Potential Multi stress tolerant Plant Growth Promoting Rhizobacteria (PGPR) as a Biofertilizers enhance sustainable Agriculture and Environment during Abiotic stresses.

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INTRODUCTION:

Biofertilizers is an Economically, environmentally beneficial for lower production cost. Plant growth promoting rhizobacteria (PGPR) can be used as biofertilizers and biocontrol agents in agriculture, forestry, and environmental rehabilitation. PGPR as Biofertilizers facilitates the overall growth and yield of crops in an eco-friendly manner.

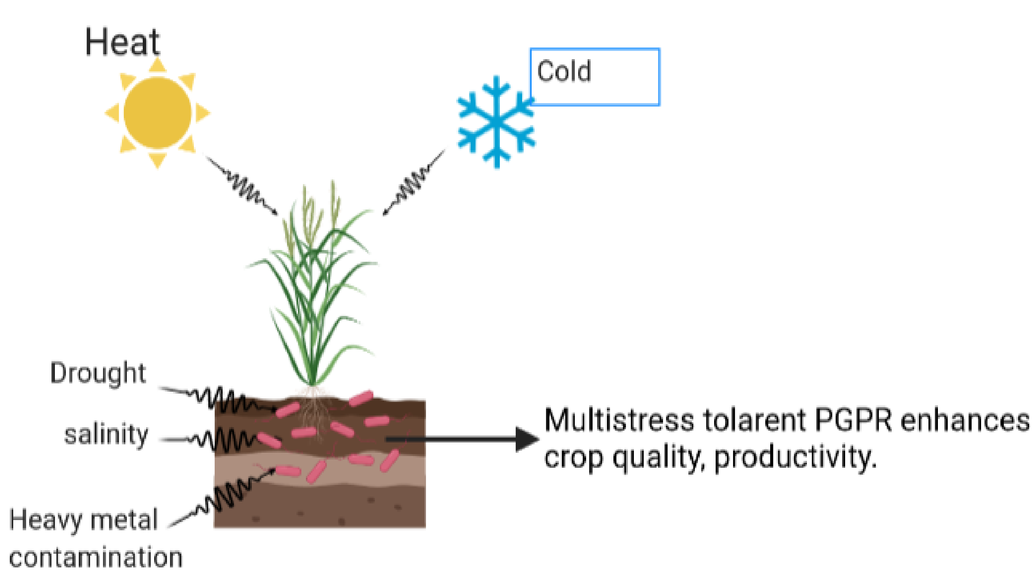

Fig: Potential PGPR enhance sustainable agriculture and environment

AIM & OBJECTIVES:

The potential multi stress tolerant PGPR as a biofertilizer is helps to replace hazardous chemical fertilizers, pesticides, and other agrochemicals and to reduce the harmful impact of various stresses on plant growth, agricultural yields, reduce chemical contaminant in soil and improve soil fertility.

METHODS:

Isolation and screening of Multi stress tolerant plant growth promoting rhizobacterial strains.

↓

Physical, Biochemical, molecular and metabolic

Characterization Multi stress tolerant of plant growth promoting rhizobacterial strains.

↓

Greenhouse Experimental Trials with incorporated Multi stress tolerant PGPR.

↓

Delineation of molecular mechanisms for Multi stress response in plants and potential PGPR strains.

RESULTS:

- The potential Multistress tolarent plant growth promoting rhizobacterial (PGPR) strains will be identified.
- The plants will be tested by incorporating PGPR for multi stress tolerant in a series of greenhouse experiments, resulting in the selection of a plant could survive in various stressful conditions.
- The combination of selected potential PGPR strain will be exposed to the Drought, salinity and chemicals agriculture field condition resulting in sustainable stress tolerant crop.
- Molecular MiRNA analysis result in a comprehensive survey of transcriptional regulation of stress tolerant PGPRs that interaction under stress (drought and salinity) and to identify stress- and tolerance-associated genes.

CONCLUSION:

The potential PGPR as a Biofertilizers will enhance sustainable Agriculture and environment during Abiotic stresses.

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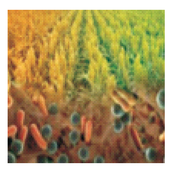


Moringa Oleifera: A Rich Source of Vitamins, Minerals, Polyphenols and Phytohormones

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Introduction:

Moringa oleifera is an evergreen tree native to the sub-Himalayan tracts of India. The whole tree has several nutrients in it and has several edible parts.

Aim:

To study the presence of several nutrients in *Moringa oleifera.*

Methods:

HPLC is performed and Spectrometer is used to obtain the presence of nutrients.

Result:

Nutrients which are present are as follows :-

Amino Acids, Methionine, Cystine, Polyphenols, Phytohormones, Vitamin A, B, C, E, Carotenoids. It also has antioxidant, anti-inflammatory, antimutagenic and anticancer properties.

It is rich in Cytokinin and Zeatin

Conclusion:

This plant has essential nutritional and medicinal properties.. Hence it is also known as **"THE TREE OF LIFE".**

Reference and Acknowledgement:

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IN VITRO STUDIES ON PLANT GROWTH PROMOTING BACTERIA FROM VARIOUS RHIZOSPHERIC SOILS IN PRESENCE OF ABIOTIC STRESS CONDITION

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Presented by: **Krishna Gupta L. & Pallavi Pandey H, Dr. Arti A.Raval (Mentor),** **No. (3.3)**

Abstract: Due to increase in both human population growth and environmental pressure it is necessary to raise agricultural productivity without enhancing environmental footprint. In particular, PGPB may improve plant growth either directly or indirectly by decreasing the inhibitory effects of various pathogenic agent. PGPB (plant growth promoting bacteria) is what at present is proving itself to be more promising better option. **Objectives:** Sample collection from rhizosphere and phyllosphere of *Carica papaya*. Bacterial isolation was done on various media. Screening of PGPB for various plant growth promoting mechanisms was carried out. Tolerance of the selected bacteria to abiotic stresses salt and heavy metals was also be observed. **Methods**: Direct Plant growth promoting characteristics like: Nitrogen fixation, phosphate Zinc and Potassium solubilization, Production of IAA and other hydrolytic Enzymatic Activities, Caseinase, Lipase, Cellulase, Amylase, Gelatinase, Pectinase Ammonia and HCN production were also studied. Growth of the selected bacteria in presence of various salt and heavy metals like Nickel chloride and Cadmium chloride are determined. **Results**: Various colonies were selected and then subjected to the analysis of PGP traits. Two out of Four bacteria showed promising results giving most of the characteristics positive. Then they were evaluated for tolerance to heavy metal and salt stresses. Out of which we found that RS a gram positive rod was able to grow in presence of (5mg/ml of NaCl concentration) and also tolerated (4 mg/ml $NiCl_2$ and 3mg/ml of $CdCl_2$concentration).**Conclusion**: The bacteria coded RS can be used to enhance plant growth in contaminated soils. and hence be applied as plant growth stimulants for better agricultural practices for a sustainable environment. Further evaluation of this strain for plant growth promotion will be carried out.

Keywords:1.Environmental footprint 2. rhizosphere 3. Phyllosphere 4. Abiotic stress

Introduction

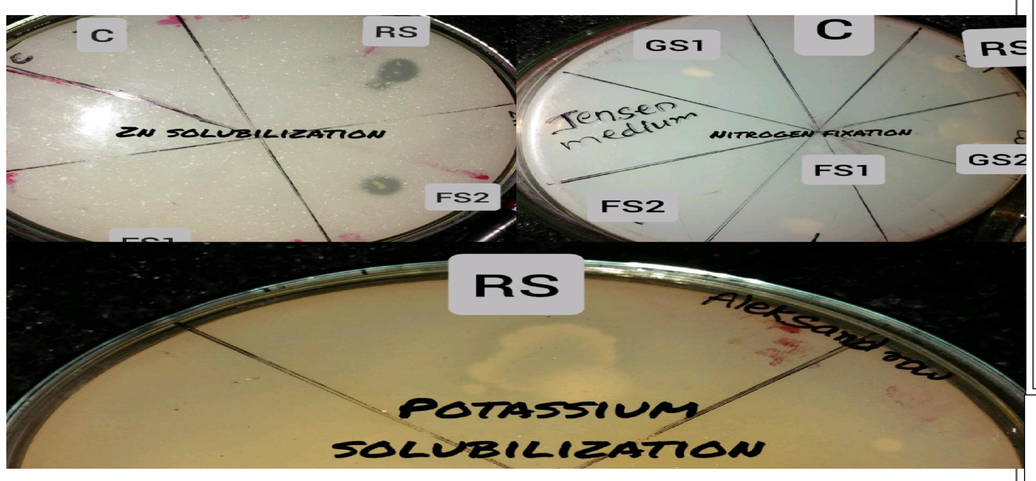
PGPB promote plant growth directly usually by either facilitating resource acquisition or modulating plant hormone levels, or indirectly by decrease the inhibitory effects of various pathogenic agents on plant growth and development.

PGPB have addressed a wide range of different mechanisms. By direct or indirect mechanisms PGPB can act on the enhancement of the performance of plant. Due to the direct action,PGPB provide the plants with some bacterial-synthesized compounds ,modulate plant hormone levels that stimulate the proliferation of plant cells and facilitate the uptake of nutrients by fixing atmospheric oxygen, solubilizing minerals such as phosphorus and producing siderophores able to sequester iron. While, indirect actions PGPB produce antagonistic substance or induce resistance against pathogens to prevent their harmful effects.PGPB can also be employed as biocontrol agents which able to kill other pathogens. The biocontrol activity of PGPB against soil borne pathogens is due to mechanisms of microbial antagonism.

Materials and methods

- **To collect the various soil samples.**
  - -Garden soil
  - -Phylosphere soil
  - -Rhizospheric soil
- **Isolation of bacterial strains.**
- **Screening of PGPB for multiple plant growth promoting activities.**
  - -Phosphate solubilization
  - -Potassium solubilization
  - -Ammonia productin
  - -HCN production
  - -Nitrogen fixation
- **Bacterial isolates for hydrolytic enzyme production.**
  - -Amylase production
  - -Caseinase[Protease] activity
  - -Lipase activity
  - -Gelatinase production
  - -Catalase test
- **Effect on abiotic stress on bacterial growt**
  - -Salinity stress
  - -Heavy metal tolerance

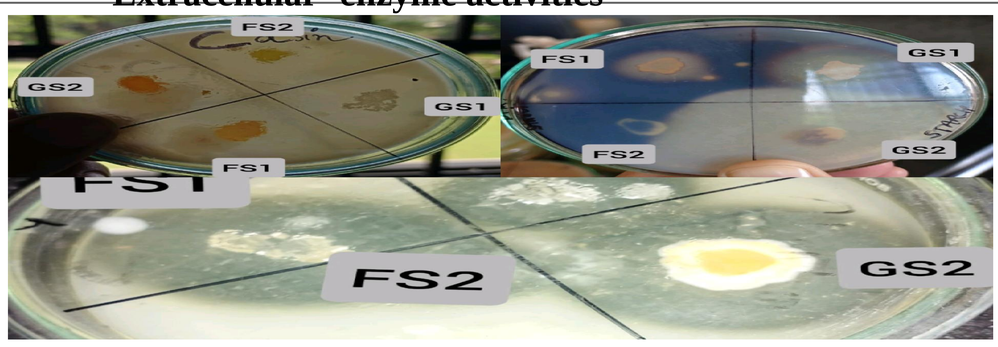
Results of PGPB characteristics



Isolate no.	Potassium solubilization	Phosphate solubilization	NH_3 production	HCN production	Zn solubilization	Nitrogen fixing bacteria
RS	++(0.8mm)	+++(0.6mm)	++	+	+++(0.4mm)	+
GS1	++(0.7mm)	++(0.4mm)	++	++	+++(1.8mm)	+
GS2	+++(1.2mm)	++(0.5mm)	+	+	+++(1.5mm)	+
FS1	-	++(0.3mm)	+	+	-	+
FS2	+++(0.9mm)	-	+	++	++(0.4mm)	-

