Original Research Article

Plant Growth Promoting Potential of Rhizobialand Non-RhizobialEndophytesAnd Their Influence on Groundnut Germination in Drought Stress Under in Vitro Conditions

ABSTRACT

Drought stress is the major limiting factor for crop production in most areas. To mitigate the adverse effects of drought in plants, we aimed to employthe drought tolerant endophytic bacteria in this present investigation. Thus, screening of drought tolerance and plant growth promoting traits (PGPR) of endophytic bacteria were carried out using polyethylene glycol (6000). All the 10 endophytic bacteria are capable of expressing plant growth promoting traits like IAA, EPS, siderophore, biofilm formation, ACC deaminase activity, mineral solubilization, and accumulation of osmolytes under normal conditions. During drought conditions, these endophytes have increased the production of EPS, biofilm formation, and osmolytes accumulation. This mechanism helps plants to hope up during drought stress.Co-inoculation of *Rhizobium puesence* S6R2and *Enterobacter cloacae*promotedgroundnutgermination under drought stress condition.

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KEYWORDS

Drought, PGPR, Endophytes, Poly Ethelene glycol, and Ground nut

1. INTRODUCTION

Drought stress is one of the agricultural challenges that affect crop productivity in dryland environments. Drought occurs when the amount of water in the plant's rhizosphere falls below the level required for development and biomass generation [1]. The intensity and exposure period determine the effects of drought on the plant system. Long-term drought exposure disrupts chloroplasts and starch granules, which directly alter photochemical activities and lower the transpiration rate of the plant. Short-term drought increases water utilization efficiency in plants by reducing stomatal aperture and transpiration rate [2]. Drought can alter soil properties significantly, limiting nutrient transport and slowing microbial processes. Reduced photosynthesis, hormonal instability, an accumulation of reactive oxygen species (ROS), and low water and nutrient uptake efficiency are all characteristics of drought-stricken plants [3]. During dry periods, plants undertake multiple copping techniques to trigger signaling responses [4].

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To combat the harmful effects of water deficiency conditions on plants, several tactics are being used, including the selection of resistant types, molecular breeding, and genetic engineering [5]. But the majority of these techniques are time-consuming, expensive, and unpopular in some places. The approaches using distinct microbial strains from different resources have lately been emphasized as an emerging and revolutionary method of plant growth promotion along with agricultural productivity insofar as the enhanced productivity of crops is concerned, under varied abiotic challenges. Plant growth promoting Rhizobacteria (PGPR) produce antioxidant and ROS-degrading enzymes in plants under field circumstances, which directly reduces the oxidative effect during drought [6]. The challenge of integrating foreign species into acclimated and established microbial communities is the fundamental reason that attempts to introduce beneficial bacteria into the rhizosphere of crops have largely failed in several ways. Endophytes have recently attracted attention because they are easily accessible to host plants and are more resistant to environmental extremes, such as high salt and drought, than soil bacteria [7, 8].

Endophytes are microorganisms that reside in plant tissues and build a relationship with the host without influencing its physiological processes [9]. Endophytes aren't responsible for any plant ailment, but they frequently make a considerable contribution to the nutrients that reach their host plant and can aid that plant in overcoming several biotic or abiotic challenges. Rhizobacterial/bacterial endophyte-mediated physical and chemical changes in plants that increase tolerance to diverse abiotic stimuli have been classified as "induced systemic tolerance" (IST) [10, 11]. Increasing host plant nutrition, promoting plant growth through phytohormone secretion, improving plant osmotic regulation, balancing the production of reactive oxygen species (ROS) and plant hormones, and inducing expression of host plant stress-responsive genes are some of the possible mechanisms of IST [12, 13].

Groundnut (*Arachis hypogea* L.) is a significant legume crop in arid and semi-arid regions as its seed contains 44–56% oil and 22–30% protein on a dry seed basis [14]. Groundnut is frequently subjected to drought stresses with the percentage deviation from the mean annual rainfall of different duration and intensities. The rise and fall in the yield and production coincided with the percentage deviation from the mean annual rainfall (DES 1990). In our previous study, a total of ninety-eight bacteria were isolated from the nodules of bunching and semi-spreading types of groundnut phenotypes. Of these 10 endophytic bacteria were selected, which comprise four rhizobialendophytes and six non-rhizobialendophytes[15]. In a previous study, we already documented rhizobial and non-rhizobialendophytes for their halotolerant capacity in both in-vitro and in vivo conditions [16]. In this present study, we documented the drought-tolerant ability of these rhizobial and non-rhizobialendophytes and their plant growth-promoting traits in moisture-induced conditions. Also, we proved the selected rhizobial and non-rhizobialendophytes' potential for mitigation of moisture stress in the germination of groundnut under in vitro condition.

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2. MATERIAL AND METHODS

2.1 Strains, Media, and Growth condition

Bacterial strains used for the study are four rhizobialendophytes which include *Rhizobium pusense* S6R2, *Rhizobium phaseoli* S10R2, *Rhizobium mayense* S11R1, *Rhizobium phaseoli* S18, and six non-rhizobialendophytes*Brevibacillusbrevis* S8R1, *Bacillus tequilensis* NBB13, *Pantoeadispersa* YBB19B, *Bacillus altitudinus* TBB5A, *Enterobacter cloacae* S23, *Paenibacillusillionoisensis* YBB20. Rhizobialendophytes were cultured in yeast extract mannitol agar medium and non-rhizobialendophytes were cultured in a nutrient medium. Unless otherwise stated overnight cultures maintained in a shaker cum incubator at 28 ± 2°C (100rpm) were used in this study.

