassium Phosphate 2.5 g/L, distilled water 1 L, pH 7.3 ± 0.2) with different water potentials (-0.05, -0.15, -

0.30, -0.49 and -0.73 MPa) was prepared by adding appropriate concentrations of polyethylene glycol (PEG 6000) [15-16] and was inoculated with 1% of overnight raised bacterial cultures in Trypticase Soy Broth (TSB). The osmotic potential of broth media was measured by a psychrometer. Three replicates of each isolate with each concentration were prepared. After incubation at 28°C under shaking conditions (120 rpm) for 24 hrs, growth was estimated by measuring the optical density at 600 nm using a spectrophotometer.

2.5.2 Exopolysaccharides (EPS) production

Bacterial strains were grown on YMG Agar (Peptone 5 g/L, Yeast extract 3 g/L, Malt extract 3 g/L, Dextrose 10 g/L, Agar 20 g/L, distilled water 1 L, pH 6.2 ± 0.2) medium were inoculated in YMG broth and preincubated at 25^{o}C for 24 hrs. 200 µl of culture broth was inoculated into 50 ml of YMG broth and incubated at 25^{o}C for 5 days at 120 rpm. Elimination of cells was followed by centrifugation (10,000 g for 20 min). The culture broth was mixed with 3 volumes of ethanol and after standing at 4^{o}C for 24 hrs, it was centrifuged (10,000 g, 4^{o}C, 20 min). The weight of the precipitated EPS was measured after drying at 80^{o}C for 3 days [17].

2.5.3 Siderophore production

Siderophore production was estimated qualitatively. Chrome azurol sulphonate (CAS) agar medium (CAS-HDTMA solution: CAS-HDTMA solution was prepared by dissolving 121 mg chrome azurol sulfate (CAS) in 100 ml of distilled water, and to this, 20 ml of 1 mM $FeCl_3.6H_2O$ solution (1 mM $FeCl_3.6H_2O$ solution prepared in 10 mM HCl) was added. It was slowly added to 20 ml hexadecyltrimethylammonium bromide (HDTMA) solution (729 mg HDTMA in 400 ml distilled water) and autoclaved at 121^{o}C for 15 minutes. To a 900 ml sterilized King's B medium) [18] was used for the detection of siderophores, isolates were grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 hrs on a rotary shaker at room temperature. CAS assay is used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar and incubated at room temperature for 24 hrs. The formation of yellow to orange colored zone around the wells indicates siderophore production.

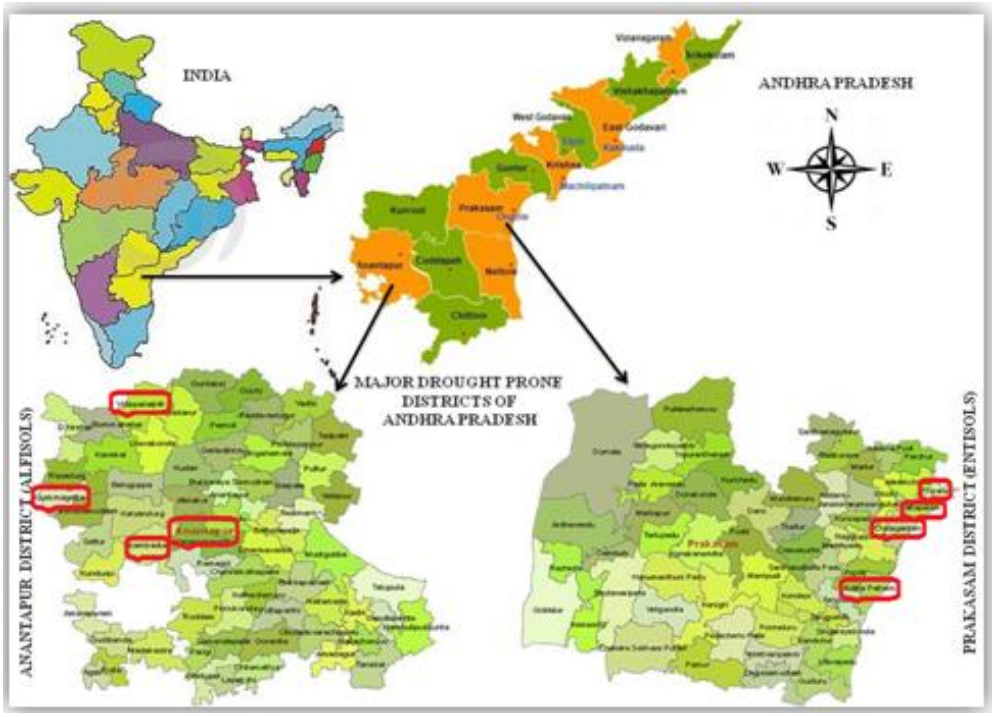

Fig. 1. Location map of soil samples collected from major drought prone districts of Andhra Pradesh, India