Note:- [Excellent(+++), Good(++), Moderate(+) and weak(-)]

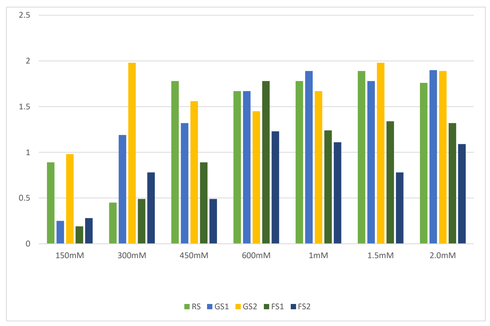
Extracellular enzyme activities



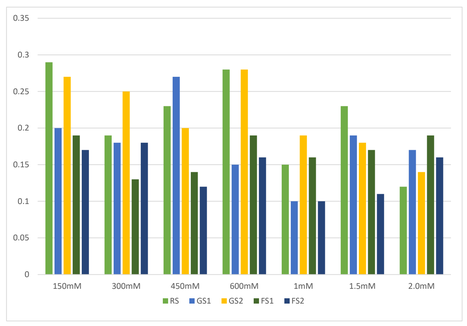
Isolate no.	Amylase	Caseinase	Lipase	Gelatinase	Catalase
RS	+++(2mm)	+(2.5mm)	++(2.5mm)	+	++
GS1	+++(2mm)	-	++(0.7mm)	+(3.5mm)	+++
GS2	-	+(3mm)	+++(1.3mm)	+(4mm)	+++
FS1	++(1.5mm)	+(2.3mm)	+	+(3.9mm)	+
FS2	+(0.7mm)	+(2.8mm)	+++(2mm)	-	+

Environmental effects of abiotic stress on bacterial growth

- **Effect of NaCl on bacterial growth**



Graph no.1 Effect of different NaCl concentration on growth of bacteria at 24 hours



Graph no.2 Effect of different NaCl concentration on growth of bacteria at 48 hours

TOLERANCE OF HEAVY METAL

- **Cadmium chloride**

Isolates no.	0.5 [mg/ml]	1.0 [mg/ml]	1.5 [mg/ml]	2.0 [mg/ml]	2.5 [mg/ml]	3.0 [mg/ml]	3.5 [mg/ml]
RS	+	++	+	++	++	-	++
GS1	++	+	-	+	++	+	-
GS2	+++	++	++	+++	+	-	++
FS1	+	-	+	+	-	+	++
FS2	+	-	+	-	+	+	-

Effect of $CdCl_2$ in [mg/ml] on the growth of bacteria

TOLERANCE OF HEAVY METAL

- **Nickel chloride**

Isolates no.	0.5 [mg/ml]	1.0 [mg/ml]	1.5 [mg/ml]	2.0 [mg/ml]	2.5 [mg/ml]	3.0 [mg/ml]	3.5 [mg/ml]
RS	+++	-	++	+	-	++	+
GS1	+	++	-	+	-	+	-
GS2	++	+	++	+	-	+	+
FS1	+	++	-	+	+	++	-
FS2	-	+	+	-	++	+	-

Effect of $NiCl_2$ in [mg/ml] on the growth of bacteria

SUMMARY OF ALL OBTAINED RESULTS

Characteristics of PGPB	Isolate RS
Phosphate solubilization	++
Potassium solubilization	+++
Ammonia production	+
HCN production	+
Zn solubilisation	+++
Nitrogen fixation	++
Amylase production	+++
Gelatinase production	++
Caseinase production	++
Lipase activity	+++
Catalase activity	+++
Salt tolerance	+++
Heavy metal tolerance	++

As per the result tables isolate RS showed positive results exhibiting many of the characteristics of PGB which was obtained from the rhizosphere of Rice and so it can be used as bioinoculant for reducing the effect of hazardous chemical fertilizers.

CONCLUSION

Present study illustrates the significance of bacteria under in vitro conditions for multiple PGPB traits. It can be concluded that from the above discussion PGPB enhance the plant growth due to the production of Phosphate solubilisation, Potassium solubilisation, Zn solubilisation, Ammonia production, HCN production and many other Extracellular enzyme activities like Amylase, Caseinase, Lipase, and Gelatinase production. Isolate RS showed all characteristics of PGPB positive which was obtained from Rice field. Such investigation is necessary as it advocates that the use of PGPB as bio-inoculant is an efficient approach to replace chemical fertilizers and these PGPB isolates may also be used as bio fertilizers to enhance the growth and productivity for commercially grown plants under local agro-climatic conditions. Metal resistant bacteria can also be utilized as Bio-controlling agent. Obtained isolates had potential to tolerate Heavy metal like Cadmium chloride and Nickel chloride. All obtained isolates were showed high salt resistance and it is adversely affect modern agriculture. Hence, understanding and manipulating this feature may be of great agro-ecological interest for future crop improvements. Based on the results it has been suggested that these strains of PGP potential and biocontrol ability which can be used as biofertilizers as well as biocontrol agent

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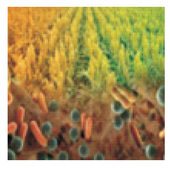


Registration number : 3.4

Antibiotic resistance among rhizospheric bacteria isolated from *Cicer arietinum* field

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Introduction

The usage of antibiotics in agriculture has been attributed to the spread of antibiotic resistant soil bacteria population including rhizospheric bacteria. The present study was undertaken to screen 170 rhizospheric bacteria isolated from chickpea field for their resistance to commonly used antibiotics viz. amphicilin , streptomycin, , Vancomycin , Kanamycin, chloramphenicol, Tetracycline, Ciprofloxacin, Tobramycine and methicilin . The present investigation was conducted to study prevalence of antibiotic resistant rhizobacteria from chickpea rhizosphere of Nagpur area and to compare incidence of antibiotic resistance among ACC Deaminase producing rhizobacteria from chickpea rhizosphere.

Method

▪The bacterial strains were tested for their resistance to different antibiotics (viz., Ampicillin, Streptomycin, Vancomycin , Kanamycin, chloramphenicol, Tetracycline, Ciprofloxacin, Tobramycine, methicilin) by agar diffusion method. The bacterial strains were inoculated in freshly prepared agar plates amended with specific antibiotic, incubated the plates at 37^0C for 24 hours and determined the antibiotic resistance by observing growth of the organisms.

▪The enzyme ACC deaminase cleaves ACC, the immediate precursor of ethylene in plants. The bacterial culture was grown in test tube containing 100 ml of liquid medium: KH2PO4 (2g), K2HPO4 (0.5g), MgSO4 (0.2g), Glucose (0.2g). The medium was supplemented with 0.3g ACC or 0.19g (NH4)2SO4 as a N source and incubated at 37°C for 24 - 72hrs.The appearance of bacterial growth indicated the ACC deaminase activity of the bacteria.

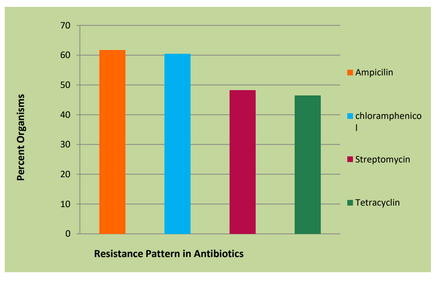
▪ Lactose fermenting activity is shown to isolate the gram negative bacteria using MacConkey agar. The selective action of this medium differentiate non-fastidious gram-negative rods and the lactose fermenting from lactose non-fermenting gram-negative bacteria.

Results

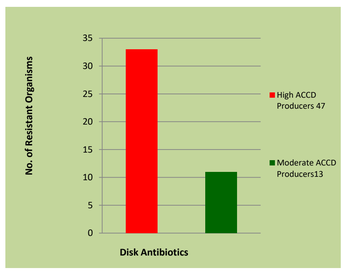
▪The results comprising 170 rhizospheric culture isolated from agricultural soil near Nagpur ,giving the results for ACC Deaminase trait , on the basis of the percentage of ACCD activity we test 9 different antibiotics for there antibiotic resistance. To check their lactose fermenting activity we inoculate culture on MacConkey agar.

▪PGP shows the ACC Deaminase trait : The total one hundred and seventy isolates, collected from chickpea rhizosphere where screen for PGP trait , ACCD activity, the bacterial isolates give high ACCD activity(35.2%), moderate ACCD activity(22.35%), low growth in ACCD(17.6%) and the negative results(24.7%) given for the ACCD trait.

▪Antibiotic resistance in chickpea rhizospheric bacteria: To check the antibiotic resistance in bacterial isolates , the isolates were grouped into 4 parts on the basis of their ACCD activity later on 9 different antibiotics were tested for their resistance pattern.
The fig. given below (a), (b) gives the information about the percentage of antibiotic resistance in isolates.



(a)



(b)

▪The resistance pattern of rhizospheric organisms, shows resistance for only one antibiotic 1R (18.23%), resistance for two antibiotic 2R (20.5%) while for multiple antibiotic resistance (MAR) (42.35%) . Results shows that most organisms were resistance for more than one antibiotic.

▪Antibiotics are classify in different classes according to their mode of action against microorganisms and chemical composition. The percentage of beta- lactam antibiotics is 68.3-73.3% , we can say that these antibiotics have the high percentage of resistance. The class ampheicol have 53.3% resistance , aminoglycoside shows 48.3%,tetracycline have 50% of resistance, while quinolone and glycopeptides have 5%and 36.6% of resistance.

▪The percentage of lactose fermenting activity in bacteria showcase the ACC Deaminase activity. From total of one hundred forty two organisms 75.9% shows lactose fermentation in highly ACCD producers, 86% in moderate ACCD producers, 93% in low ACCD producers and 93.9% in non- ACCD producers.

Conclusion

▪In the present study we observed rich bacterial diversity in soil in terms of their functional (PGP) trait of production of ACC Deaminase (ACCD) 39.29% of organisms showed high ACCD activity, 22.35% showed moderate activity, while 17.6% showed low ACCD activity. ACCD activity was not detected in 24.7% organisms. ACCD producing bacteria facilitate the growth of plants by decreasing plant ethylene level and thus increase the productivity of the crops.

▪The antibiotic resistance of the isolated strains showed a high level of resistance against antibiotics in the order : Methicillin (75.8), Ampicilin (61.7%), Chloramphenicol (60.5), Streptomycin (48.23), Tetracyclin (46.47), Kanamycin (17), Tobramycin (17) and ciprofloxacin(6.38).

Functional validation of OsNAM gene in Arabidopsis shows its crucial role in plant-PGPR interaction by providing tolerance to abiotic stress and phytohormone crosstalk.

Registration no. 3.5

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Background

- Salinity is one of the major constraints that affect crop development and yield.
- Plants respond and adapt to salt stress via complex mechanisms that involve morpho-physiological, biochemical, and molecular changes.
- The expression of numerous genes is known to alter during various abiotic stresses and impart stress tolerance.
- Recently, some known rhizospheric microbes have also been used to mitigate the effects of abiotic stresses; however, the molecular basis of such interactions remains elusive.

Objective

To elucidate the plant growth-promoting rhizobacteria (PGPR; *Bacillus amyloliquefaciens*-SN13) -induced crosstalk among salinity and phytohormones in *OsNAM*-overexpressed *Arabidopsis* plants.

