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Study on Plant Growth Promoting Traits of PGPR Isolates from Semi-Arid Kachchh, Gujarat under Salt Stress Conditions

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Abstract

Salinity is a major problem in the agricultural sector, as it turns productive agronomical land to become unproductive. Therefore, Plant-growth-promoting rhizobacteria (PGPR), that live in the plant root zone named the rhizosphere, is one of the prominent solutions to overcome this problem in an eco-friendly manner as Rhizobacteria responds to osmotic stress and support plant development. Thus, the present study was aimed to characterize various traits of the PGPR strains isolated from saline soils of Kachchh. The characterization of the traits in the presence and absence of sodium chloride was assessed including IAA production (76.97±1.68 mg/l), Ammonia production (38.59±0.19 mg/l), Siderophore production (49.21±1.83%), Phosphate solubilization (4897.73±25.53 mg/l). When assessed for the salt tolerance of the strains in the presence of NaCl between 20-50 gm/L, the strain D6 exhibited a better growth even at 5% NaCl concentration (2.047OD). Further, the effect of PGPR on the growth of *V. radiata* was 100% in all the experimental setup, whereas in case of *B. juncea*, the highest germination of 96.67% was observed only in T2 and T1+T2+T3. Further, the molecular sequencing of the strains revealed the identification of the strains as *Klebsiella quasipneumoniae*, *Bacillus paralicheniformis* and *Klebsiella pneumoniae*.

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Arid Region; Crops; Molecular Sequencing; Morphometric Characteristics; Rhizosphere; Salinity.

Introduction

One third of the worldwide gross domestic product is contributed by the agriculture sector. The world's population, however, is expected to reach 9.5 billion people by 2050 due to the tendency toward population growth, creating a significant increase in the demand for food (Green *et al.,* 2005). The production of various crops is severely limited by

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factors such as the urbanization, scarcity of fertile land, unexpected weather conditions linked to climate change, abiotic and biotic pressures (Glaser and Lehr, 2019). Salinity is one of the most serious factors reducing the productivity of agricultural crops, with adverse effects on germination rate, plant vigour and crop yield (Munns and Tester, 2008). Soil salinity is predicted to become a serious issue in the coming decades reducing the - agriculture area by 1–2 % every year, hitting hardest in the arid and semi-arid regions (FAO, 2002). In mung bean salt stress significantly reduces root and shoot nitrogen characteristics (Shafique *et al.,*2019). In mustard plant height, branching pattern, pod formation and dry matter yield are negatively affected by higher levels of salinity in the irrigation water used before sowing and during flower initiation (Shekhawat *et al.,* 2012). It has been estimated that on a world-wide scale, the production by approximately 400 million hectares of arable land is being severely limited by salinity (Bot *et al.,* 2000). Therefore, application of beneficial microbiomes as biofertilizers in sustainable agriculture practices has developed as an innovative and environment-friendly technology for improving soil fertility and plant growth (Ullah *et al.,* 2019). Plant growth promoting rhizobacteria (PGPR) comprises bacteria, which are free living in nature and found from the rhizosphere having the ability to produce and secrete metabolites that help plant growth after colonizing on their roots and to combat biotic and abiotic stress (Beneduzi *et al.,* 2012). Around 24 countries were commercially engaged in producing PGPR biofertilizers both in large and small scales (Bharti *et al.,* 2017).

Material and Methods

Sampling Site and Sampling Location

A total of 34 soil samples were collected with the help of auger from the rhizosphere region of crops from 17 villages sites Gaduli, Vanku, Arikhana, Nara, Dhanavada, Halapar, Khavda, Nirona, Samagoga, Andhau, Anjar, Kidana, Khengarpar, Bhachau, Adesar, Gagodar and Bhuj in Kachchh district, Gujarat during pre-monsoon and post-monsoon seasons. The rhizospheric soil was collected in sterilized containers for microbiological analysis.

Isolation and Identification of Isolates

Bacteria were isolated by following serial dilution techniques on nutrient agar medium, *Rhizobium* media and Kings B medium (Himedia). The bacterial isolates having different morphological appearance on agar plates were maintained on nutrient slants at 4°C.