2.5.4 Indole acetic acid production

The production of Indole acetic acid was done according to Dubey and Maheswari, [19]. LB broth (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, distilled water 1000 ml, pH to 7.0 ± 0.2) was prepared and 24 hrs old cultures were inoculated into the broth and incubated at 28 °C for 72 hrs. After the incubation period, the cultures were centrifuged at recommended rpm and time. 2 ml of supernatant was collected into a test tube and two drops of O-phosphoric acid were added. Salkowski reagent was prepared and added into the test tube double the amount of supernatant. To prepare the Salkowski reagent 0.4 gms of ferric chloride was added into 5 ml of distilled water and 17.5 ml of perchloric acid was added into 32.5 ml of distilled water and mixed the ratio of 1:150. Incubate the tubes for 30 min in dark. Development of pink color after the respective incubation period indicates the positive test for IAA production.

2.5.5 ACC (1-Aminocyclopropane-1-carboxy-late) deaminase activity

Screening for ACC deaminase activity of drought-tolerant PGPR isolates was done based on their ability to use ACC as a sole nitrogen source. All drought tolerant PGPR isolates were grown in 5 ml of trypticase soy broth (TSB) medium incubated at 28^{o}C at120 rpm for 24 hrs. The cells were harvested by centrifugation at 3000 g for 5 minutes and washed twice with sterile 0.1 M Tris-HCl (pH 7.5) and resuspended in 1 ml of 0.1 M Tris-HCl (pH 7.5) and spot inoculated on petri plates containing modified DF (Dworkin and Foster) salts minimal medium 10 ml and distilled water 990 ml, supplemented with 3 mM ACC as sole nitrogen source. Plates containing only DF salts minimal medium without ACC as negative control and with $(NH_4)_2$ SO_4 (0.2% w/v) as a positive control. The plates were incubated at 28^{o}C for 72 hrs. The growth of isolates on ACC supplemented plates was compared to negative and positive controls and was selected based on growth by utilizing ACC as a nitrogen source [20].

2.5.6 Phosphate solubilizing activity

Phosphate solubilization activity was determined using Pikovskaya's agar (Glucose 10 g/L, $Ca_3(PO_4)_2$ 5 g/L, $(NH_4)_2SO_4$ 0.5 g/L, NaCl 0.2 g/L, $MgSO_4$ $7H_2O$ 0.1 g/L, KCl 0.2 g/L, Yeast Extract 0.5 g/L, $MnSO_4$ H_2O 0.002 g/L and $FeSO_4$ $7H_2O$ 0.002 g/L, Agar 20 g/L, distilled water 1 L, pH 6.2 ± 0.2) medium containing 0.5% (W/V) $Ca_3(PO_4)_2$ [21]. Pikovskayas agar plates were prepared and sterilized. The inoculums were spot inoculated on the pikovskayas plate. 24 hrs old culture was used for the inoculation. The plates were incubated for 72-96 hrs at room temperature. The clear zone was observed around the spotted area after the incubation period.

2.5.7 Potassium releazing ability

Potassium solubilization was determined using Aleksandrov medium containing 0.3% potassium aluminum silicate [22]. KMB (Glucose 5 g/L, $MgSO_4$. $7H_2O$ 0.5 g/L, $CaCO_3$ 0.1 g/L, $FeCl_3$ 0.006 g/L, $Ca_3(PO_4)_2$ 2 g/L, Potassium aluminium silicate 3 g/L, Agar 20 g/L, distilled water 1 L, pH 7.2 ± 0.2) media was prepared and sterilized. The 24 hrs old culture was spot inoculated on the KMB plates and incubated for 72 hrs at room temperature. Plates were observed for the clear zone around the spotted area after the incubation period.

3. RESULTS

3.1 Rhizosphere Soil Samples Collection

Bacterial isolates acquired from soil samples of various drought prone locations of groundnut are identified and calculated distribution of bacterial isolates from various locations. Among all the eight mandals highest number of efficient bacterial isolates are drawn from chirala (21%) followed by kothapatnam (18%), anantapur (17%), chinnaganjam (14%), vetapalem (12%), gummagatta (8%), vidapanikal (6%) and kambadur (4%) Fig. 2. Bacterial isolates were further studied under *In-vitro* conditions to understand their plant growth promoting properties with the following multiple beneficial activities.