Materials and Methods

- Amplification and cloning of *OsNAM and Agrobacterium*-mediated transformation of *A. thaliana*
- Bacterial inoculation and stress treatment
- Morpho-physiological and biochemical analyses of *OsNAM* overexpression lines to salt stress.
- Expression analysis of stress-responsive genes in *OsNAM*-overexpressing lines
- Chromatographic analysis

Results

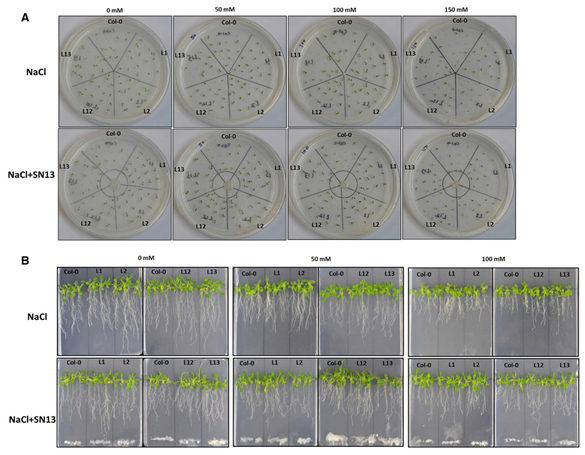

Table 1 Comparison of phenotypic parameters of WT (Col-0) and *OsNAM* transgenic lines exposed to 100 mM of NaCl stress

	Biomass (mg)		Rosette diameter (major) (cm)		Rosette diameter (minor) (cm)		Leaf length (cm)		Leaf width (cm)		Petiole length (cm)	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Col-0 (WT)	569.1 ± 11.89^{bc}	145.85 ± 3.86^{a}	4.38 ± 0.28^{a}	5.10 ± 0.08^{a}	3.48 ± 0.30^{a}	4.50 ± 0.24^{a}	1.53 ± 0.23^{a}	2.10 ± 0.08^{a}	1.00 ± 0.08^{a}	1.08 ± 0.05^{a}	0.73 ± 0.12^{ab}	0.60 ± 0.00^{a}
L1	980.4 ± 114.8^{d}	296.3 ± 38.79^{ab}	6.20 ± 0.13^{c}	6.18 ± 0.19^{b}	5.58 ± 0.16^{c}	5.08 ± 0.32^{a}	2.13 ± 0.09^{b}	2.48 ± 0.12^{b}	1.50 ± 0.08^{c}	1.40 ± 0.17^{b}	0.53 ± 0.05^{a}	0.88 ± 0.09^{b}
L2	783.55 ± 206.2^{cd}	425.3 ± 171.3^{ab}	6.20 ± 0.52^{c}	5.90 ± 0.47^{b}	5.23 ± 0.71^{c}	4.85 ± 0.36^{a}	2.33 ± 0.18^{b}	2.33 ± 0.23^{ab}	1.33 ± 0.09^{bc}	1.43 ± 0.05^{b}	0.85 ± 0.16^{b}	0.53 ± 0.05^{b}
L12	838.05 ± 87.0^{cd}	328.65 ± 8.25^{ab}	5.38 ± 0.55^{b}	5.80 ± 0.42^{b}	4.53 ± 0.44^{b}	4.70 ± 0.62^{a}	2.05 ± 0.23^{b}	2.30 ± 0.13^{ab}	1.38 ± 0.22^{bc}	1.43 ± 0.06^{b}	0.73 ± 0.19^{ab}	0.65 ± 0.16^{b}
L13	776.2 ± 46.07^{cd}	260.9 ± 0.46^{ab}	5.15 ± 0.18^{b}	5.60 ± 0.63^{ab}	4.50 ± 0.27^{b}	4.40 ± 0.55^{a}	2.20 ± 0.08^{b}	2.18 ± 0.12^{a}	1.23 ± 0.09^{b}	1.33 ± 0.09^{b}	0.63 ± 0.05^{a}	0.58 ± 0.05^{b}

Data represent the means ± SD of six independent experiments. Different alphabets indicate significant differences according to Duncan's test ($P \leq 0.05$)

Fig. 1 a, b Assessment of growth parameters of WT (Col-0) and overexpressed lines (L1, L2, L12 and L13): germination assay (a) and root length analysis (b) under various concentrations of salt stress

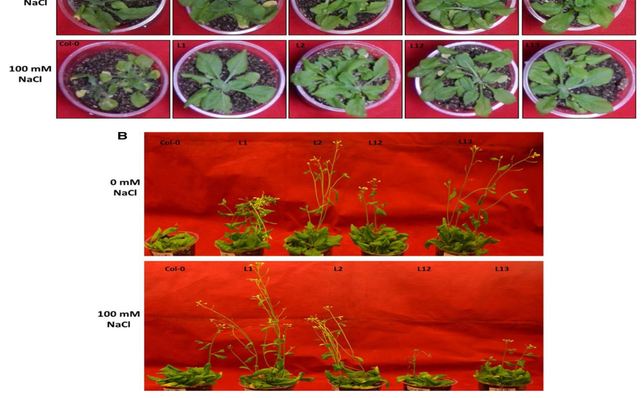

Fig. 2 a, b Illustration of phenotypic changes: rosette (a), and floral transition (b) in WT (Col-0) and transgenic *A. thaliana* plants overexpressing *OsNAM* under 100 mM of NaCl stress

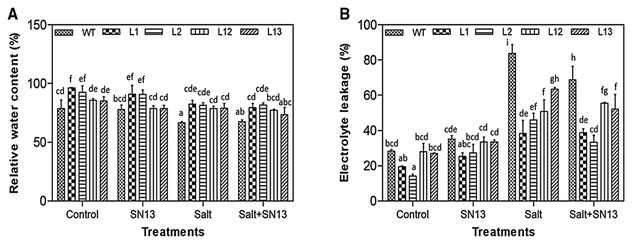

Fig. 3 a–d Determination of physiological and biochemical parameters: RWC (a), EL (b), Lipid peroxidation (c), and Proline (d) in WT (Col-0) and transgenic plants exposed to 100-mM NaCl stress in the presence and absence of SN13.

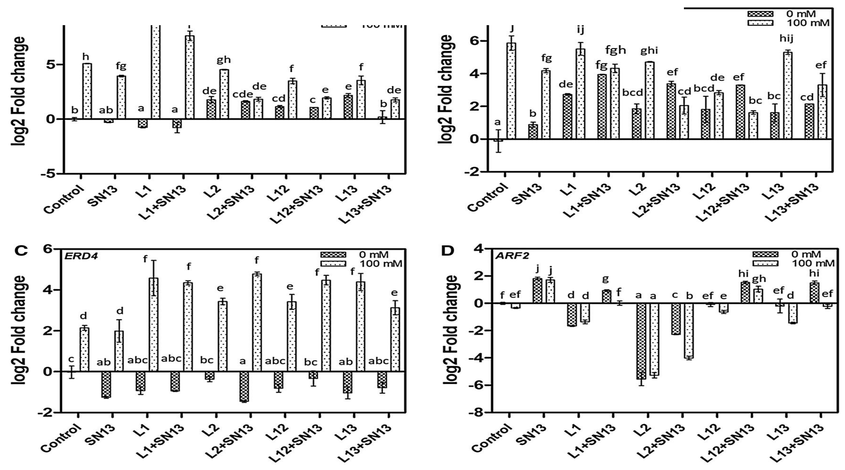

Fig. 4 Expression analysis of *AP2/ERF* (a), *GST* (b), *ERD4* (c), and *ARF2* (d) genes in WT and transgenic plants exposed to 100 mM of NaCl in the presence or absence of SN13.

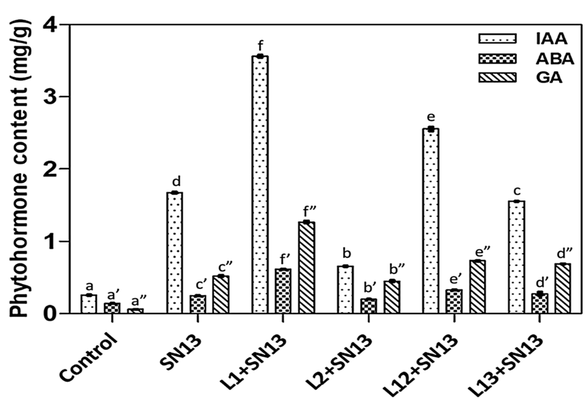

Fig. 5 Analysis of phytohormones content in WT (Col-0) and transgenic lines in the presence of SN13

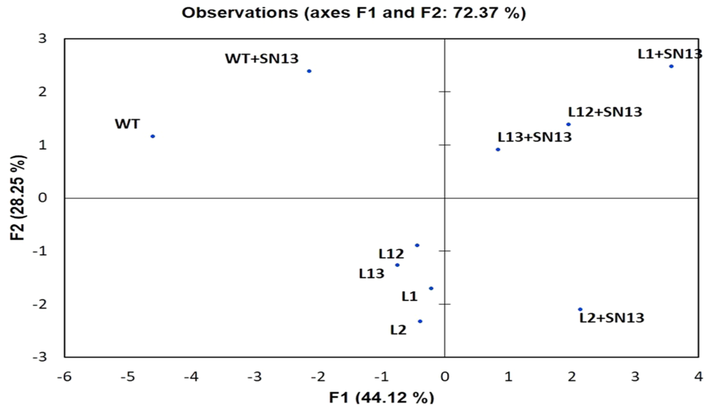

Fig. 6 PCA between all measured parameters in WT (Col-0) and transgenic lines in the presence and absence of SN13

Fig. 7 A schematic representation summarizing the role of SN13-induced *OsNAM* in salt stress tolerance in transgenic *Arabidopsis*

Conclusions

- The overexpression of SN13-responsive *OsNAM* gene in *Arabidopsis* helps it to better tolerate salt stress by modulating various morpho-physiological, biochemical, and molecular parameters as well as phytohormone content.
- The present study also helps to establish the role of this gene in rice–SN13 interaction in stress alleviation thus paving way for its inclusion in crop improvement programs directed towards conferring abiotic stress tolerance.

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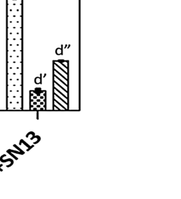

Mitigation of nutrient stress through modulating carbohydrate metabolism by PGPR *Bacillus amyloliquefaciens* in rice

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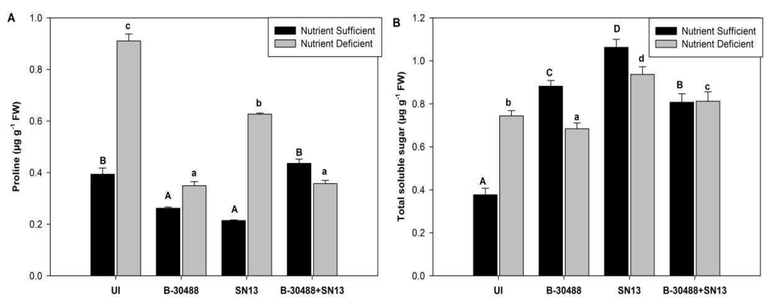
Introduction

- Deficiency of mineral nutrients in plants causes several abnormalities.
- Plant growth promoting rhizobacteria were used to improve rice rhizosphere soil health under nutrient starvation.
- Present study illustrates the role of two PGPR strains viz. *Baccilus amyloliquefaciens* SN13, *Paenibacillus lentimorbus* B-30488 and their consortium in alleviation of nutrient stress in rice (*Oryza sativa* L. var. IR-36).