2.2Screening of selected endophytes for polyethylene glycol tolerance

The bacterial isolates' osmotic tolerance was assessed by observing their growth in nutrient broth and yeast extract mannitol broth modified with two different concentrations of PEG 6000 (0% & 40 %) and incubated the cultures at 28 ± 2°C for 48 h under constant shaking (200 rpm). By measuring the cultures' optical density at 600 nm with a spectrophotometer (M/s. Shimadzu, Japan), the growth and their drought-tolerant capacity were analyzed. Based on Optical Density values, these endophytes were classified as susceptible (OD<0.1), Tolerant (OD = 0.1-0.25), and completely Tolerant (OD >0.252).

2.3 PLANT GROWTH PROMOTING TRAITS

2.3.1 Indole-3-Acetic Acid Production

Bacteria were inoculated in 5ml of nutrient and yeast extract mannitol broth with two different concentrations of PEG (0% & 40%) in a test tube supplemented with 0.1% tryptophan and incubated for 2 days in shaking conditions at 120 rpm at 28 ± 2°C. Supernatants were collected by centrifugation of broth at 12,000 rpm for 10 min. The reaction mixture for the auxin production consists of 500µl of cell-free supernatant, 50 µl of 0.1mM orthophosphoric acid, and 2ml of Salkowski reagent (1ml of 0.5M $FeCl_3$ in 50 ml of 35% perchloric acid). Then the reaction mixture was kept in the dark for 30 mins. The pink to red coloration is a positive indication of auxin production. Colour intensity was measured at an absorbance of 530nm. A standard curve is prepared using IAA and expressed as µg ml^{-1}[17].

2.3.2 ACC Deaminase Activity

In order to estimate 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, endophytic bacteria were inoculated in minimal Dworkin and Foster (DF) salt medium (DF salt

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contains 6.0 g Na_2HPO4, 4.0 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 2.0 g citric acid, 2.0 g glucose, and 2.0 g gluconic acid per litre with the following trace elements: 124.6 mg $ZnSO_4 \cdot 7H_2O$, 78.22 mg $CuSO_4 \cdot 5H_2O$, 11.19 mg $MnSO_4 \cdot H_2O$, 10 mg MoO_3, 10 mg H_3BO_3, 1 mg $FeSO_4 \cdot 7H_2O$; pH 7.2) supplemented with 3 mM ACC instead of $(NH_4)_2SO_4$ as a sole nitrogen source in two different concentration of PEG (0% &40%). After incubation for 3 days, a quantitative estimation of ACC deaminase activity was done at 540nm. α-ketobutyrate was used for the standard curve preparation. The Bradford test was used to estimate the protein. The amount of -ketobutyrate released in nmol per milligram of cellular protein per hour was used to quantify the ACC deaminase activity [18].

2.3.3 Exopolysaccharide (EPS) production

EPS was extracted from three days old culture broth through centrifugation at 10,000 rpm for 10mins. In the ratio of 1:2, supernatant and ethanol were mixed and incubated at -20°C for 24hrs. The precipitated EPS was dissolved in 2ml of water. 200 µl of 5% phenol and 1 ml of 93% sulphuric acid were also added, and the mixture was incubated at room temperature for 10 minutes. EPS production is indicated by the formation of yellow colour and colour intensity was observed at 490nm. Values were compared with the standard value of glucose [19].

2.3.4 Biofilm formation assay

Bacterial endophytes were allowed to grow in 96 well microtiter plates for biofilm formation assay. 10 µl (1×10^8cfu/ml) of a 24-hour-old culture was inoculated into the 96 well microtiter plate with 150 µl of yeast extract mannitol and nutrient broth supplemented with PEG (0% & 40%). After 2 days of incubation, the plate was washed thoroughly two or three times with distilled water and dried. Then 150 µl of 1% crystal violet stain was added. After 45 mins plate was again rinsed with distilled water 2-3 times. Purple ring formation on the good edge was correlated with biofilm formation. 200 µl of 95% ethanol was added to the wells for quantitative analysis and absorbance was measured at 590nm [20].

2.3.5 Siderophore production

The cell-free supernatant from 48hrs old culture amended with PEG (0% and 40%) in nutrient and yeast extract mannitol broth was collected by centrifugation at 10,000 rpm for 10 min. 0.5 ml of CAS reagent were added to 0.5ml of cell-free supernatant. Optical density was measured after 20 mins incubation at 630 nm [21]. The percent siderophore unit (psu) was calculated as follows:

$$\text{Siderophore production (psu)} = \frac{(A_r - A_s) \times 100}{A_r}$$

Here, A_r= absorbance of reference (CAS solution and uninoculated broth)

A_s= absorbance of the sample (CAS solution and cell-free supernatant of sample)

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2.3.6 Phosphate solubilization

Solubilization of insoluble phosphate was quantitatively estimated by inoculating bacterial strain in 10 ml of Pikovskaya's broth supplemented with 0.5% tricalcium phosphate with and without PEG (40%). The broth was kept in a shaker (120rpm) at 28±2°C for 5 days. 1ml of supernatant and 0.8ml of color reagent were added to the test tube and made up to 5ml. The color developed was observed after 15mins at 660nm. For standard curve preparation, potassium dihydrogen phosphate was used [22].