3.2 Screening for Drought Tolerance

In the present study a total of 51 bacterial isolates were identified and screened against moisture stress conditions. Isolated bacteria tested for the surviving potentiality to tolerate abiotic stress such as drought tolerance using nutrient broth along with polyethylene glycol 6000 at different concentrations. All the isolates showed growth at (0%, 10%, 20%, 30% and 40% PEG) -0.05, -0.65, -1.57, -2.17 and -2.70 matric potential (MPa). Bacterial isolates against

polyethylene glycol 6000 and their growth is expressed in terms of optical density (OD) at different drought stress conditions Table 1 & Fig. 3. This showed the potential of these isolates to tolerate drought stress at -0.05 MPa. However, the isolates which are recorded with the highest optical density were taken and screened for further plant growth promoting characteristics.

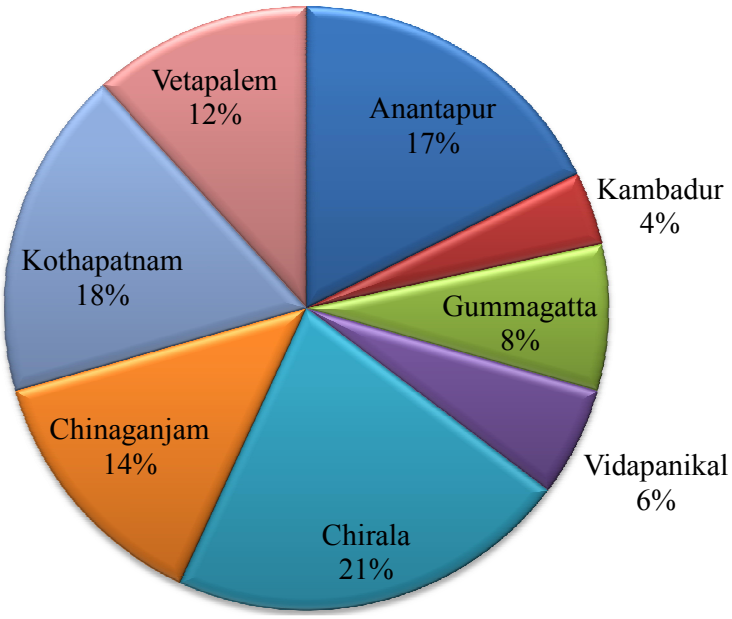

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Fig. 2. Distribution of screened bacterial isolates over drought prone locations of Andhra Pradesh (for 51 isolates)

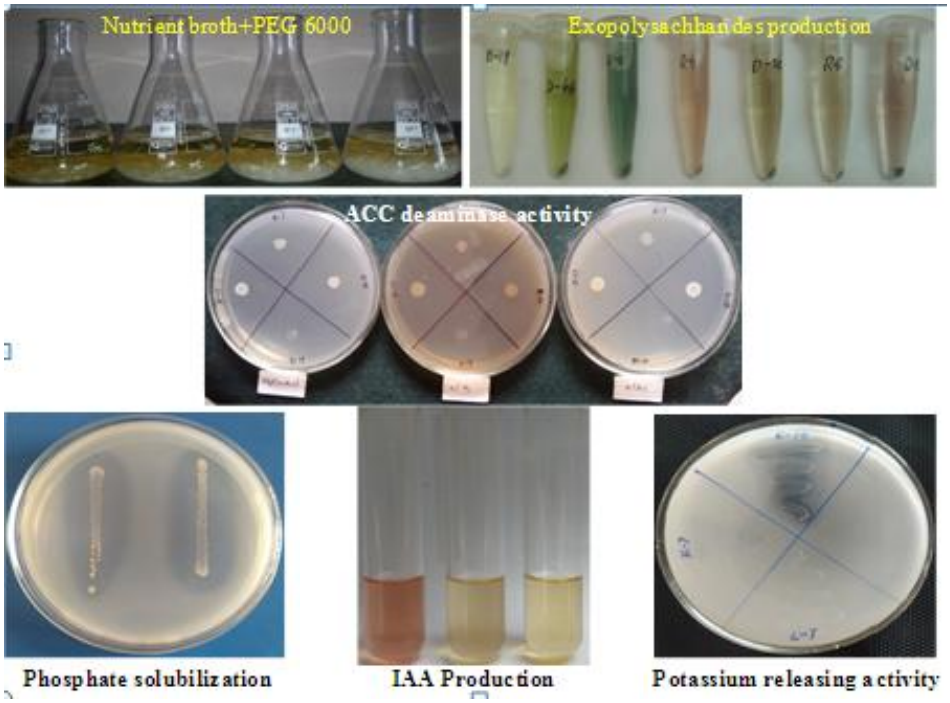

Fig. 3. Plant growth promoting characteristics of potential isolates

Table 1. Growth of bacterial isolates against moisture stress under *In-vitro* conditions