Microscopic Observation for Morphological Characteristics

Morphological characters of the colonies like the colour, shape, size, surface and gram staining etc. were recorded. Based on the colony morphological characteristics and staining properties the isolates were screened for their growth promoting activities like Indole acetic acid (IAA) production, ammonia production, phosphate solubilization, HCN production.

Screening of PGPR Traits for Multiple Plant Growth Promoting Activities

IAA production

Indole acetic acid (IAA) production were quantitatively determined by Salkowski method. (Gordon and Weber, 1951).

Hydrogen cyanide production (HCN)

Bacterial isolates were screened for production of hydrogen cyanide by adapting the method (Bakker *et al.,* 1987).

Production of Ammonia

Bacterial strains were tested for production of ammonia in peptone water as described by (Cappuccino *et al,.*1992).

Siderophore Estimation

Quantitative estimation of siderophore production was measured, in Luria Bertani broth medium (Arora and Verma, 2017).

Phosphate Solubilization

Quantitative estimation of Phosphate employing NBRIP broth was done using molybdophosphoric acid blue method (Jackson, 1973).

Salt Tolerance

The bacterial cultures were selected and enriched in LB broth and grown in different salt concentrations ranging between 20 – 50gm/land the optical density was measured at 600 nm (Sarkar *et al.,* 2017).

Effect of salt on the PGPR Activities

The strains were tested for the ability to solubilize phosphate, siderophore, ammonia and IAA production

in the presence of varying salt concentrations (10-30gm/l NaCl) was done in triplicates following Wang *et al* (2021).

Haemolysis Test

Blood agar medium incorporating5% defibrinated sheep blood was used and incubated at 37°C for 18-24 hrs. for observation of haemolysis (α, β, or γ) by the bacterial strains were performed as per the method of Buxton (2016).

16S rRNA and Phylogenetic Analysis

The molecular sequencing of the strains was done by Gujarat Biotechnology Research Centre where 16S rRNA sequences of the isolates were compared with sequences available in the BLAST tool in the NCBI database. Further, a phylogenetic dendrogram was constructed using the neighbor-joining method.

Germination Experiment using Petri Dish method of *Vigna radiata* and *Brassica juncea*

Seeds of green gram (*Vigna radiata*) were obtained from a local market whereas Mustard seeds (*Brassica juncea*) were procured from Gujarat State Seed Corporation Limited, Bhuj, Gujarat. Prior to the assay, the seeds of uniform size were sorted out for surface sterilization using 0.1% sodium hypochlorite followed with sterile distilled water. The seeds were then soaked in flasks having Freshly grown 18hr old bacterial cultures *viz*., single strain, combination of two strains, consortium and in control as well for 2 hrs at 30 °C in case of *V. radiata* and for 6 hrs at 30ºC in case of *B. juncea.* The triplicate Petri dishes were arranged in a Complete Randomized Design (CRD). Ten treated seeds were placed on petri dishes with sterilized filter paper. The number of germinated seeds was counted on the 10th day to record the germination percentage, length of shoots and roots, vigour index and relative water content (Tang *et al.,*2019; Sinha and Jee 2020).

Data Analysis

All the results were analyzed for in descriptive statistics using Microsoft Office Excel.