2.3.7 Zinc Solubilization

For the quantitative solubilization of zinc, bacterial cultures were inoculated in Bunt and Rovira broth amended with 0.1% zinc oxide at pH 7. The flasks were shaken at 150 rpm while being incubated at 28±2° C for 72 hours. The culture broth was centrifuged at 10,000 rpm for 10 minutes, after 72 hours of incubation. Atomic absorption spectrophotometer (AAS Model: GBC Avanta PM) was used to quantify the concentration of Zn in the cell-free supernatant [23].

2.3.8 Estimation of accumulated osmolytes

The bacterial culture was inoculated in nutrient and yeast extract mannitol broth and kept in a shaker (120rpm) at 28±2°C for 24 h. Proline estimation was done by centrifugation at 10,000 rpm for 10 min to harvest the cell pellet. 80% ethanol was added to the cell pellet and kept at 60°C in a water bath for 45 mins and 1ml of supernatant was collected by centrifugation of the suspension at 8,000 rpm for 15mins. 1 ml of acid ninhydrin and 1 ml of glacial acetic acid were added to the supernatant and boiled at 100°C for 1 hour. Then, the tubes were cooled inan ice box. Proline from the supernatant was extracted by the addition of 2ml of toluene and the OD value was measured at 520nm. The proline was used to prepare a standard curve. The result is given as µg of proline per milliliter of bacterial culture [24].

To estimate glycine betaine, the cells were extracted by centrifugation at 10,000 rpm for 10 minutes. An aliquot of 0.5 ml of the supernatant was taken into a test tube and chilled in cold water for an hour after being diluted to 1:1 with 2 N sulphuric acid. After adding 0.2 ml of cold iodine reagent, the liquid was gently vortexed. Following 16 hours of storage at 4°C, the samples were moved to a centrifuge tube and centrifuged at 10,000 rpm for 15 minutes at 0°C. A 1 ml micropipette was used to carefully aspirate the supernatant, and the centrifuge tube was maintained on ice. The precipitate was obtained, and it was mixed rapidly with 9 ml of 1, 2-dichloroethane to dissolve it. In the spectrophotometer, absorbance was detected at 365 nm after 2.0–2.5 hours. The glycine betaine standards (50–200 mg ml^{-1}) were made in 2N sulphuric acid. The data is given as µg of glycine betaine per milliliter of bacterial culture.

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Cells were harvested by centrifugation at 10,000 rpm for 10 minutes, and trehalose was extracted from the cell pellet overnight in ethanol at a 70 percent (w/v) concentration. To eliminate cell debris, the ethanol extract was centrifuged at 10,000 rpm for 10 min. The supernatant was then dried at 70°C. 10 ml of distilled water was used to dissolve the dry residue. One milliliter of the extract was transferred to a test tube after it had been carefully diluted in 10 milliliters of distilled water. This was mixed with 2 ml of anthrone reagent (200 mg l^{-1}anthrone in 95% sulphuric acid) and incubated for 15 minutes in a hot water bath. The assay mixture was then incubated and chilled on ice for five minutes, and the absorbance was measured at 630 nm. The standard curve was generated using 0–50 g ml^{-1}trehalose, to determine the trehalose concentration [25].

2.4 In-vitro germination study

Best drought-tolerant and well-performing plant growth-promoting rhizobacteria were selected and treated with groundnut seed (cv., VRI 2). Groundnut seeds were surface sterilized with 70% ethanol and 0.5 % sodium hypochlorite solution. Then the seeds were soaked in the cell suspension (1×10^{8}cfu/ml) for 45 mins as follows T1-*Enterobacter cloacae* S23, T2 - *Bacillus tequilensis*NBB 13, T3 - *Rhizobium pusense*S6R2, T4 - *Rhizobium pusense*S6R2 + *Enterobacter cloacae* S23, T5 - *Rhizobium pusense*S6R2 + *Bacillus tequilensis*NBB 13, T6 - Control. After treatments, the seeds were placed in Petri plates on two sheets of germination paper previously moistened with distilled water amended with PEG (0%, 20%, and 30%). The seeds in plates were incubated for five days from the day the experiment was deployed, the number of seeds that had germinated was counted every day, and seeds that had at least a primary root were considered germinated [26]. At the end of the experiment the percentage of germination [27] and the germination speed index (GSI) [28],. GSI was calculated by the formulae: GSI = E1 /N1 + E2 /N2 + ... + En/Nn, where: E1, E2, En = number of germinated seeds, computed in the first, second, ..., last count; N1, N2, Nn = number of days after the test deployment. The germination percentage was calculated by dividing the number of seeds germinated by the total number of seeds. The experiment was conducted in a completely randomized design with three replications.