S. No.	Name of the isolate	Moisture stress				
		(-1.57 MPa)	(-2.17 MPa)	(-2.70 MPa)	(-0.05 MPa)	(-0.65 MPa)
1.	AKPN	0.475	0.151	0.100	0.055	0.073
2.	PKRN	0.907	0.117	0.145	0.099	0.087
3.	PVKN-1	0.597	0.144	0.136	0.090	0.078
4.	PVKN-2	1.315	0.124	0.134	0.063	0.060
5.	AGPS	1.111	0.144	0.178	0.082	0.208
6.	AVVS-1	1.459	0.460	0.261	0.126	0.214
7.	AVVS-2	1.340	0.591	0.659	0.311	0.228
8.	PCMS-1	1.004	0.572	0.259	0.121	0.216
9.	PCKS-1	1.558	0.357	0.143	0.095	0.218
10.	PCKS-2	0.284	0.096	0.100	0.094	0.083
11.	PKRS-1	0.415	0.097	0.087	0.073	0.074
12.	PKRS-2	0.497	0.096	0.067	0.072	0.078
13.	PVAS-1	1.205	0.099	0.100	0.096	0.072
14.	PVAS-2	0.791	0.099	0.097	0.077	0.070
15.	AAAS	0.151	0.079	0.051	0.000	0.158
16.	AAKS	0.245	0.124	0.129	0.026	0.179
17.	AGVS	0.690	1.380	0.170	0.042	0.157
18.	PCKS-3	1.171	1.224	0.596	0.015	0.149
19.	PKRS	1.825	1.632	1.290	0.401	0.182
20.	PVAS	1.462	1.361	0.882	0.577	0.591
21.	AKPL	2.037	0.286	0.523	0.782	0.257
22.	PCTL-1	2.016	1.190	0.984	0.905	0.245
23.	PCTL-2	2.097	0.664	0.974	0.953	0.199
24.	PCKL-1	1.918	0.253	0.529	0.233	0.241
25.	PCKL-2	1.810	0.172	0.767	1.025	0.166

S. No.	Name of the isolate	Moisture stress				
		(-1.57 MPa)	(-2.17 MPa)	(-2.70 MPa)	(-0.05 MPa)	(-0.65 MPa)
26.	PCKL-3	2.085	0.209	0.849	0.406	0.225
27.	PCKL-4	1.927	0.202	1.362	0.762	0.233
28.	PCML	1.823	0.188	0.815	0.306	0.453
29.	AAAR-1	1.458	1.550	0.879	0.536	0.297
30.	AAAR-2	1.124	1.115	0.577	0.316	0.245
31.	AAKR-1	0.890	0.724	0.538	0.310	0.226
32.	AAKR-2	1.384	0.901	0.432	0.074	0.088
33.	PCKR-2	0.570	1.415	0.278	0.213	0.095
34.	PCMR-2	1.738	1.345	0.876	0.414	0.119
35.	PVAR	0.150	0.079	0.043	0.103	0.382
36.	PKRS-1	0.307	0.576	0.414	0.102	0.238
37.	PKRS-2	1.228	1.244	0.943	0.640	0.407
38.	PKRS-3	1.423	1.213	0.657	0.522	0.398
39.	AGVS	0.965	0.896	0.878	0.107	0.245
40.	AGPS	0.982	1.166	0.830	0.631	0.176
41.	PCTS-1	1.409	0.713	0.701	0.373	0.148
42.	PCTS-2	0.673	0.629	0.598	0.360	0.151
43.	PCKS-4	1.569	1.021	0.672	0.262	0.184
44.	PCMS	1.048	0.860	0.862	0.382	0.167
45.	PCKS-5	0.618	0.189	0.208	0.053	0.182
46.	AVVS	0.426	0.749	1.001	0.504	0.191
47.	PKES-1	1.307	0.618	0.412	0.475	0.204
48.	PKES-2	0.837	0.536	0.710	0.478	0.184
49.	PKES-3	0.970	0.735	0.785	0.530	0.199
50.	PKRS-1	0.920	0.751	0.528	0.376	0.220
51.	PKRS-2	1.070	0.786	0.881	0.439	0.249

**Moisture Stress Levels: MPa (MegaPascal)*

3.3 Exopolysaccharide Production

The bacterial strains which are screened from drought tolerance were further tested for EPS production. Among fifty one isolates maximum amount of EPS production was observed in the isolate AKPL (18 mg ml^{-1}) and PVAR (13 mg ml^{-1}). No EPS production was observed in the isolates PCKS-2, AAAS, AGVS, PCTL-1, PCTL-2, AAKR-1, PCKR-2 and PCMR-2 Table 2 & Fig. 3. The formation of EPS in bacteria is triggered by stress and EPS possess unique cementing and water holding properties through the resulting biofilm formation.