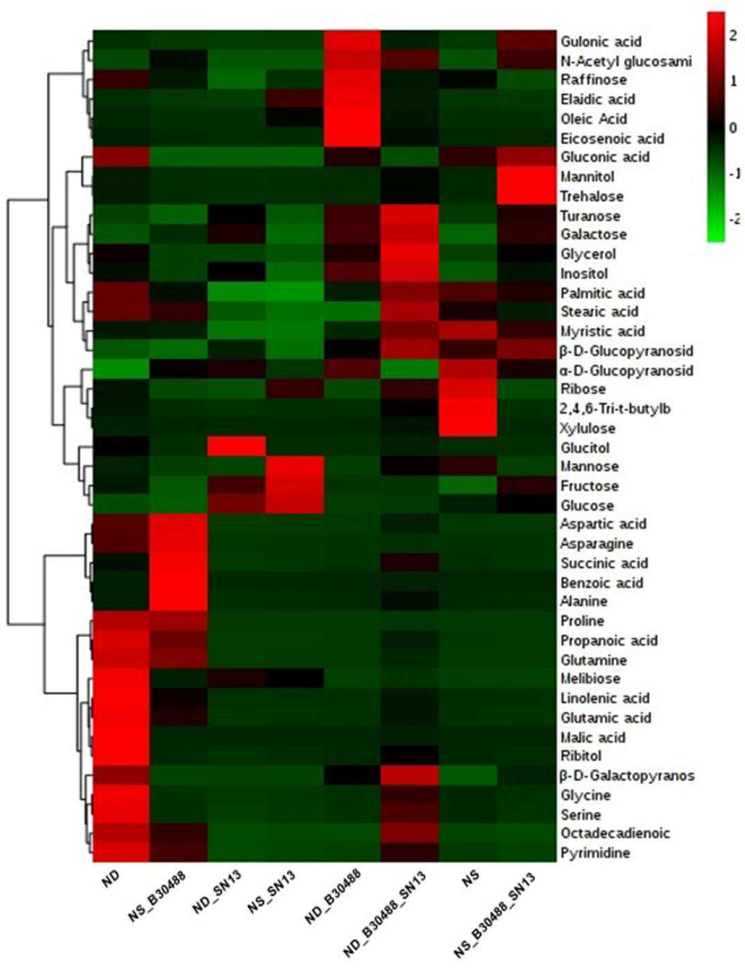
Materials and Methods

- Plants were grown in vermiculite (inert substrate) and bacterial treatment was given prior to stress treatment.
- Plant phenotyping, photosynthetic pigment analysis and stress related parameters were analysed.
- The relative metabolite abundances were determined using GC-MS and further validated using qRT-PCR to explore the PGPR induced metabolic pathways under low nutrient stress condition in rice.

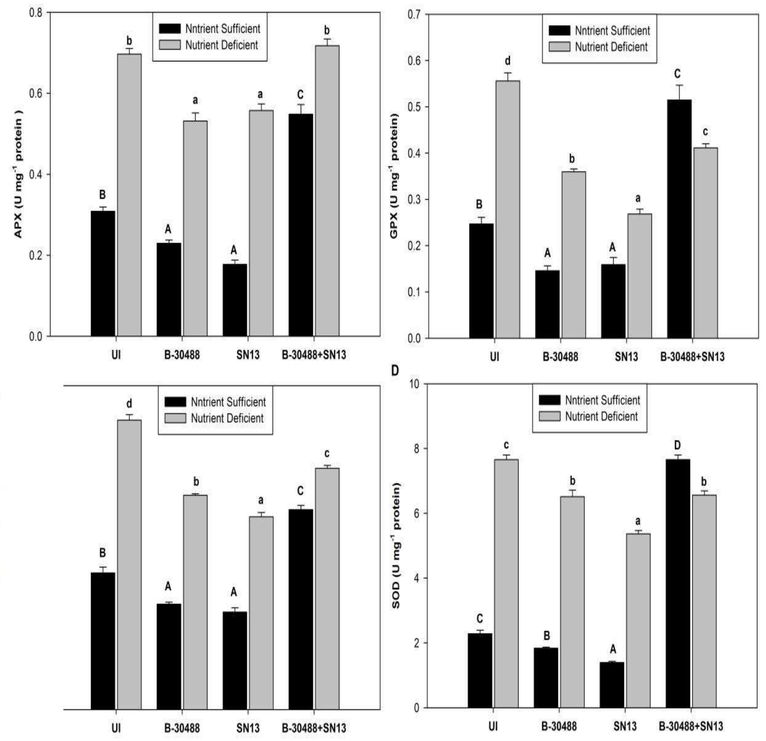
Results



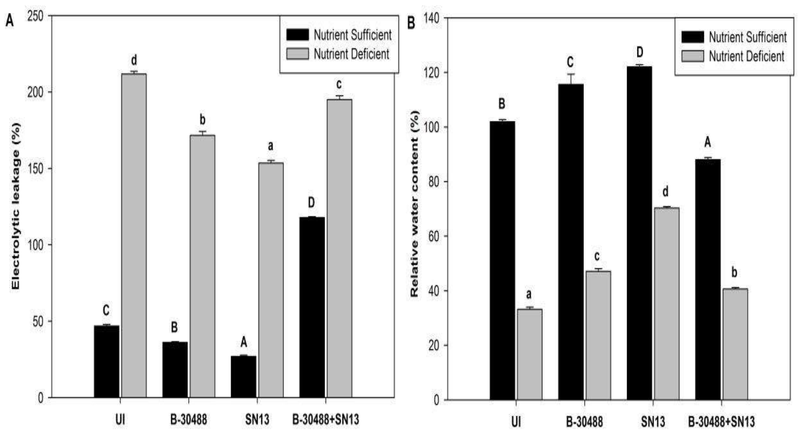
Proline and total soluble sugar in rice seedlings



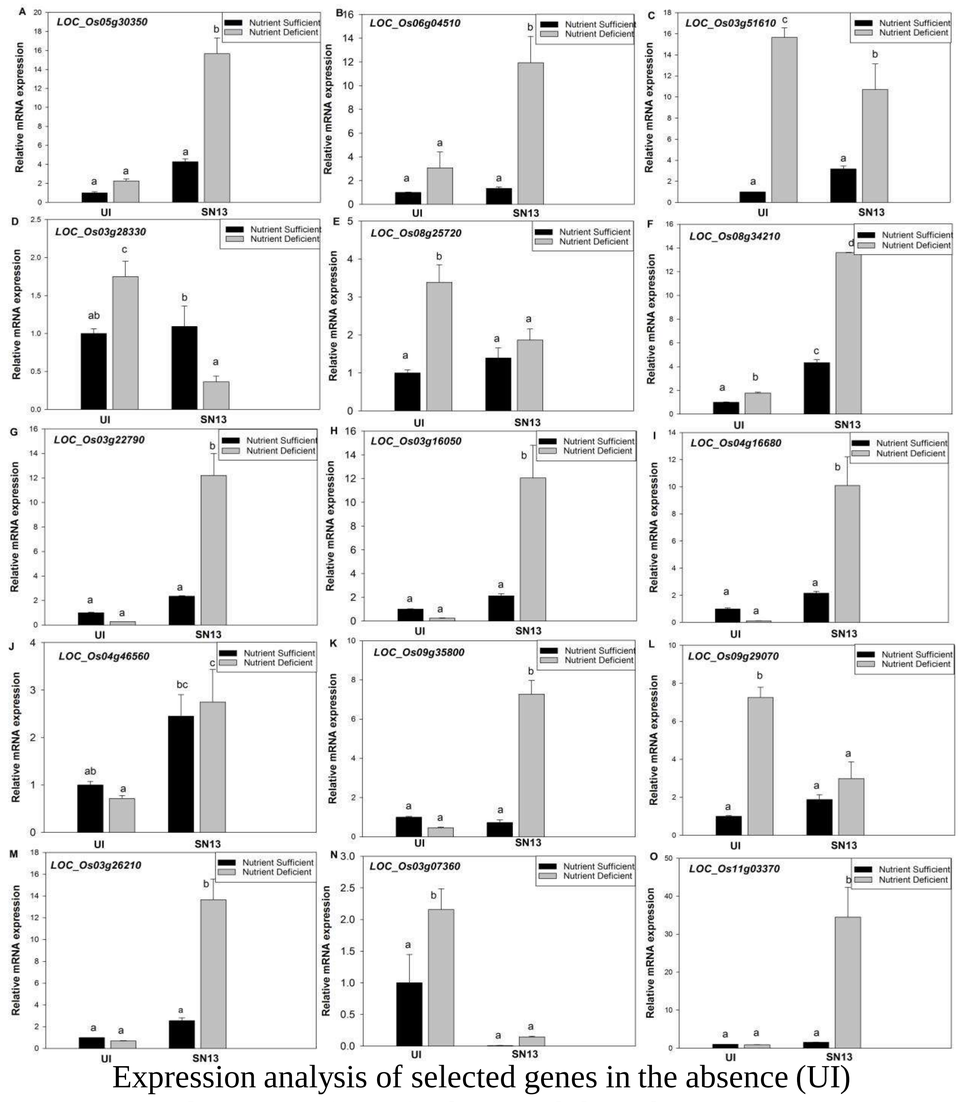
Relative concentrations of metabolites in rice seedlings determined using GC-MS



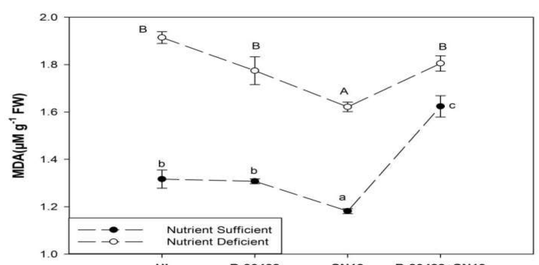
Antioxidant enzyme status in rice seedlings



Electrolytic leakage (EL) and relative water content (RWC) of rice seedlings



Expression analysis of selected genes in the absence (UI) and presence (SN13) of *B. amyloliquefaciens* SN13



MDA content of rice seedlings

Conclusions

- Plant-PGPR interactions are host and environment specific, which eventually influences molecular and metabolic paradigms in host plant
- GC-MS and gene expression analysis affirmed the fact that PGPR reprograms plant metabolism for deficiency-induced nutrient stress amelioration.
- PGPR *Bacillus amyloliquefaciens* SN13 modulates carbohydrate metabolism in rice seedlings under suboptimum nutrient level.

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Holistic approach of plant growth promoting rhizobacteria (*Ochrobactrum* sp.) on Maize (*Zea mays*) tolerance towards water stress

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Background

- Water-stress is one of the major abiotic stresses globally and secondary impact of climate change to the plants in particular, of agro-ecosystems.
- PGPR having multiple plant growth promoting attributes proved to be one of the alternatives in mitigating the drought stress in an eco-friendly manner.
- A multidisciplinary approach, involving morphological, physiological, biochemical, metabolical, molecular, and ecological help to understand the plant response to drought stress under NBRISH6 treatment in a holistic manner.

Objectives

- To investigate the impact of NRISH6 (*Ochrobactrum* sp.) on the response to drought stress (WS) in Maize.
- To explore the phytobeneficial impact of NBRISH6 using holistic approach.

Materials and Methods

- Effect of NBRISH6 inoculation on plant vegetative parameters, nutrient content, biochemical, and antioxidant enzyme assays.
- Impact of NBRISH6 inoculation on gas exchange parameters, chlorophyll content, phytohormones, stress responsive genes, and histology of maize plant.
- Impact of NBRISH6 on metabolite changes in plants and functional diversity and soil enzymes in rhizosphere under drought stress.