2.5 Statistical Analysis

All the experiments were carried out in triplicate and the data were mentioned with mean and standard error. The experimental data were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test using SPSS software for identifying the significant difference between treatments at the 5% level. Wherever statistical significance was observed, the critical difference was worked out at a 5 percent probability level and the values were furnished in the respective tables.

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3. RESULTS

3.1 Drought tolerance ability of the endophytes

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Bacterial culture growth was reduced with the increasing concentration of polyethylene glycol. The results revealed that *Bacillus tequilensis*NBB 13, *Enterobacter cloacae* S23 and *Rhizobium pusense*S6R2 were completely tolerant to the drought stress.

Table 1. Screening of drought tolerance capacity of RE and NRE

S. No.	Bacteria	Drought tolerant capacity (40 % PEG)
	Rhizobialendophytes	
1	*Rhizobium phaseoli*S18	S
2	*Rhizobium pusense*S6R2	CT
3	*Rhizobium phaseoli*S10R2	S
4	*Rhizobium mayense*S11R1	S
	Non-rhizobialendophytes	
1	*Bacilusaltitudinus*TBB 5A	MT
2	*Bacillus tequilensis*NBB 13	CT
3	*Enterobacter cloacae* S23	CT
4	*Brevibacillusbrevis*S8R1	S
5	*Pantoeadispersa*YBB20	S
6	*Paenibacillusillionoisensis*YBB19B	S

CT – completely tolerant (OD value >0.25); MT- moderately tolerant (OD value 0.25-0.1);
S – Sensitive (OD value <0.1)

3.2 PLANT GROWTH PROMOTING TRAITS

3.2.1 Indole acetic acid production

All the bacteria were capable of producing indole acetic acid in both unstressed and stressed conditions. Maximum production was recorded in*Rhizobium phaseoli* S18 (RE) (23 µg/ml), followed by*Paenibacillusillionoisensis* YBB19B (NRE) (27 µg/ml), and minimum production was observed in *Rhizobium mayense*S11R1 (RE) (0.94 µg/ml), followed by *Bacilusaltitudinus*TBB5A (NRE) (2.5 µg/ml) under unstressed condition. In the case of drought conditions, maximum production was recorded in *Rhizobium phaseoli*S18 (RE) (1.18 µg/ml), followed by *Bacillus tequilensis*NBB13 (NRE) (2.03 µg/ml). Minimum production was observed in*Rhizobium mayense* S11R1 (RE) (0.43 µg/ml) followed by *Brevibacillusbrevis*S8R1 (NRE) (0.20 µg/ml).

Table 2. IAA production of RE and NRE under normal and stressed conditions

S. No.	Bacteria	IAA production (µg/ml)	
		Without stress	With stress (40% PEG)
	Rhizobialendophytes		
1	*Rhizobium phaseoli*S18	23.43 ± 6.89^{a}	1.18 ± 0.71^{abc}
2	*Rhizobium pusense*S6R2	20.05 ± 0.47^{a}	0.57 ± 0.03^{cd}
3	*Rhizobium phaseoli*S10R2	2.19 ± 0.3^{c}	0.78 ± 0.03^{cd}
4	*Rhizobium mayense*S11R1	0.95 ± 0.27^{c}	0.44 ± 0.17^{cd}
	Non-rhizobialendophytes		
1	*Bacilusaltitudinus*TBB 5A	2.58 ± 0.2^{bc}	1.84 ± 0.2^{ab}

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2	*Bacillus tequilensis*NBB 13	3±0.17^{bc}	2.03±0.22^{a}
3	*Enterobacter cloacae* S23	5.14±0.09^{bc}	1.82±0.04^{ab}
4	*Brevibacillusbrevis*S8R1	10.26±2.77^{b}	0.2±0.2^{d}
5	*Pantoeadispersa*YBB20	10.06±1.28^{b}	0.37±0.17^{cd}
6	*Paenibacillusillionoisensis*YBB19B	27.48±2.16^{a}	1.11±0.3^{bc}
	CD (0.05)	7.836	0.8818

Values are mean ± standard error with 3 replicates. Means followed by the same letter do not differ by DMRT at a 5% probability

3.2.2 ACC deaminase activity

The result indicated that all the selected isolates were capable of ACC deaminase activity with maximum activity in *Enterobacter cloacae*S23 (NRE) with 433.05 followed by 119.47nmol α-ketobutyrate mg protein^{-1}h^{-1} and *Rhizobium mayense*S11R1 (RE) with 72.13 and 43.66 nmol α-ketobutyrate mg protein^{-1} h^{-1} both in a normal and drought stress condition. And the minimum activity was observed in *Brevibacillusbrevis*S8R1 (NRE) followed by *Rhizobium phaseoli*S10R2 (RE).