3.4 Siderophore Production

Among all the fifty one isolates siderophore production was observed in the isolates of AVVS-1, AVVS-2, PCMS-1, PKRS-2, PVAS-1, PVAS-2, AGVS, PCKS-3, PVAS, AKPL, PCKL-1, PCML, AAAR-1, AAAR-2, AAKR-2, PCKR-2, PCMR-2, AVVS, PKES-1and PKES-2 Table 2.

3.5 Indole Acetic Acid Production

All the fifty one bacteria exhibiting positive for IAA production. Among all the isolates AKPN, AGPS, AVVS-1, PCMS-1, AGVS, PKRS, AAKR-1, AAKR-2, PCTS-1, PCKS-1, PCKS-2 and PKES-3 exhibited strong (**) IAA production activity, whereas remaining isolates showing weak (*) IAA production Table 2 & Fig. 3. IAA production by varying different physiological parameters such as pH, temperature, carbon and nitrogen sources of culture media. So that the intended conditions at which the IAA production is maximized.

3.6 ACC deaminase Activity

All the bacterial strains were screened against ACC deaminase based on the enrichment method under drought stress conditions (-2.70 MPa) where ACC is used for the sole nitrogen source Table 2 & Fig. 3. Among fifty one bacterial isolates, thirty four isolates showed strong (+++) ACCd production by utilized sole nitrogen source as ACC, six isolates were moderate (++) in ACCd production, remaining eleven isolates showed weak (+) and no ACC deaminase production.

3.7 Phosphate Solubilization

Bacterial isolates were screened for PO_4 solubilization potential in Pikovskayas broth supplemented with 0.1% tricalcium phosphate. Among all the isolates 36 isolates recorded positively for phosphate solubilization by qualitatively among these the highest solubilization was recorded in the isolate PCKS-2 (4.67 mm), followed by the isolate AAAS (4.60 mm) and least by PCMS-1 and PVAS (2.29 mm) Table 2 & Fig. 3. All the bacterial isolates were recorded positively for phosphate solubilization quantitatively, among these the highest solubilization was recorded in the isolate PCKS-5 (11.3% of Pi), followed by the isolate PKES-1 (9.5% of Pi) and least in AVVS-1 (2.60% of Pi).

3.8 Potassium Releasing Activity

All the isolates recorded positively for potassium releasing activity by quantitatively among these the highest amount was recorded in the isolate PCKS-2 (3.60 mm), followed by the isolate PCKR-2 (3.50 mm), PVAR (3.50 mm) and AVVS (3.50 mm) and the least was recorded by PCKS-1 and PKRS-1 (2.20 mm) Table 2 & Fig. 3. Among the isolates highest amount of potassium releasing activity was recorded in the isolate PCMS (1875.0 µg ml-1), followed by the isolate PCKL-4 (1812.5 µg ml-1) least by PVKN-2 (500.0 µg ml-1).

4. DISCUSSION

PGPR colonizes the roots of the plant and promotes plant growth and development through a variety of mechanisms. The exact mechanism by which PGPR stimulates plant growth is not known, although several mechanisms such as the production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion [23-24]. Little information about screening and using of drought stress tolerant PGPR with groundnut is available. In the present study, beneficial bacteria were isolated from groundnut rhizosphere soils from drought prone areas of Andhra Pradesh. The highest number of bacteria are found in the rhizosphere of a plant due to exudates secreted by the plant which could have made rhizosphere samples richer in nutrients thereby increasing the diversity of microbes isolated. Specific constituents of soil nutrients and pH may impose physiological constraints on microorganisms' survival and growth, thereby directly altering bacterial community composition. Of these constituents, soil pH has been proven to be the most influential factor. The rhizosphere is a dynamic

Table 2. Performance of bacterial isolates against plant growth promoting characteristics