Results

A total of 130 bacterial strains were isolated from the soil samples, among this, 61 morphologically different isolates were selected and found 19 isolates having better PGPR traits confirmed via qualitative analysis. Further characterization revealed that three isolates D6, E11 and G14 revealed maximum number of PGP traits and all the three cultures were reconfirmed for PGPR activity in the absence and presence of salt (up to 30g/l). The experiment revealed that in the absence of salt, the Phosphate solubilization ranged from 4897.73±25.53 - 4131.20±30.17 mg/l, the IAA production ranged between 76.97±1.68 -64.59±1.53mg/l. In case of Siderophore production, the strains exhibited 49.21±1.83 - 22.22±0.92% and the Ammonia production was between 7.50±0.17 - 3.50±0.11mg/l, whereas in the presence of salt, the Phosphate solubilization ranged between 4034.16±25.77 - 3566.70±29.22 mg/l, IAA production was between 48.15±1.70 - 19.92±0.69 mg/l, Siderophore production between 32.24±0.24 - 12.62±0.715%, Ammonia production between 4.78±0.13 to2.23±0.10 mg/l. During the initial qualitative examination, HCN production was absent in the presence and absence of NaCl by all the strains. The order of IAA production in the absence of salt was D6>G14>E11, whereas in case of Phosphate solubilization, Siderophore production, Ammonia production, it was in the order of G14>D6>E11. Whereas in the presence of salt, IAA production and phosphate solubilization was exhibited by the strains as D6>G14>E11 and for Siderophore and Ammonia production, it was G14>D6>E11 as shown in Table 1. When the growth of strains in the absence and presence of NaCl (5%) is concerned, it was 1.514OD and 2.047OD by D6 strain, 0.775OD and 1.574OD by E11 strain and in case of G14 strain it was 1.556 OD and 2.013 OD respectively.

When all the bacterial strains were subjected for haemolysis test, it exhibited γ hemolysis pattern which represents that all the strains are non-pathogenic in nature. The molecular sequencing followed by BLAST of the strains, the isolate D6 is identified as *Klebsiella quasipneumoniae* (Accession no OP257241), strain E11 as *Bacillus paralicheniformis* (Accession no OP257271) and strain G14 as *Klebsiella pneumoniae* (Accession no OP257277) through 16SrRNA gene sequencing. A phylogenetic dendrogram was constructed by partial 16S rRNA gene sequencing following BLAST analysis to search for the homology using the neighbor-joining method (Fig. 1). The germination assay revealed that *B. juncea* showed the highest germination percentage in T2 and consortia treatments compared to the control ranging from 83.33 to 96.67%. Conversely

ranging SVI (654.66±14.67 to 1112.33±49.05), RWC (86. 89 ±0.86 % to 91.45 ±0.77 %) and shoot length (5.6±0.10 cm to 7.23±0.18 cm) values were high in consortia treatments than the others while Root length (2.26±0.12 cm to 5.36±0.13 cm) was the highest in T1 (Table2). Whereas in case of *V. radiata*, the germination rate was 100% in all the treatments including control with SVI (1680±5.77 to 2693.33±6.67), root length (4.56±0.03 cm to 7.13±0.38 cm) were highest in T1 compared to control and other treatments, RWC (86. 89 ±0.86 % to 92.56±0.45 %) and shoot length (12.23±0.09 cm to 20.43±0.03 cm) were highest in T2 set compare to control and other treatments as shown in Table 2.