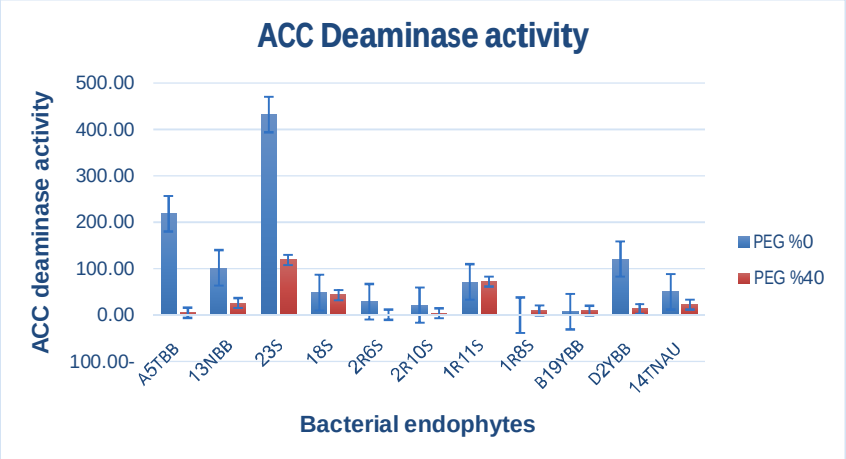

Figure 1. Clustered column chart showing ACC deaminase activity of RE and NRE in both normal (0%) and stressed conditions (40% of PEG)

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3.2.3 EPS production

The amount of EPS production was increased during stress conditions. The maximum production was noted in TBB 5A (NRE) (148.4 µg/ml) followed by *Rhizobium mayense*S11R1 67.59 µg/ml; *Bacillus tequilensis*NBB13 (56.25 µg/ml) and *Rhizobium mayense*S11R1 (113.5 µg/ml) under normal and water-deficient condition. The least EPS production was observed in *Bacillus tequilensis*NBB13 (21.9 µg/ml) and *Rhizobium phaseoli*S18 (26.465 µg/ml) under normal conditions. While in stressed conditions the least EPS production was recorded in *Brevibacillusbrevis*S8R1 and *Rhizobium phaseoli*S10R2.

Table 3. EPS production by bacterial endophytes under stressed and normal conditions

S. No.	Bacteria	EPS production (µg/ml)	
		Without stress	With stress (40% PEG)
	Rhizobialendophytes		
1	*Rhizobium phaseoli*S18	26.465±3.99^{b}	69.38±0.62^{c}
2	*Rhizobium pusense*S6R2	49.22±1.67^{b}	81.64±2.10^{b}
3	*Rhizobium phaseoli*S10R2	60.33±5.51^{b}	54.13±18.51^{def}
4	*Rhizobium mayense*S11R1	67.59±1.05^{d}	113.52±0.578^{a}
	Non-rhizobialendophytes		
1	*Bacilusaltitudinus*TBB 5A	148.4±23.7^{a}	42.53±7.32^{fg}
2	*Bacillus tequilensis*NBB 13	21.97±0.8^{d}	56.25±5.59^{d}
3	*Enterobacter cloacae* S23	22.99±0.72^{bc}	54.83±4.25^{de}
4	*Brevibacillusbrevis*S8R1	64.61±5.60^{d}	29.98±1.988^{gh}
5	*Pantoeadispersa*YBB20	24.77±3.1^{d}	43.60±2.96^{efg}
6	*Paenibacillusillionoisensis*YBB19B	33.89±1.98^{cd}	38.78±6.98^{h}
	CD value (0.05%)	20.56	11.84

Values are mean ± standard error with 3 replicates. Means followed by the same letter do not differ by DMRT at 5% probability.

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3.2.4 Biofilm formation ability

Strong biofilm formation ability was observed in *Rhizobium pusense*S6R2in both stress and unstressed condition; *Enterobacter cloacae*S23 also formed strong biofilm in unstressed conditions and had moderate biofilm ability in a stressed condition.

Table 4. Biofilm forming ability of RE and NRE

S. No.	Bacteria	Biofilm formation capacity	
		Without stress	With stress (40%PEG)
	Rhizobialendophytes		
1	*Rhizobium phaseoli*S18	Weak	Negative
2	*Rhizobium pusense*S6R2	Strong	Strong
3	*Rhizobium phaseoli*S10R2	Strong	Strong
4	*Rhizobium mayense*S11R1	Strong	Negative
	Non-rhizobialendophytes		
1	*Bacilusaltitudinus*TBB 5A	Moderate	Weak
2	*Bacillus tequilensis*NBB 13	Strong	Moderate
3	*Enterobacter cloacae* S23	Strong	Moderate
4	*Brevibacillusbrevis*S8R1	Moderate	Moderate
5	*Pantoeadispersa*YBB20	Weak	Negative
6	*Paenibacillusillionoisensis*YBB19B	Strong	Negative

OD >0.3 – strong; 0.2-0.3 – moderate; 0.1-0.2 – weak; <0.1- negative

3.2.5 Siderophore formation

Siderophore formation ability is usually expressed in the percent siderophore unit (psu). *Rhizobium phaseoli*S10R2 and *Brevibacillusbrevis*S8R1 exhibited the maximum siderophore formation ability in normal conditions and *Enterobacter cloacae*S23 and *Rhizobium phaseoli*S18 showed the maximum siderophore formation ability under -10.7 bars of stress.