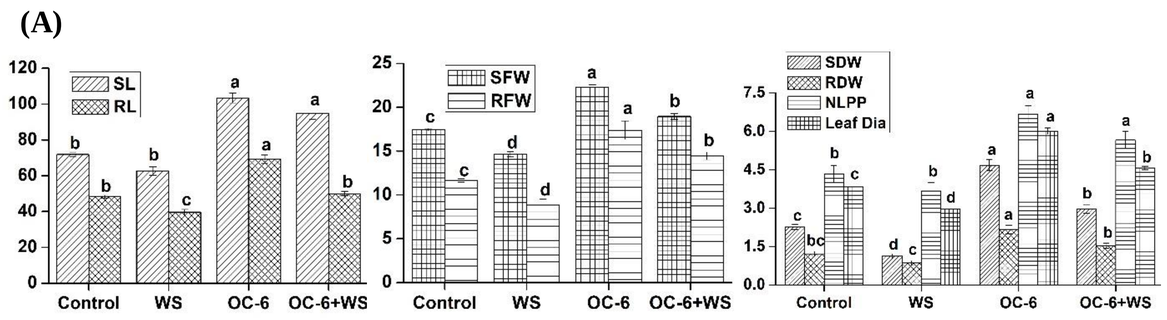
Results

Nutrient content in Maize shoot

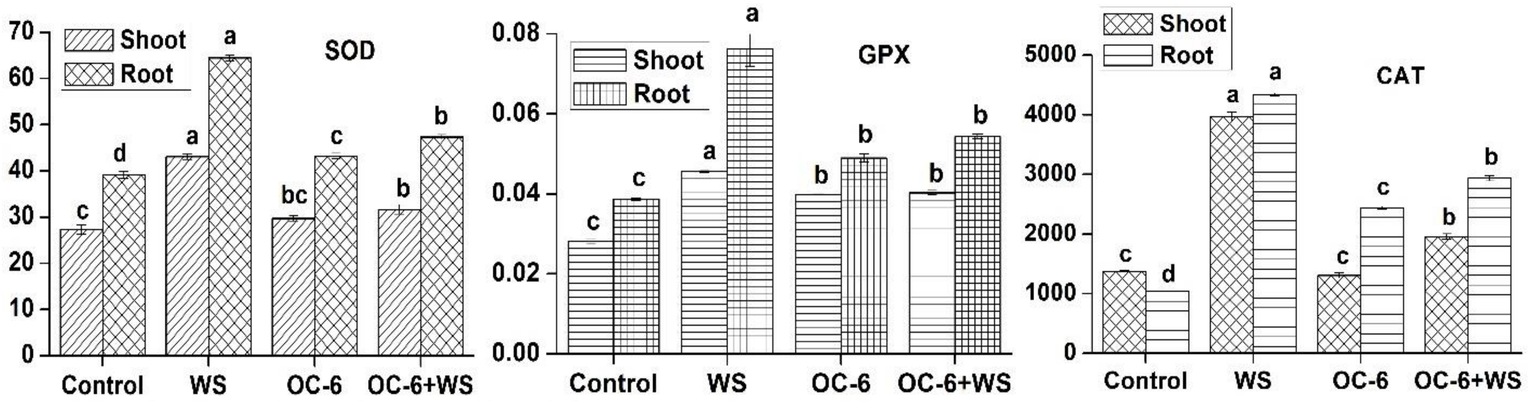
Shoot nutrient	Control	WS	OC-6	OC-6+WS
Potassium (K; µg gm^{-1})	3540.00±30.55^{c}	2851.7±4.41^{d}	4551.7±15.89^{b}	5266.7±7.26^{a}
Nitrogen (N; %)	3.79±0.10^{c}	0.7±0.011^{d}	5.7±0.09^{a}	4.6±0.166^{b}
Phosphorus (P; µg gm^{-1})	437.83±5.94^{c}	310.2±3.44^{d}	645.3±7.55^{a}	560.0±4.24^{b}
Sodium (Na; µg gm^{-1})	3095.00±20.81^{c}	2960.0±7.63^{d}	3455.0±7.63^{b}	4008.3±6.01^{a}
Calcium (Ca; µg gm^{-1})	4960.00±7.63^{c}	3315.0±10.40^{d}	6771.7±11.66^{a}	5783.3±10.14^{b}
Manganese (Mn; ppm)	43.73±0.30^{c}	32.3±0.12^{d}	127.6±0.23^{a}	86.5±0.38^{b}
Iron (Fe; ppm)	89.24±1.82^{c}	54.0±1.30^{d}	205.1±1.21^{a}	121.0±1.15^{b}
Zinc (Zn; ppm)	17.27±0.54^{c}	11.1±0.35^{d}	54.5±1.59^{a}	30.7±0.25^{b}

Measurement of gas exchange parameters and chlorophyll content in leaves

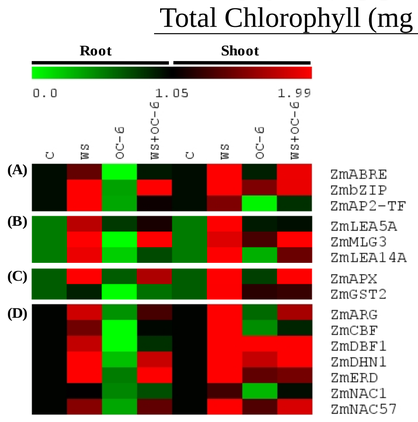
Parameters	Control	WS	OC-6	OC-6+WS
Stomatal Conductance (g_s; mmol m^{-2} s^{-1})	0.03±0.0004^{b}	0.01±0.003^{c}	0.04±0.002^{a}	0.01±0.002^{c}
Intercellular CO_2 concentration (Ci; µmol CO_2mol air^{-1})	454.33±2.91^{b}	383.0±2.51^{c}	673.0±3.15^{a}	233.3±2.02^{d}
Transpiration rate (E; mmol m^{-2} s^{-1})	2.74±0.002^{b}	0.1±0.01^{d}	3.3±0.06^{a}	0.4±0.001^{c}
Vapor pressure deficit (VpDL; kPa)	2.64±0.04^{d}	3.1±0.05^{b}	2.9±0.02^{c}	3.2±0.01^{a}
Intrinsic water use efficiency (iWUE; µmol CO_2 /mmolH_2O)	8.77±0.1^{c}	12.6±0.9^{b}	9.6±0.1^{c}	21.6±0.4^{a}
Photosynthetic rate (A_N; µmol m^{-2} s^{-1})	24.07±0.20^{b}	1.5±001^{d}	32.0±0.30^{a}	9.0±0.10^{c}
Respiration (µmol m^{-2} s^{-1})	-1.74±0.001^{ab}	-3.4±0.60^{b}	-0.5±1.00^{a}	-1.7±0.40^{ab}
Chlorophyll a (mg g-1 FW)	1.08±0.0145c	0.77±0.0029d	1.85±0.0049a	1.25±0.034b
Chlorophyll b (mg g-1 FW)	1.84±0.0042b	1.17±0.0088d	3.23±0.0063a	1.31±0.0039c
Total Chlorophyll (mg g-1 FW)	8.47±0.0221b	5.45±0.0362d	14.80±0.0295a	6.50±0.051c



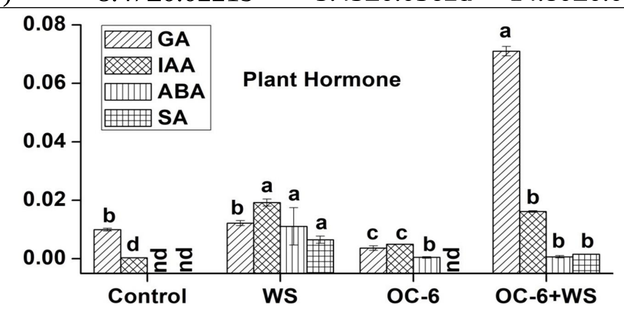
Impact of NBRISH6 inoculation on (A) plant growth parameters (B) biochemical parameters



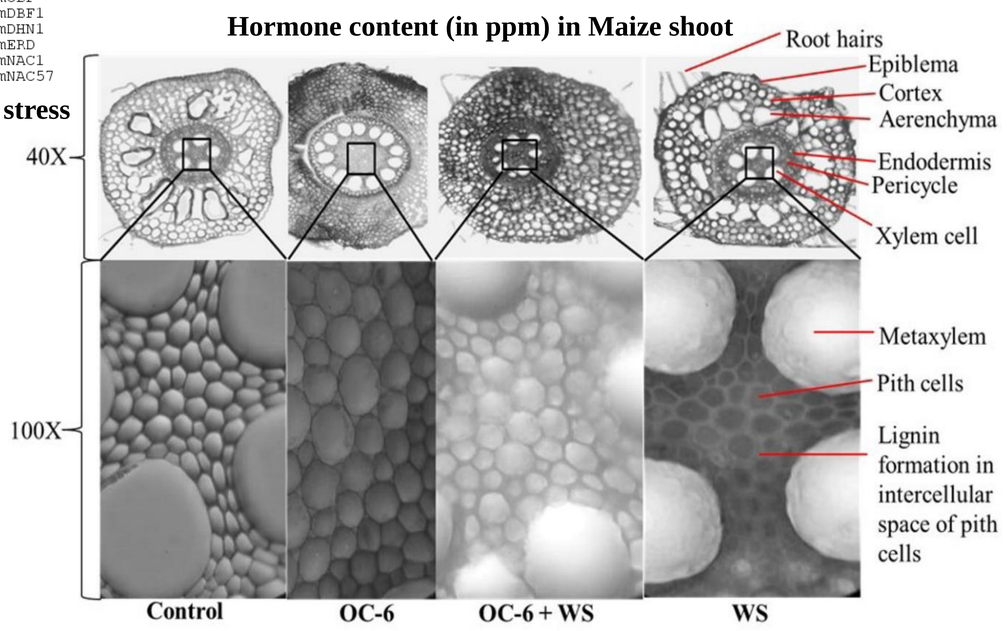
Impact of NBRISH6 inoculation on plant defense enzyme assay



Relative expression of drought stress responsive genes



Hormone content (in ppm) in Maize shoot



Histology of cross-section of Maize roots under different conditions

Conclusions

- NBRISH6 promotes plant growth under drought stress by various mechanisms including improving vegetative growth, nutrient uptake, photosynthetic parameters, root anatomical features, lowering oxidative stress, phytohormone level, and expression of genes of defense enzymes
- NBRISH6 impacts the ABA life cycle in plant under WS by influencing several components of ABA biosynthesis/degradation pathway

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Exploring the potential of microbes in drought stress alleviation in wheat

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Registration No. 3.10

Introduction: Drought stress is considered as one of the major agricultural problem. Generation of free radicals and reactive oxygen species (ROS) such as super oxide radicals, hydrogen peroxide, singlet oxygen and hydroxyl radicals in plant cells are major consequences of drought.

Objective: Herein, present study was designed to elucidate the role of plant growth promoting microbes for amelioration of water stress in wheat.

Methods: A one month pot experiment was conducted using randomised complete block design with 3 replications for exploring the effect of inoculation of BioNPK (microbial product) and *Archaea* on osmoprotectant and MDA (malondialdehyde) in wheat under water stress (30% F.C.). Total soluble sugar (Dubois et al., 1951), protein (Bradford, 1976), proline (Bates et al. 1973), Glycine betaine (Grieve and Grattan, 1983) and Malondialdehyde (MDA) content (Heath and Packer, 1968) in wheat root and leaves were determined.

Results

Table 1. Response of microbial inoculation in relation to osmoprotectant and MDA content in wheat leaves and roots under drought stress.