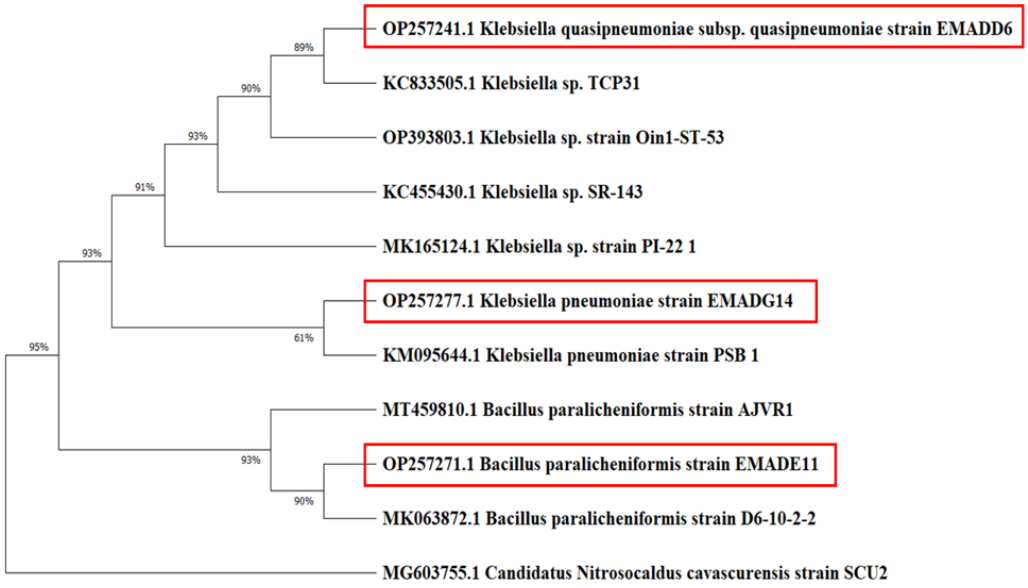

Fig. 1: Neighbor joining Phylogenetic tree of the bacterial strains

Table 1: PGPR traits of the three isolates in the presence and absence of NaCl (30 gm/l)

Sr.	PGPR Character	Bacterial strains					
		D6		E11		G14	
		No NaCl	NaCl (30 gm/l)	No NaCl	NaCl (30 gm/l)	No NaCl	NaCl (30 gm/l)
1	Phosphate solubilization(mg/l)	4356± 39.86	4034.16 ±25.77	4131.20 ±30.17	3566.70 ±29.22	4897.73 ±25.53	3890.61 ±35.11
2	IAA production (mg/l)	76.97± 1.68	48.15± 1.70	64.59 ±1.53	19.92 ±0.69	69.46± 1.14	43.78± 0.60
3	Siderophore production (%)	33.33 ±1.83	29.70± 0.47	22.22 ±0.92	12.62 ±0.71	49.21 ±1.83	32.24 ±0.24
4	Ammonia production(mg/l)	38.59 ±0.19	32.84 ±0.16	36.33 ±0.49	37.06 ±0.16	35.36 ±0.16	33.92 ±0.01
5	HCN production (Qualitative)	-	-	-	-	-	-

Average± standard error from three separate replicates (– not detected)

Table 2: Germination assay of Vigna radiata and Brassica juncea in Petri dish method

Treatments	Germination % (GP)		SVI (Mean ± S.E.)		RWC % (Mean ± S.E.)		Shoot Length (cm) (Mean ± S.E.)		Root Length (cm) (Mean ± S.E.)	
Crop	***V.r***	***B.j***	***V.r***	***B.j***	***V.r***	***B.j***	***V.r***	***B.j***	***V.r***	***B.j***
Distilled water	100	83.33	1680±5.77	654.66±14.67	82.42±6.0	86. 89 ±0.86	12.23±0.09	5.6±0.10	4.56±0.03	2.26±0.12
Luria Bertani broth	100	86.67	1933.33±6.67	719±26.85	87.75±0.87	89.65±0.23	14.6±0.06	5.86±0.09	4.73±0.09	2.43±0.18
Control 1	100	86.67	2233.33±8.82	759±74.28	89.95±0.79	88.61 ±1.63	16.5±0.06	5.9±0.56	5.83±0.07	2.83±0.12
T1	100	93.33	2433.33±28.48	1014.66±27.36	92.56±0.45	89.83 ±2.11	17.2±0.15	6.83±0.33	7.13±0.38	5.36±0.13
T2	100	96.67	2693.33±6.67	908.33±28.04	90.32±1.03	91.04 ±1.15	20.43±0.03	5.6±0.12	6.5±0.06	3.8±0.06
T3	100	86.67	2463.33±12.02	807.66±63.07	89.25±3.23	89.17 ±0.87	17.63±0.09	5.53±0.52	7±0.06	3.83±0.41
T1+T2	100	93.33	2330±5.77	1000±11.27	87.44±3.07	88.75 ±0.85	16.9±0.06	6.66±0.28	6.4±0.06	4.06±0.03
T2+T3	100	93.33	1933.33± 14.53	859.33±86.75	87.59±0.81	90.44 ±0.41	14.5±0.06	6.03±0.39	4.8±0.09	3.13±0.20
T1+T3	100	86.67	2193.33±26.03	947.33±31.42	90.04±2.48	89.34 ±0.84	16.03±0.30	6.7±0.12	5.9±0.06	4.3±0.55
T1+T2+T3	100	96.67	2116.66±12.02	1112.33±49.05	88.27±1.15	91.45 ±0.77	15.9±0.06	7.23±0.18	5.26±0.12	4.26±0.32