Table 5. Siderophore production of RE and NRE

S. No.	Bacteria	Siderophore production (psu)	
		Without stress	With stress (40% PEG)
	Rhizobialendophytes		
1	*Rhizobium phaseoli*S18	40.33±0.76^{de}	16.98±1.14^{b}
2	*Rhizobium pusense*S6R2	67.88±0.4^{er}	6.74±0.25^{f}
3	*Rhizobium phaseoli*S10R2	81.89±2.36^{b}	1.15±0.07^{h}
4	*Rhizobium mayense*S11R1	45.21±0.28^{d}	2.55±0.05^{g}
	Non-rhizobialendophytes		
1	*Bacilusaltitudinus*TBB 5A	43.62±1.34^{c}	15.34±0.99^{b}
2	*Bacillus tequilensis*NBB 13	49.42±0.24^{g}	7.58±0.35^{e}
3	*Enterobacter cloacae* S23	36.07±0.01^{h}	18.41±0.89^{a}
4	*Brevibacillusbrevis*S8R1	4.36±3.33a	12.55±0.81^{d}
5	*Pantoeadispersa*YBB20	19.99±0.57^{d}	13.13±0.54^{c}
6	*Paenibacillusillionoisensis*YBB19B	1.75±0.28^{f}	13.71±0.41^{c}
	CD(0.05%)	3.92	1.097

Values are mean ± standard error with 3 replicates. Means followed by the same letter do not differ by DMRT at a 5% probability

3.2.6 Phosphate solubilization

In normal conditions, *Bacilusaltitudinus*TBB5A and *Rhizobium pusense*S6R2 expressed the maximum phosphate solubilization efficiency *i.e*., 10.25 and 14.23 µg/ml. Under drought stress conditions *Rhizobium mayense*S11R1 and *Pantoeadispersa*YBB20 performed well with the efficiency of 0.26 and 1.89 µg/ml. Most of the tested RE and NRE could solubilize the insoluble phosphate under stressed conditions.

Table 6. Phosphate and Zinc solubilization of RE and NRE

S. No.	Bacteria	Phosphate solubilisation (µg/ml)		Zinc solubilization(µg/ml)	
		Without stress	With stress (40%)	Without stress	With stress (40%)
	Rhizobialendophytes				
1	*Rhizobium phaseoli*S18	1.54±0.16^{d}	ND	101.54±5.11^{ab}	0.61±0.03^{f}
2	*Rhizobium pusense*S6R2	14.23±0.05^{e}	ND	105.93±3.24^{ab}	0.44±0.01^{f}
3	*Rhizobium phaseoli*S10R2	9.09±0.27^{b}	ND	105.19±4.39^{ab}	1.28±0.05^{e}
4	*Rhizobium mayense*S11R1	12.42±0.07^{c}	0.26±0.004^{b}	101.54±1.55^{ab}	4.6±0.07^{c}
	Non-rhizobialendophytes				
1	*B.altitudinus*TBB 5A	10.25±0.35^{a}	ND	105.93±3.82^{ab}	3.09±0.11^{d}
2	*Bacillus tequilensis*NBB 13	5.92±0.08^{e}	ND	108.85±3.92^{a}	0.11±0^{f}

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3	*Enterobacter cloacae* S23	5.98±0.01^{f}	ND	105.19±3.69^{ab}	0.53±0.02^{f}
4	*Brevibacillusbrevis*S8R1	3.46±0.21^{c}	0.03±0.001^{c}	85.44±3.85^{c}	14.31±0.65^{a}
5	*Pantoeadispersa*YBB20	17.25±0.21^{b}	1.89±0.063^{a}	82.52±2.71^{c}	3.7±0.12^{d}
6	*Paenibacillusillionoisensis*YBB19B	17.4±0.17^{b}	ND	98.61±2.61^{b}	11.2±0.3^{b}
	Mean	9.15±0.15	0.155±0.006	99.14±3.31	3.69±0.12
	CD (0.05%)	0.541	0.0567	10.215	0.6515

ND- Not detected; Values are mean ± standard error with 3 replicates. Means followed by the same letter do not differ by DMRT at a 5% probability

3.2.7 Zinc Solubilization

*Bacillus tequilensis*NBB13 and *Rhizobium pusense*S6R2 possessed the high solubilization efficiency of Zinc under unstressed conditions and were capable of solubilizing 108.851 and 105.925 µg/ml while *Rhizobium mayense*S11R1 and *Paenibacillusillionoisensis*YBB19B exhibited the maximum solubilization efficiency of 4.601 and 11.199 µg/ml, respectively under stressed conditions.

3.2.8 Estimation of osmolytes accumulation

Under drought stress *Rhizobium pusense*S6R2 accumulated increased proline content,*Enterobacter cloacae* S23 and *Rhizobium phaseoli*S10R2 showed higher trehalose, and *Bacilusaltitudinus*TBB5A and *Rhizobium mayense*S11R2 accumulated high glycine betaine.