Treatments	Total soluble sugar (mg/g F.W.)	Protein (mg/gF.W.)	Proline (mg/gF.W.)	Glycine betaine (mg/gF.W.)	MDA (mg/gF.W.)
Leaves					
30% FC- Uninoculated stressed control	21.23±0.90^{b}	7.17±0.90^{c}	2.11±0.22^{a}	33.00±3.00^{a}	62.00±6.24^{a}
50% FC- Uninoculated un-stressed control	11.57±0.81^{c}	3.67±0.50^{d}	0.79±0.10^{c}	8.60±1.01^{c}	16.28±2.20^{d}
30% FC + Bio-NPK	23.33±3.06^{b}	10.75±1.43^{b}	1.42±0.28^{b}	24.00±2.00^{b}	45.10±5.15^{b}
30% FC + Archaea	32.00±2.65^{a}	17.00±1.00^{a}	1.13±0.25^{bc}	21.33±3.06^{b}	30.98±2.64^{c}
Roots					
30% FC- Uninoculated stressed control	11.58±1.29^{bc}	5.90±1.00^{b}	0.84±0.07^{a}	8.47±1.15^{a}	11.90±2.13^{a}
50% FC- Uninoculated un-stressed control	8.37±0.74^{c}	2.73±0.42^{c}	0.28±0.03^{c}	2.93±0.50^{c}	3.59±0.16^{b}
30% FC + Bio-NPK	12.00±2.00^{ab}	5.20±1.00^{b}	0.61±0.04^{b}	6.48±0.68^{ab}	10.45±0.92^{a}
30% FC + Archaea	15.13±1.03^{a}	10.20±1.00^{a}	0.48±0.11^{b}	4.93±0.61^{bc}	6.11±0.69^{b}

Conclusion: Microbial inoculation created multifarious modulation in osmoprotective substance in wheat plant under water stress (30% F.C.).

References

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Paenibacillus lentimorbus reduces nutrient deficiency in Cicer arietinum L.

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Reg No. 3.11

Background

- **Utilization of PGPR as an alternate and efficient technology in nutrient limited conditions for sustainable chickpea production.**
- **Alteration in gene expression and metabolic profile under nutrient stress condition in chickpea.**

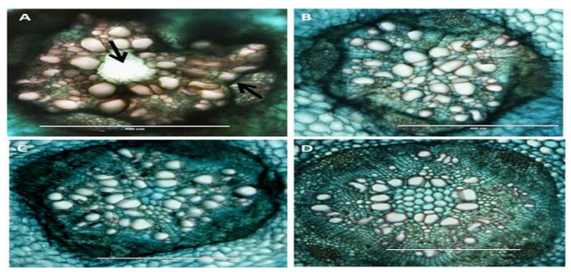
Objective

- **To evaluate nutrient deficiency induced stress amelioration ability of B-30488 on chickpea.**

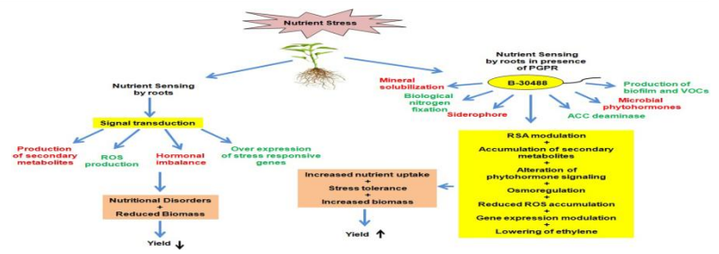
Material and Method

- **To determine the optimum nutrient concentration for chickpea growth, the screening experiment was performed under hydroponic conditions.**
- **An extended plant test was conducted under green house with four different treatments in chickpea plants.**
- **N+ for nutrient sufficient, N+ + B for nutrient sufficient inoculated with B-30488, N− for nutrient deficiency, and N− + B for nutrient deficiency inoculated with B-30488.**

Result



Effect of different treatments on chickpea root anatomy. (A) limited nutrients (N), (B) limited nutrients in presence of B-30488 (N−+B), sufficient nutrients (N)+ and sufficient nutrients along with B-30488 inoculation (N++B). Scale bar: 400 μm.



A hypothetical model created based on the differential response of the enzyme assays, physiological, and molecular analysis under nutrient deficient condition in chickpea in presence and absence of PGPR and other well-known concepts.

Conclusion

- **Chickpea plants inoculated with *P. lentimorbus* B-30488 displayed greater flexibility and tolerance to nutrient deficiency due to PGPR induced alteration in gene expression and metabolic pathways**

Extensive alteration in gene expression of Bacillus amyloliquifaciens-rice under salt stress

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Reg No. 3.12

Background

- **Salinity is one of the major stress factor that pose a serious threat to sustainable agricultural production and global food security by causing yield loss of cereals crop.**
- **The Bacillus amyloliquifaciens –SN13 and model crop rice (Oryza sativa) were chosen to understand the complex regulatory networks that govern plant-PGPR interaction under salt stress.**

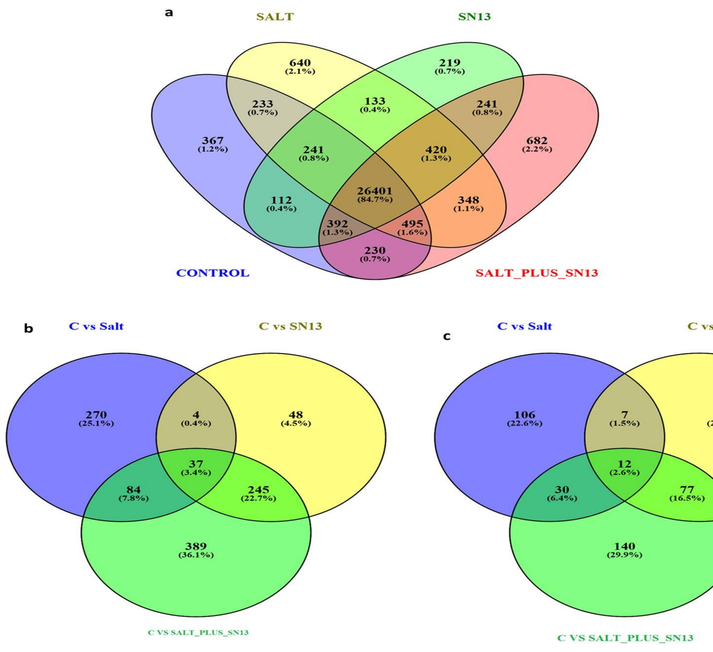
Objective

To decipher the molecular mechanism underlying beneficial microbe-plant interaction in rice crop exposed to salt stress.

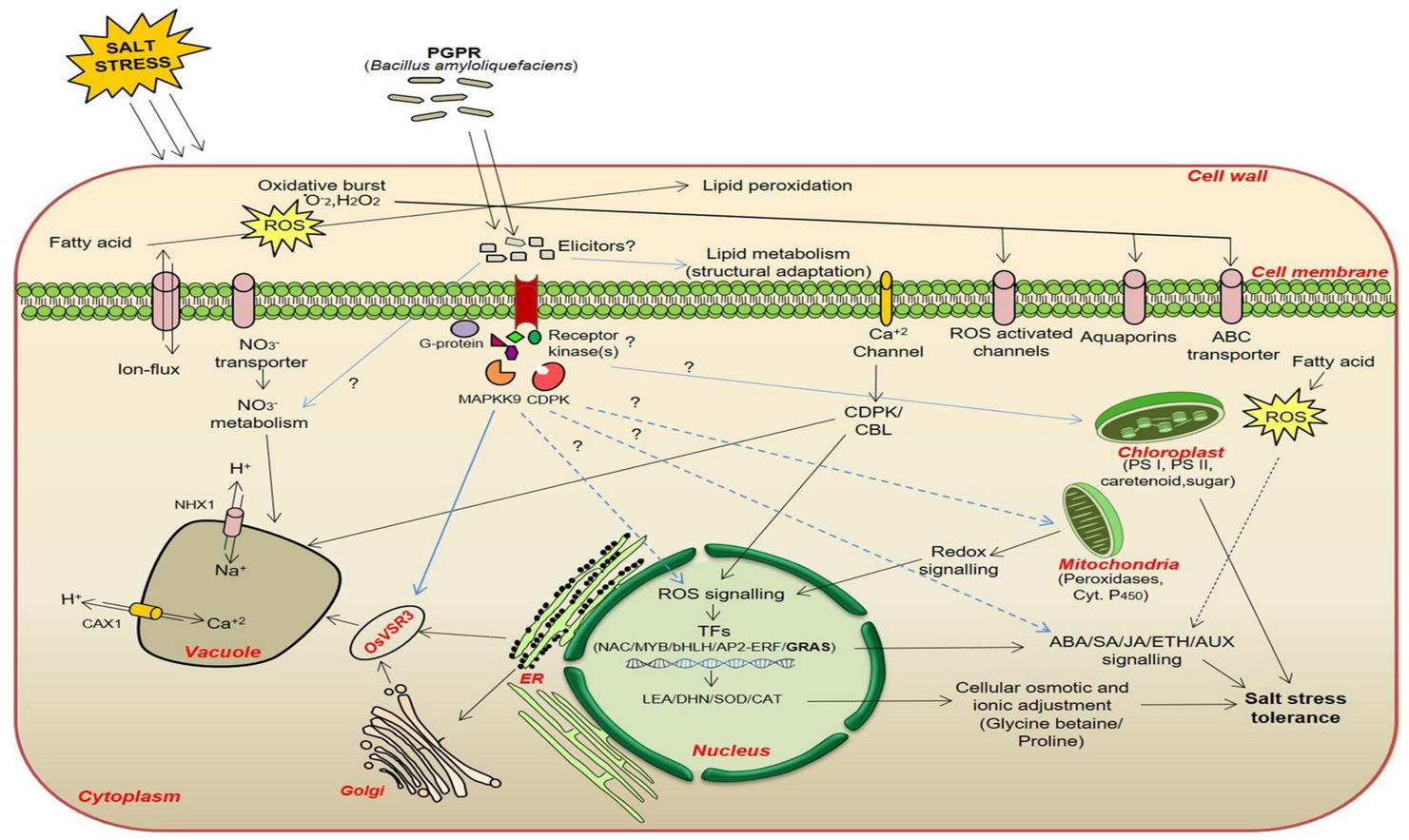
Materials and Methods

- **Phenotypic characterization of rice seedlings based on important abiotic stress marker such as relative water content and electrolyte leakage were determined in leaf and root samples.**
- **Root samples were used for total RNA extraction using Spectrum plant total RNA kit (sigma, USA).**
- **RNA-seq libraries were prepared using the True-seqTM RNA sample preparation kit (illumina CA, USA).**