S. No.	Name of the isolate	EPS Production (mg ml^{-1})	Siderophore Production	IAA Production	ACC Deaminase			Phosphate solubilization		Potassium releasing activity	
					Control	Absolute Control	ACC	Solubilization Index	% of Inorganic Phosphorus Released	Solubilization Index	µg K ml^{-1}
1.	AKPN	-1.0	-	**	-	+	+	2.30	6.70	2.22	1375.0
2.	PKRN	-2.0	-	*	-	-	+	3.80	6.10	3.00	1625.0
3.	PVKN-1	-2.0	-	*	+	+	+	2.50	4.90	2.25	1000.0
4.	PVKN-2	4.0	-	*	-	-	-	3.80	5.60	3.20	500.0
5.	AGPS	1.0	-	**	-	-	-	4.00	5.40	2.50	750.0
6.	AVVS-1	1.0	+	**	+	+	+	2.67	2.60	2.29	1125.0
7.	AVVS-2	6.0	+	*	-	-	-	2.63	5.40	-	1000.0
8.	PCMS-1	6.0	+	**	+	+	+	2.29	7.90	-	1250.0
9.	PCKS-1	2.0	-	*	+	+	+	3.00	5.00	2.20	937.5
10.	PCKS-2	0.0	-	*	-	-	-	4.67	4.55	3.60	1625.0
11.	PKRS-1	-1.0	-	*	-	-	+	4.33	4.95	3.40	1250.0
12.	PKRS-2	5.0	+	*	+	+	+	4.50	3.75	3.20	1125.0
13.	PVAS-1	5.0	+	*	+	+	+	2.86	5.20	3.40	1437.5
14.	PVAS-2	1.0	+	*	+	+	+	5.83	4.20	3.40	1625.0
15.	AAAS	0.0	-	*	-	-	-	4.60	5.50	2.22	1312.5
16.	AAKS	4.0	-	*	+	+	+	2.83	4.75	2.33	1250.0
17.	AGVS	0.0	+	**	+	+	+	4.33	5.95	2.83	1625.0
18.	PCKS-3	3.0	+	*	+	+	+	4.13	6.10	3.17	1437.5
19.	PKRS	2.0	-	**	+	+	-	3.50	5.60	3.00	1000.0
20.	PVAS	1.0	+	*	+	+	+	2.29	4.70	2.50	1375.0
21.	AKPL	13.0	+	*	+	+	+	4.00	5.40	2.75	1312.5
22.	PCTL-1	0.0	-	*	+	+	+	-	6.30	3.20	1500.0
23.	PCTL-2	0.0	-	*	+	+	+	-	7.60	-	1312.5
24.	PCKL-1	1.0	+	*	+	+	+	2.80	4.20	2.33	1500.0
25.	PCKL-2	1.0	-	*	+	+	+	3.67	6.50	2.50	1468.8

S. No.	Name of the isolate	EPS Production (mg ml^{-1})	Siderophore Production	IAA Production	ACC Deaminase			Phosphate solubilization		Potassium releasing activity	
					Control	Absolute Control	ACC	Solubilization Index	% of Inorganic Phosphorus Released	Solubilization Index	µg K ml^{-1}
26.	PCKL-3	1.0	-	*	+	+	+	3.00	6.70	2.50	1437.5
27.	PCKL-4	-2.0	-	*	+	+	+	-	7.85	-	1812.5
28.	PCML	3.0	+	*	+	+	+	-	6.05	2.70	1625.0
29.	AAAR-1	2.0	+	*	+	+	+	4.00	5.45	3.20	1468.8
30.	AAAR-2	1.0	+	*	-	-	-	4.33	6.00	2.57	1625.0
31.	AAKR-1	0.0	-	**	-	-	-	2.75	5.60	2.33	1437.5
32.	AAKR-2	1.0	+	**	+	+	+	2.88	6.90	2.38	1437.5
33.	PCKR-2	0.0	+	*	+	+	+	3.80	6.80	3.50	1625.0
34.	PCMR-2	0.0	+	*	+	+	+	2.50	5.00	2.50	1375.0
35.	PVAR	-18.0	-	*	-	-	-	3.63	7.15	3.50	1500.0
36.	PKRS-1	1.0	-	*	+	+	+	2.71	4.30	3.20	1750.0
37.	PKRS-2	-5.0	-	*	+	+	+	-	5.60	2.40	875.0
38.	PKRS-3	1.0	-	*	-	-	-	-	8.80	3.00	1687.5
39.	AGVS	-5.0	-	*	+	+	+	3.20	5.60	3.20	1250.0
40.	AGPS	-5.0	-	*	+	+	+	-	5.55	3.20	1500.0
41.	PCTS-1	-2.0	-	**	+	+	+	-	6.05	2.50	1375.0
42.	PCTS-2	-2.0	-	*	+	+	-	2.38	7.20	2.22	1250.0
43.	PCKS-4	-2.0	-	**	+	+	-	2.71	5.70	2.29	1687.5
44.	PCMS	-2.0	-	*	+	+	+	-	8.00	-	1875.0
45.	PCKS-5	-1.0	-	**	+	+	+	2.71	11.30	-	1750.0
46.	AVVS	-5.0	+	*	+	+	+	-	5.30	3.50	1468.8
47.	PKES-1	-2.0	+	*	+	+	+	-	9.45	3.20	1437.5
48.	PKES-2	-5.0	+	*	+	+	+	-	4.80	2.40	1375.0
49.	PKES-3	-8.0	-	**	+	+	-	-	6.70	2.33	1625.0
50.	PKRS-1	-1.0	-	*	+	+	-	-	6.01	2.20	1312.5
51.	PKRS-2	-1.0	-	*	+	+	+	-	5.20	2.80	1437.5