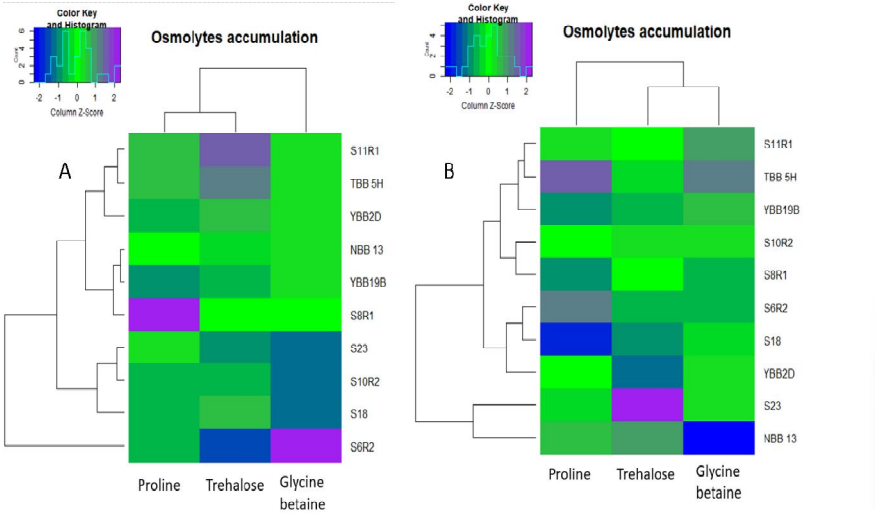

Figure 2. Heatmap for the accumulation of osmolytes under normal (A) and stressed condition (B).

3.3 InvitroGermination study

All the treatments showed a 100% of germination percentage under normal conditions and there was no significant difference among the treatments observed. In the case of the 20% PEG condition, T3, T4, and T5 showed 100 % of germination percentage, whereas T1, T2, and T6

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showed 83.3 % of germination percentage. And no germination was observed in the 30% PEG condition. Speed of germination was also the same for all the treatments under normal conditions, whereas in the 20 % PEG condition the maximum speed of germination was observed in T4 and the least was noted in T1.

Table 7. Germination percentage and germination speed index (GSI) obtained from groundnut seeds subjected to germination under drought conditions

S. No.	Treatments	Treatments details	Germination percentage		Germination speed index	
			Without stress	With stress (20%PEG)	Without stress	With stress (20%PEG)
1	T1	*Enterobacter cloacae* S23	100	83.3[b]	3.5	1.5[d]
2	T2	*Bacillus tequilensis*NBB 13	100	83.3[b]	3.5	2.83[c]
3	T3	*Rhizobium pusense*S6R2	100	100[a]	3.2	4[a]
4	T4	*Rhizobium pusense*S6R2 + *Enterobacter cloacae* S23	100	100[a]	3.5	4.3[a]
5	T5	*Rhizobium pusense*S6R2 + *Bacillus tequilensis*NBB 13	100	100[a]	3.5	3.4[b]
6	T6	Control	100	100[a]	3.5	1.5[d]
		CD (0.05%)		5.343	0.413	0.386

Means followed by the same letter do not differ by DMRT at a 5% probability

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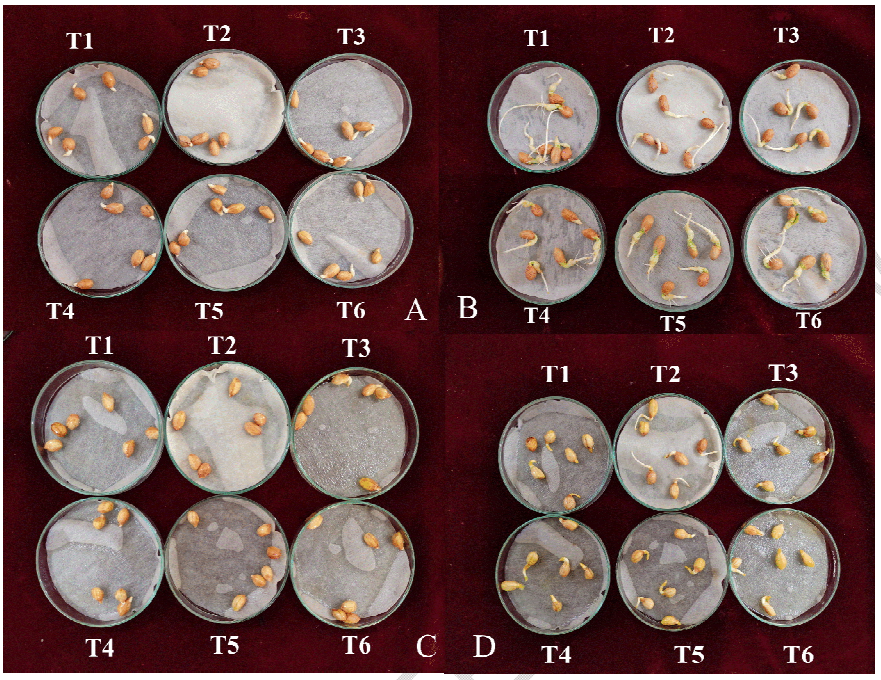

Figure 3. Groundnut seed germination at 3rd day (A&C) and 5th day (B&D) with 0% PEG (A&B) and 20% PFG (C&D) T1 – S23; T2 – NBB13; T3- S6R2; T4 – S6R2 + S23; T5 – S6R2 + NBB13; T6 – Uninoculated