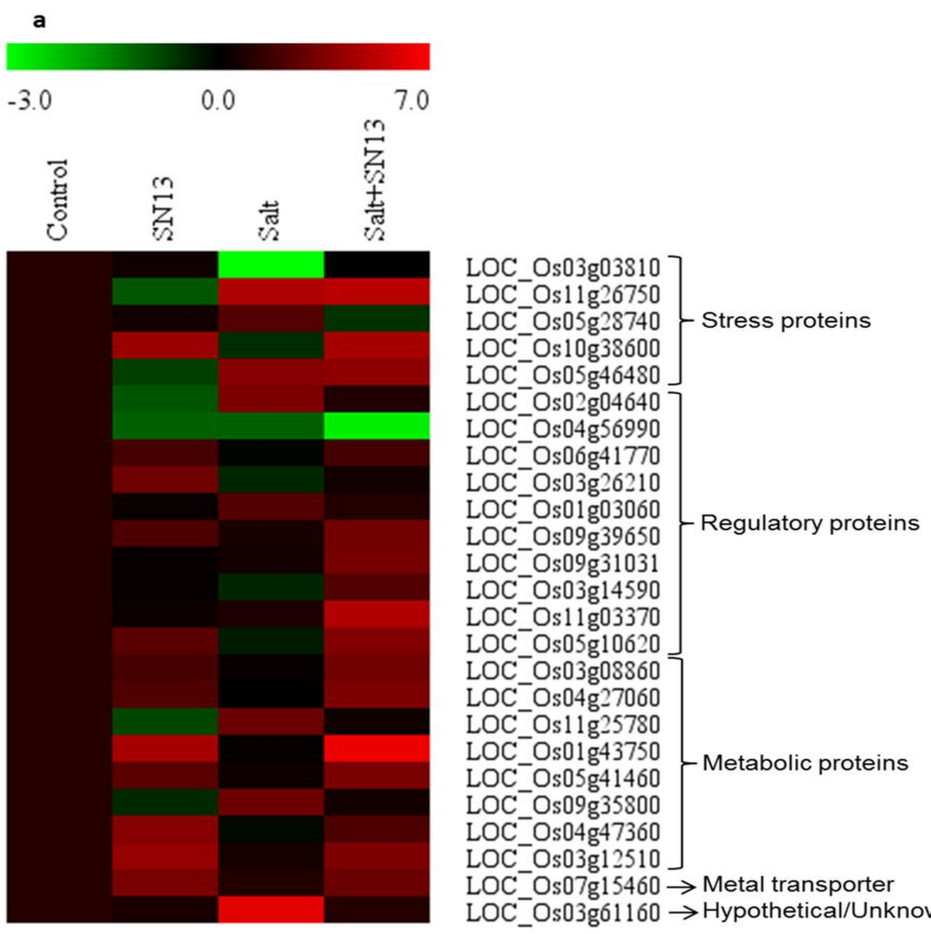
Results

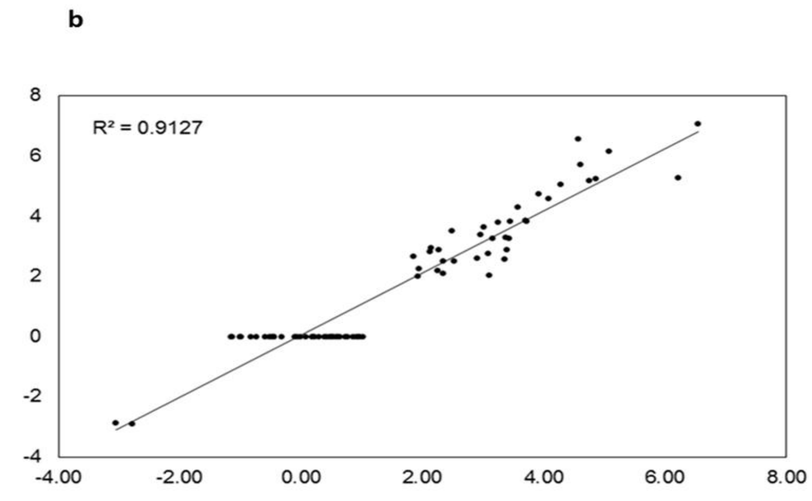


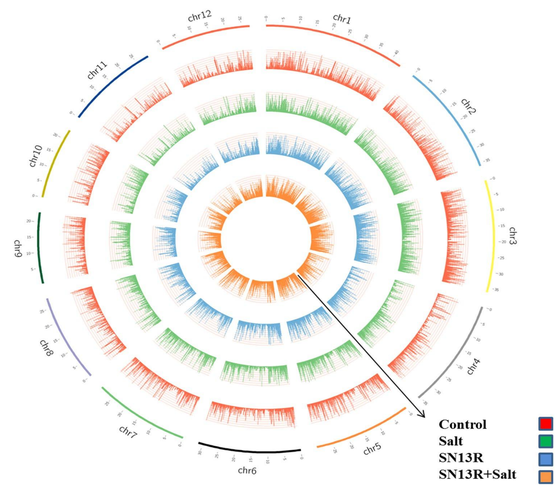
Venn diagram of the total number of expressed non-redundant transcript in control, salt SN13 and salt +SN13 samples (a). Venn diagram of the differential expressed up regulated genes (b). And downregulated genes (c). In salt, SN13 and +SN13 samples as compare to control.



A model depicting SN13-mediated salt stress regulation in rice seedlings.







Circos shows the histogram of FPKM of genes mapped onto 12 rice chromosome

Differential expression of 25 genes through qRT-PCR analysis in rice exposed to salt stress in the presence or absence of SN13. (a). Scatters plot with correlation value of 0.91 between RNA-seq and qRT-PCR data.1

Conclusions

- **The comparative transcriptome study revealed that the plant response at the molecular level on B. amyloliquifaciens inoculation suggesting it as a potential microbe to be used as bioinoculants.**

Characterization of plant growth-promoting alkalotolerant *Alcaligenes* and *Bacillus* strains for mitigating the alkaline stress in *Zea mays*

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Background

- Intensification of sodic soil due to increasing pH is an emerging environmental issue and severely affects agricultural crops.
- Alkaline soil pH disturbs biological properties of soil and therefore influences the functioning of plant-microbe interaction.
- Apart from genetic engineering and molecular marker assisted breeding technologies, use of alternate technologies like utilization of plant growth promoting bacteria (PGPB) for ameliorating abiotic stress is gaining importance and momentum for consideration.

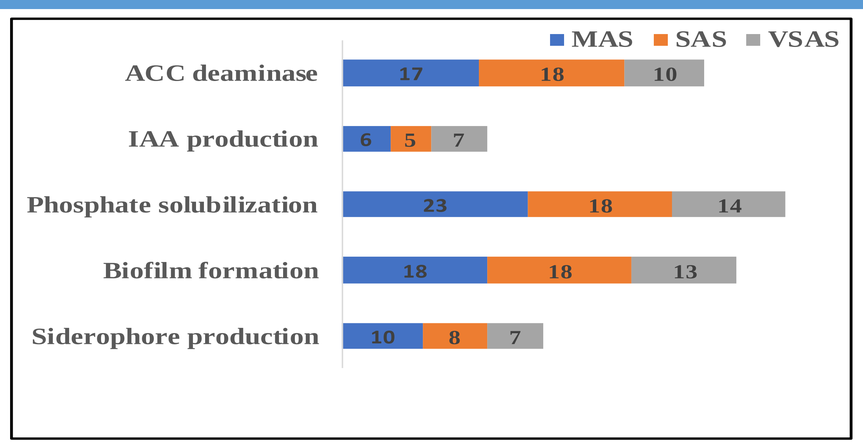
Objectives

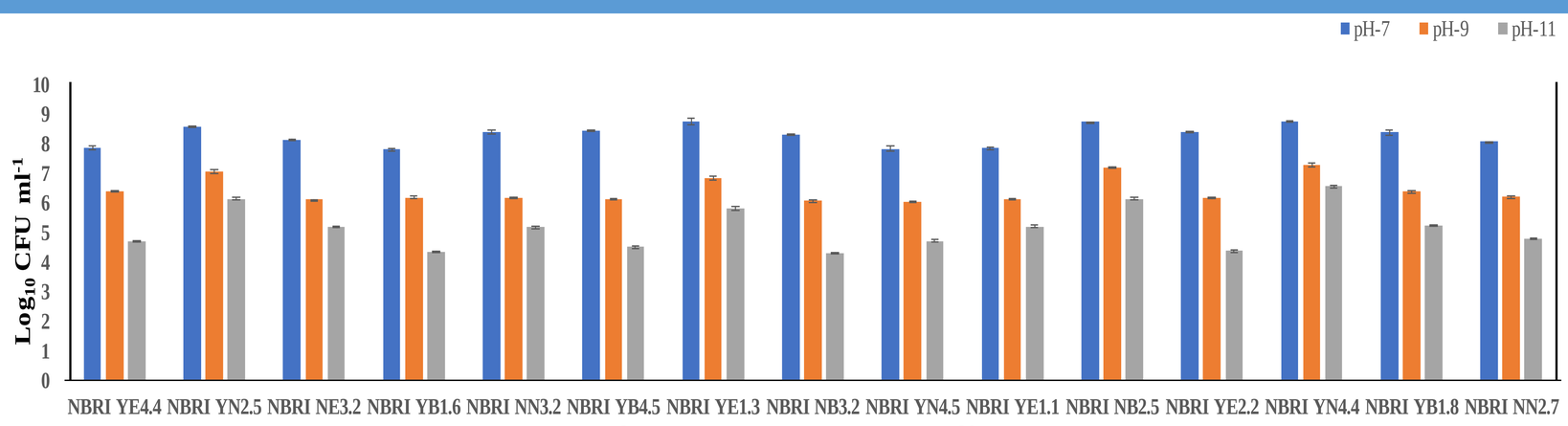
- Isolation and characterization of alkalotolerant PGPR from alkaline soil.
- Evaluation of PGPR for alkaline stress amelioration using maize (*Zea mays* L.) as the model plant.

Materials and Methods

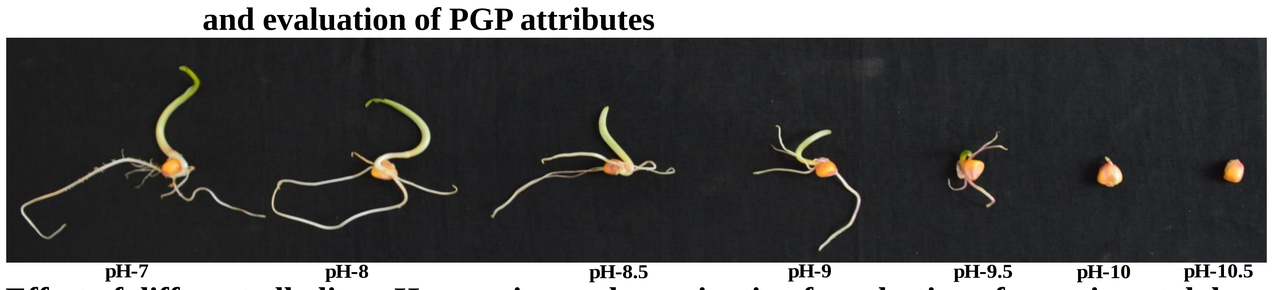
- Isolation and characterization of PGPR from moderately alkaline soil (MAS; pH 8-9), strongly alkaline soil (SAS; pH 9-10), and very strongly alkaline soil (VSAS; pH >10).
- Screening of PGPR on the basis of various PGP traits, alkaline stress tolerance, and *in vitro* seed germination test in maize.
- Inoculation of selected PGPR strains for evaluation of vegetative and biochemical parameters of maize
- Statistical analysis was performed using SPSS 16.0

Results





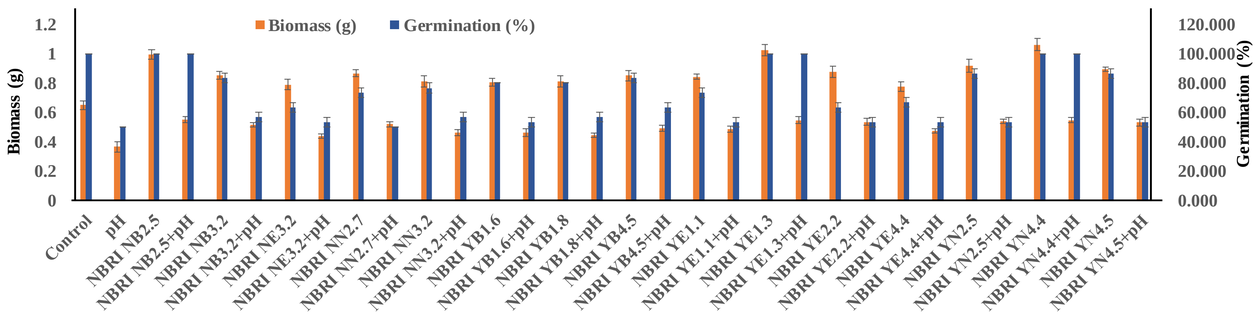
Isolation of bacterial strains from MAS, SAS, and VSAS and evaluation of PGP attributes