**EPS: Exopolysachharide; IAA: Indole Acetic Acid; ACC: 1-aminocyclopropane-1-carboxylate deaminase.*

region governed by complex interactions between plants and the organisms that are in close association with the root. The composition and pattern of root exudates affect microbial activity and population numbers, which in turn have an impact on the nematodes and microarthropods that share this environment. Beneficial or harmful relationships exist between rhizosphere organisms and plants, which ultimately affect root function and plant growth. In addition, the rhizosphere may include organisms that do not directly benefit or harm plants but influence plant growth and productivity. Plant growth promoting microorganisms may increase drought tolerance of plants growing in arid or semiarid areas because under drought stress conditions bacterial cells accumulate compatible solutes such as amino acids, quaternary amines, and sugars that prevent degenerative processes and improves cell growth under adverse osmotic conditions.

The isolates may undergo a cellular mechanism of osmotic adaptation through compatible solute and osmolyte productions. Exopolysaccharides possess unique water holding and cementing properties, thus play a vital role in the formation and stabilization of soil aggregates and regulation of nutrients and water flow across plant roots through biofilm formation [25]. The EPS production of these selected isolates was higher under stressed than under no stress conditions, indicating that EPS production in bacteria occurs as a response to the stress [26]. Siderophores are produced and utilized by bacteria as iron (Fe)-chelating agents which are produced in response to iron deficiency which normally occurs in neutral to alkaline pH soils. Although siderophore production is mainly achieved under iron deficiency, other factors such as carbon source, nitrogen source, pH, and temperature are essential to the synthesis of siderophores. It has been reported that higher concentrations of phosphate solubilizing bacteria are commonly found in the rhizosphere soil as compared to non rhizospheric soil [27]. IAA is one of the most important phytohormones and functions as an important signal molecule in the regulation of plant development. It has been reported that IAA production by PGPR can vary among different species and strains, and is also influenced by culture conditions, growth stage and substrate availability [28]. Plants are constantly exposed to abiotic stress, such as drought, which is one of the most serious problems associated with plant growth and development affecting agricultural demands. Drought tolerant microorganisms could survive in these habitats and bound to seed coat or root of developing seedlings, and cause deamination of ACC the immediate precursor of ethylene in plants by ACC deaminase leading to lowering of plant ethylene level through the activity of enzyme ACC-deaminase that hydrolyzes ACC into α-ketobutyrate and ammonia, instead of ethylene and thereby facilitating the growth and development of plants [29]. Insoluble phosphate compounds can be solubilized by organic acids and phosphatase enzymes produced by plants and microorganisms. Phosphate solubilization is also has shown by most of the PGPR. Phosphorous is essential for plant health and is typically insoluble or poorly soluble in soils under salt stress conditions. Some of the bacteria improves the solubilization of unavailable phosphorous and applied phosphates, resulting in higher yields even under stress conditions. Potassium releasing activity is the most important mechanism for the microorganisms to solubilize a fixed form of potassium in the soil. The main mechanism of potassium releasing bacteria is acidolysis, chelation, exchange reactions, complexolysis and production of organic acids. The utilization of potassium releasing bacteria to increase the soluble form of potassium and has been regarded as a desirable pathway to increase plant yields [30]. The outcome obtained from this study provided insights into the relationship between the rhizosphere bacteria and their different plant growth promoting characteristics with regard to the root zone of groundnut plants under drought prone areas of Andhra Pradesh.

5. CONCLUSION

It is inferred from the present study that bacterial isolates are exhibited the highest tolerance to moisture stress under *In-vitro*. Among all 51 bacterial isolates were identified and screened against moisture stress conditions. The peanut roots will have the source for rhizobacteria that are capable of directly protecting plants from drought stress. The maximum amount of EPS production was observed in the isolate AKPL (18 mg ml^{-1}) and PVAR (13 mg ml^{-1}). The isolation and characterization of stress-tolerant bacteria are not only essential for understanding their characteristics within the rhizosphere but also their utilization in eco-friendly and sustainable agro-technologies. The screened bacterial isolates have the greatest potential as showing different levels of moisture stress tolerance at 0% (-0.05 MPa), 10% (-0.65 MPa), 20% (-1.57 MPa),