4. DISCUSSION

All the bacterial endophytes taken for the study exhibited a variety of plant growth-promoting traits such as IAA, EPS production, biofilm-forming ability, ACC deaminase, siderophore, osmolytes accumulation, zinc, and phosphate solubilization, which interact either directly or indirectly to enable faster growth rate of plants [29]. The root system infected with IAA-producing rhizobacteria changes root architecture by growing in size and weight, in several branches, and surface with an increase in the contact area with the soil. All these adjustments strengthen the plant's capacity to probe the soil for nutrient exchange, which enhances the plant's nutritional reserve and growth potential [30]. Our research showed that all bacterial endophytestaken for the study can synthesize IAA even in drought-stressed environments, which may aid plants in strengthening roots and survival. The precursor to the phytohormone ethylene is 1-aminocyclopropane-1-carboxylic acid (ACC). Although ethylene is typically produced by plants, abiotic circumstances like drought, salinity, or water floods significantly boost its production. PGPR can produce the ACC deaminase enzyme, which degrades ACC and prevents it from being

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metabolized to ethylene. Abiotic stress stimulates plants to produce greater amounts of ACC, which is then broken down by bacterial ACC deaminase into ammonia and α-ketobutyrate, preventing the formation of ethylene. By releasing ACC deaminase, PGPR shields plant enduring abiotic stress from the deleterious effects of ethylene. Each of the endophytic bacteria used in this study expressed ACC deaminase activity. Even while most bacterial endophytes expressed relatively lower levels of ACC deaminase production under extreme drought (40 percent PEG - 17.5 bars), the development of this enzyme may still be crucial for the host plants' ability to withstand drought stress. Under extreme environments, EPS production has been recorded as a stress response and survival mechanism [31]. *Rhizobium* spp. had been used to explain the origin of EPS in soil bacteria because of their main function in soil aggregation, which optimizes moisture retention in the rhizosphere[32, 33]. We can infer that the selected bacterial endophytes have the flexibility to cope with both themselves and their host in a tough environment because they have illustrated an enhanced production of EPS under stress conditions with a range of 30-110 g/ml. All 10 endophytes, showed increased EPS production, and they formed biofilms even under abiotic stress. EPS production and biofilm formation are therefore directly correlated.

Mineral solubilization is the one key factors of all plant growth-promoting bacteria, our endophytic isolate could solubilize phosphorous and zinc. Some isolates were unable to solubilize the insoluble phosphate (tricalcium phosphate) under drought conditions (-17.5 bars). In the case of Zinc solubilization, the concentration of soluble zinc under stress was lower when compared to the solubilized zinc under normal conditions. Under drought stress, maintaining membrane integrity is necessary to alleviate protein denaturation [34, 35]. To counteract the negative consequences of drought, proline synthesis causes osmotic adjustment, free radical scavenging, and stabilization of subcellular structures in plant cells [36]. In this investigation, proline accumulation was significantly increased for all the bacterial endophytes, this implied these plant growth-promoting bacteria helps in the survival of plants under abiotic stress condition. Trehalose, a non-reducing disaccharide, acts as an osmoprotectant by stabilizing dehydrated enzymes and membranes; thus, its biosynthesis imparts osmoprotectant in bacteria [11]. Trehalose production was slightly lowered compared to the unstressed condition. Glycine betaine content was also increased under stressed conditions. This acclimation of osmolytes was the major mechanism to cope with the drought stress.

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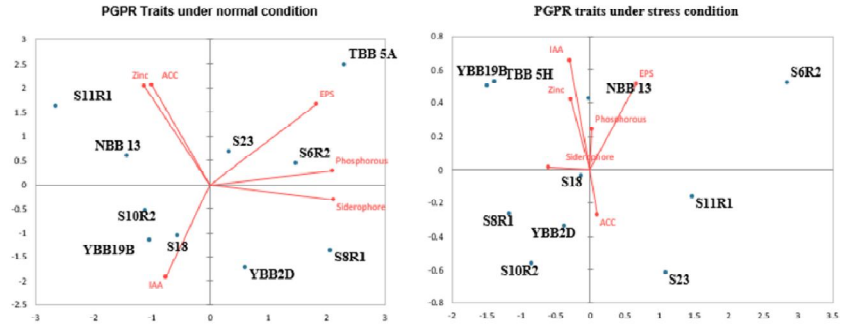

Figure 4. Principle component analysis (PCA) of PGPR under normal and stressed conditions

PCA charts were used for the identification of the best cultures to fix treatments. The RE and NRE performing well under stress was taken as the best and further germination studies were carried out with those efficient isolates. *Rhizobium pusense* S6R2 (RE) and *Bacillus tequilensis*NBB13;*Enterobacter cloacae* S23 (NRE) fell under the positive quadrant in PCA and were selected for germination assay. EPS, phosphorous, and siderophore were the major influencing factors for the selection of the best isolates. A germination study revealed that co-inoculation of S6R2 (*Rhizobium puesence)* and S23 (*Enterobacter cloacae)* have performed well.

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4. CONCLUSION

The application of drought tolerant endophytic bacterial strains in crops is an environment-friendly strategy that ameliorates the adverse effects of drought stress in plants. These endophytes adopt different mechanisms like osmolyte production, phytohormones production, and antioxidants synthesis which induce drought tolerance in plants. The bacterial combination is the best choice for improving morphological, physiological, and biochemical parameters of crops under drought and it can be used as a potential inoculant in arid agro-ecologies after validation under field conditions.

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