T1=D6, T2=E11 and T3=G14

Discussion

Soil salinization has been experienced worldwide and it is estimated that the gradual increase in salt content in irrigated soils has been considered as one of the main threats against crop production (Bacilio *et al.,*2004). PGPR bacteria may provide a natural and harmless means to improve the growth and yield of crops especially under environmental stresses (Zahir *et al.,*2004). The present study assessed the PGP traits such as IAA production, HCN production, Ammonia production, Siderophore production, Phosphate solubilization of the isolates. Among the total of 19 isolates, 18 isolates showed positive for solubilization of phosphate and production of IAA which was also confirmed by reduction in pH in the NBRIP liquid medium. Several studies have shown different bacterial genera, such as *Bacillus, Pseudomonas*, *Rhizobium*, *Klebsiella*, *Agrobacterium* and *Erwinia*, have the potential to solubilize and release phosphorus and potassium from soil (Shrivastava *et al.,*2018).IAA synthesis by PGPR helps in root growth, cell division, stem elongation and increasing the surface area of roots such that plants can obtain additional water and nutrients (Zhang *et al.,*2005), similarly Phosphate solubilization nature of PGPR facilitates nutrition for plant growth and development (Etesami and Alikhani,2017).Similar reports by Patel *et al*. (2012) showed that out of a total of 176 isolates, 82 isolates were screened on the basis of their fast growth potential from two different regions in Gujarat in which, five strains of the 82 strains were selected based on their PGP traits.

Microbes producing siderophore function as an effective PGPR with multifunctional potential of plant growth and disease suppression (Sayyed *et al.,* 2013) and Ammonia production is a significant for nitrogen accumulation because it promotes root, shoot, and biomass growth, which accelerates plant growth (Masclaux-Daubresse *et al.,* 2010).and the present study also confirmed the Siderophore and Ammonia production by the PGPR isolates. Qualitative estimation of HCN revealed a negative result by all the isolates. In addition, all the isolates were confirmed for their salt tolerance nature in the presence of NaCl at 50g/l. Further, the strains showed maximum positive traits (D6, E11 and G14) were assessed further which revealed that in the presence and absence of salt, E11 strain displayed minimum PGPR traits compared to D6 and G14strains. After this, the three strains were tested for germination studies of *V. radiata* and *B. juncea* in the different treatments considering single strains, double strains and as consortium (Table2). Study byRamadoss *et al.,*(2013) found that a total of three isolates (SL3, SL32 and PU62), out of 84, from different hypersaline lakes of India produced siderophores and none of the isolates produced HCN traits .Similarly, large number of studies have been reported on the role of PGPR in promoting crop growth (Wang *et al.,* 2020) and PGPR can have multiple impacts on the phytohormone status including shoot hormone concentrations and modifying root-to-shoot signaling, under salt stress (Dodd *et al.,* 2010). Hence, it is inferred that the PGP bacterial isolates involved in the present study can be recommended for reclamation of salinized agricultural lands.

Conclusion

In the present study, total of 130 strains were isolated from the rhizospheric region of various crops and amongst which three strains showing better PGPR traits in the presence and absence of Sodium chloride were selected for germination of *Vigna radiata* and *Brassica juncea*. Three potential bacterial strains namely, D6, E11 and G14 strains were identified by 16SrRNA sequencing and tested for their salt tolerance up to 5% NaCl. In addition, Hemolysis assay was performed to check the pathogenic nature of the strains and found to be non-pathogenic in nature. However, further work is needed to evaluate the efficacy of these PGPR bacterial strains under pot experiments and field conditions.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

All the data sets generated and analyzed during this study has been included in the manuscript.

Ethics Approval Statement

Not applicable.

Authors' Contribution

The author K. Karthikeyan has conceptualized and designed the experiments, author M. R. Sharma has conducted the experiments and wrote the original draft. Authors G. Jayanthi and Kalpesh. D. Sorathia has reviewed and edited the manuscript. All the authors read and approved the final manuscript for publication. All the authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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