30% (-2.17 MPa) and 40% (-2.70 MPa) and also having different plant growth promotion. For more specific identification of bacteria from the results of this study, it is recommended to carry out 16S rRNA sequencing can also be done to get a complete sequence for molecular identification. Moreover, the bacterial strains and their consortium formulation require field evaluation and validation before being confirmed as bio-inoculants to combat various abiotic stresses in various agroecosystems.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lau JA, Lennon JT. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences. 2012; 109(35):14058-14062.
2. Ahemad M, Kibret M. Review, Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective Journal of King Saud University-Science. 2014; 26:1-20.
3. Yadav JJP, Verma KN, Tiwari. Effect of plant growth promoting Rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum* L.) under *In-vitro* condition Biological Forum-An International Journal. 2010;2(2):15-18.
4. Bashan Y, Holguin G. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol PGPB (plant growth promoting bacteria) and PGPB. Soil Biology and Biochemistry. 1998;30(8-9):1225-1228.
5. Cassan F, Maiale S, Masciarelli O, Vidal A, Luna V, Ruiz O. Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. European Journal of Soil Biology. 2009;45(1):12-19.
6. Nelson LM. Plant growth promoting rhizobacteria (PGPR): Prospect for new inoculants. Online. Crop Management; 2004.
DOI: 10.1094/CM-2004-0301-05-RV.
7. Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. 2006;163:173-181.
8. Khan N, Ali S, Tariq H, Latif S, Yasmin H, Mehmood A, et al. Water Conservation and Plant Survival Strategies of Rhizobacteria under Drought Stress. Agronomy, 2020;10(11):1683-1695.
9. Hasanuzzaman M, Bhuyan MHM, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, et al. Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. Antioxidants. 2020;9(8): 681-690.
10. Bashan Y, Holguin G, Bashan LE. Azospirillum plant relationship: Physiological, molecular, agricultural and environmental advances. Canadian Journal of Microbiology. 2004;50(8):521-577.
11. Vlassak KL, Van H, Duchateau L. Isolation and characterization of fluorescent Pseudomonas associated with the roots of rice and banana grown in Srilanka. Plant and Soil. 1992;145:51-63.
12. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology, 9th ed, Willams and Wilkins Co. Baltimore; 1994.
13. Cappucino JG. Microbiology: A Laboratory Manual. Addison Wesley Publishing Company; 1983.
14. Sundaram NM, Murali SR. Isolation and characterization of bacteria from rhizospheric soils of Curcuma longa for different plant growth promotion (PGPR) activities. World Journal of Pharmaceutical Research. 2018;7:692-700.
15. Michel BE, Kaufmann MR. The osmotic potential of polyethylene glycol 6000. Plant Physiology. 1973;51:914-916.
16. Sandhya V, Ali SZ, Grover M, Reddy G, Bandi V. Drought-tolerant plant growth

promoting Bacillus spp. effect on growth, osmolytes and antioxidant status of maize under drought stress. Journal of Plant Interactions. 2009;6:1-14.
17. Ali SZ, Sandhya V, Rao LV. Isolation and characterization of drought tolerant ACC deaminase and exopolysaccharide producing fluorescent Pseudomonas sp. Annals of Microbiology. 2013;5:1-10.
18. Schwyn B, Neilands JB, Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry. 1987;160(1):47-56.
19. Dubey RC, Maheshwari DK, Aeron A, Kumar B, Kumar S. Integrated approach for disease management and growth enhancement of Sesamum indicum L. utilizing Azotobacter chroococcum TRA2 and chemical fertilizer. World Journal of Microbiology and Biotechnology. 28, 3015. Hydroxamate siderophores. 2012;3024.
20. Honma M, Shimomura T. Metabolism of 1-Aminocyclopropane-1- carboxylic acid. Agricultural and Biological Chemistry. 1978;42:1825-1831.
21. Pikovskaya RI. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologia. 1948;17:362-370.
22. Prajapati MC, Modi HA. Isolation of two potassium solubilizing fungi from ceramic industry soils. Life sciences Leaflets. 2012;5:71-75.
23. Glick B. The enhancement of plant growth by free living bacteria. Microbiology. 1995; 41:109-117.
24. Lalande R, Bissonnette N, Coutlee D, Antoun H. Identification of rhizobacteria from maize and determination of their plant-growth promoting potential. Plant and Soil. 1989;115:7-11.
25. Tisdall JM, Oades JM. Organic matter and water stable aggregates in soils. Journal of Soil Science. 1982;33:141-163.
26. Roberson EB, Firestone MK. Relationship between desiccation and exopolysaccharide production in soil Pseudomonas sp. Applied Environmental Microbiology. 1992;58:1284-1291.
27. Reyes VA, Valduz Z. Phosphate solubilizing microorganisms isolated from the rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. Plant and Soil. 2006;287:69-75.
28. Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, et al. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane *In-vitro*. Plant and Soil. 2001;237:47-54.
29. Glick BR, Bashan Y. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of fungal phytopathogens. Biotechnology Advances. 1997;15:353-378.
30. Dong X, Lv L, Wang W, Liu Y, Yin C, Xu Q, et al. Differences in distribution of potassium solubilizing bacteria in forest and plantation soils in myanmar. International Journal of environmental Research and Public Health. 2019;16(5): 2-14.

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