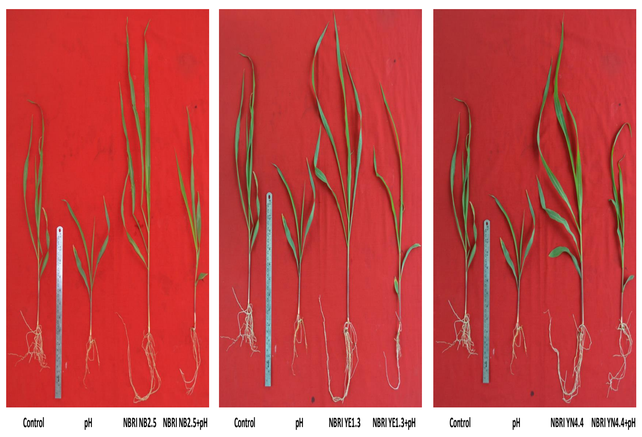
Growth assessment (log10 CFU ml-1) of selected strains under different alkaline pH (7, 9, 11) at day seven

Isolates	Accession No.	Identification	ACC deaminase activity	IAA production	Phosphate solubilization	Biofilm formation	EPS production	Alginate production	Siderophore production
NBRI NB2.5	MK168633	*Alcaligenes faecalis*	17.61±0.38[d]	3.09±0.16[a]	1.87±0.18[a]	2.22±0.01[m]	425.40±1.72[d]	271.84±1.67[h]	-
NBRI NB3.2	MK168630	*Bacillus subtilis*	6.52±0.11[a]	7.39±0.06[de]	14.23±0.24[d]	1.48±0.04[k]	712.61±1.69[j]	185.28±0.55[d]	+
NBRI YB1.6	MK168626	*Staphylococcus cohnii*	34.86±1.21[h]	6.60±0.11[cd]	16.01±0.41[e]	0.43±0.00[d]	406.03±4.35[c]	346.72±4.24[l]	+
NBRI YB1.8	MK168636	*Bacillus aryabhattai*	22.49±1.02[e]	7.79±0.22[e]	17.81±0.19[f]	0.83±0.00[e]	573.73±5.10[f]	152.00±2.00[b]	-
NBRI YB4.5	MK168628	*Bacillus altitudinis*	28.66±0.71[f]	6.30±0.00[c]	14.79±0.20[d]	1.00±0.01[i]	412.31±5.06[c]	274.24±3.48[h]	+
NBRI NN2.7	MK168637	*Bacillus cereus*	54.25±0.79[i]	11.06±0.38[f]	22. 36±0.40[h]	0.05±0.00[a]	504.83±3.03[f]	271.04±1.36[h]	-
NBRI NN3.2	MK168627	*Bacillus subtilis*	54.34±0.86[i]	5.95±0.10b[c]	17.09±0.29[f]	0.11±0.01[b]	266.28±5.33[b]	170.88±5.13[c]	+
NBRI YN2.5	MK168624	*Bacillus aryabhattai*	30.53±0.73[fg]	52.12±0.49[j]	17.13±0.34[f]	0.97±0.01[f]	637.86±3.01[h]	307.68±2.99[j]	+
NBRI YN4.4	MK168635	*Bacillus aryabhattai*	32.00±0.65[g]	15.30±0.52[g]	22. 94±0.46[h]	2.13±0.01[j]	737.53±7.24[k]	253.60±2.88[g]	+
NBRI YN4.5	MK168631	*Bacillus paralicheniformis*	9.65±0.20[b]	6.13±0.22[c]	12.35±0.23[c]	1.30±0.00[j]	654.33±0.94[j]	215.20±3.53[e]	+
NBRI NE3.2	MK168625	*Bacillus flexus*	14.50±0.51[c]	6.18±0.19[c]	12.89±0.27[c]	0.24±0.01[c]	578.71±5.69[f]	236.96±2.38[f]	+
NBRI YE1.1	MK168632	*Bacillus cereus*	35.51±0.35[h]	6.65±0.33[cd]	14.77±0.43[d]	0.59±0.00[f]	238.76±1.88[a]	125.12±2.15[a]	+
NBRI YE1.3	MK168629	*Bacillus subtilis*	22.15±0.82[e]	20.06±0.26[h]	20.75±0.25[g]	0.88±0.00[h]	471.88±5.32[e]	340.00±2.2[l]	-
NBRI YE2.2	MK168634	*Bacillus aryabhattai*	18.14±0.46[d]	22.19±0.16[i]	9.01±0.26[b]	0.07±0.00[ab]	599.30±3.20[g]	234.08±3.15[f]	-
NBRI YE4.4	MK168623	*Bacillus aryabhattai*	8.63±0.53[b]	5.22±0.14[b]	14.73±0.43[d]	0.49±0.01[e]	729.08±6.39[k]	246.08±2.24[g]	+

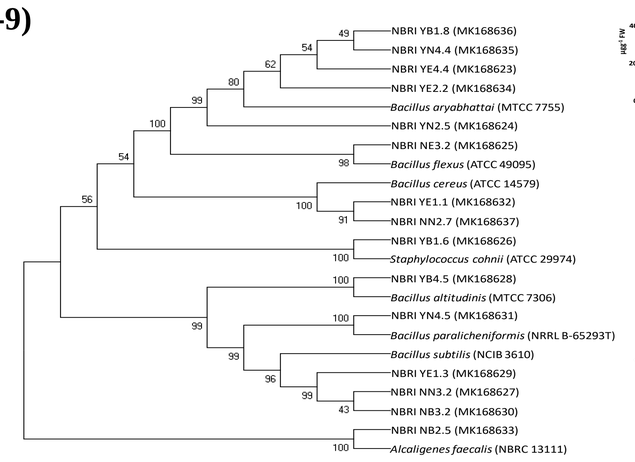
Effect of different alkaline pH on maize seed germination for selection of experimental dose



Effect of PGPR inoculation on seed germination and biomass under selected alkaline pH (pH-9)

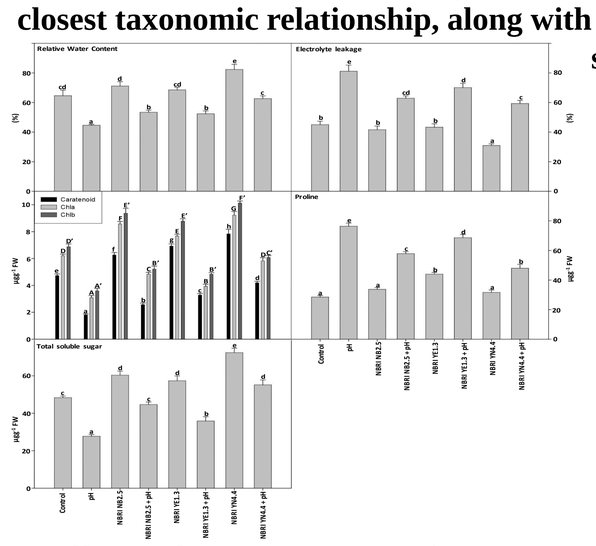


Evaluation of PGPR inoculation on growth promotion of Maize (*Zea mays*)

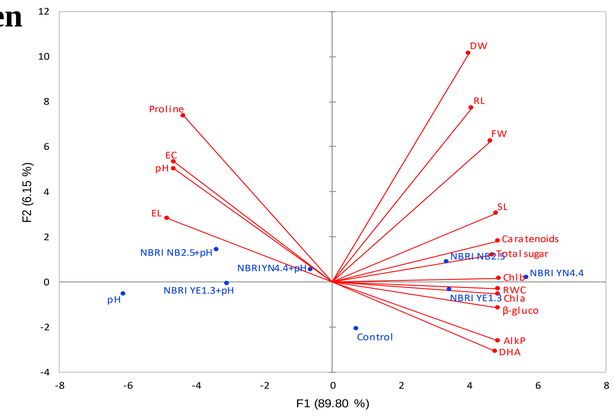


Phylogenetic relationships based on the 16S rRNA gene

Isolates with their corresponding 16S rRNA gene GenBank nucleotide accession numbers, closest taxonomic relationship, along with their plant growth promoting attributes at day seven



Effect of PGPR on maize vegetative parameters under normal and stress condition



Represents the Pearson correlation biplot between the F1 and F2.

Conclusion

- Alkalotolerant bacterial isolates exhibiting multiple PGP attributes and alkaline stress revealed *Bacillus* spp. as dominant genera among MAS, SAS, and VSAS.
- NBRI NB2.5, NBRI YE1.3, and NBRI YN4.4 can be used as bioinoculant for alkaline environment.

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(3-4, September 2021)

Abiotic stress tolerant *Jeotgalicoccus huakuii* NBRI 13E confers plant growth promotion and salt stress amelioration

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Background

- Land degradation due to salinization has drastically affected the global crop production.
- PGPR has been proved to be one of the alternatives in mitigating the effect of several abiotic stresses in an eco-friendly manner.
- PGPR having ACC deaminase activity reduces the level of ethylene stress and thereby, enhances growth of plant under various stresses.
- The present study aimed to demonstrate the potential of abiotic stress tolerant *Jeotgalicoccus huakuii* NBRI 13E for plant growth promotion and salt stress amelioration.

Objectives

- To demonstrate the potential of *Jeotgalicoccus huakuii* NBRI 13E for plant growth promotion and salt stress amelioration.
- To assess the ability of NBRI 13E inoculation for improving growth and yield of maize crop in alkaline soil.

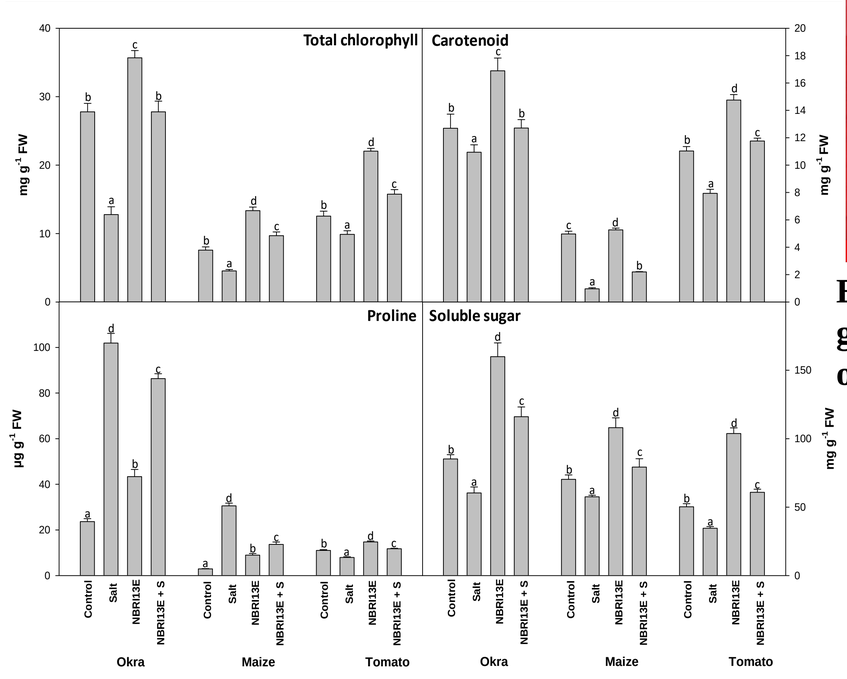
Materials and Methods

- Quantitative estimation of PGP and other attributes under normal and salt stress condition of NBRI 13E.
- Assessment of NBRI 13E for its ability of plant growth promotion under greenhouse condition.
- Tracking of NBRI 13E in the rhizosphere of okra, maize, and tomato.
- Evaluation of NBRI 13E for its ability of enhancing growth and yield of maize in microplot condition under alkaline stress.

Results

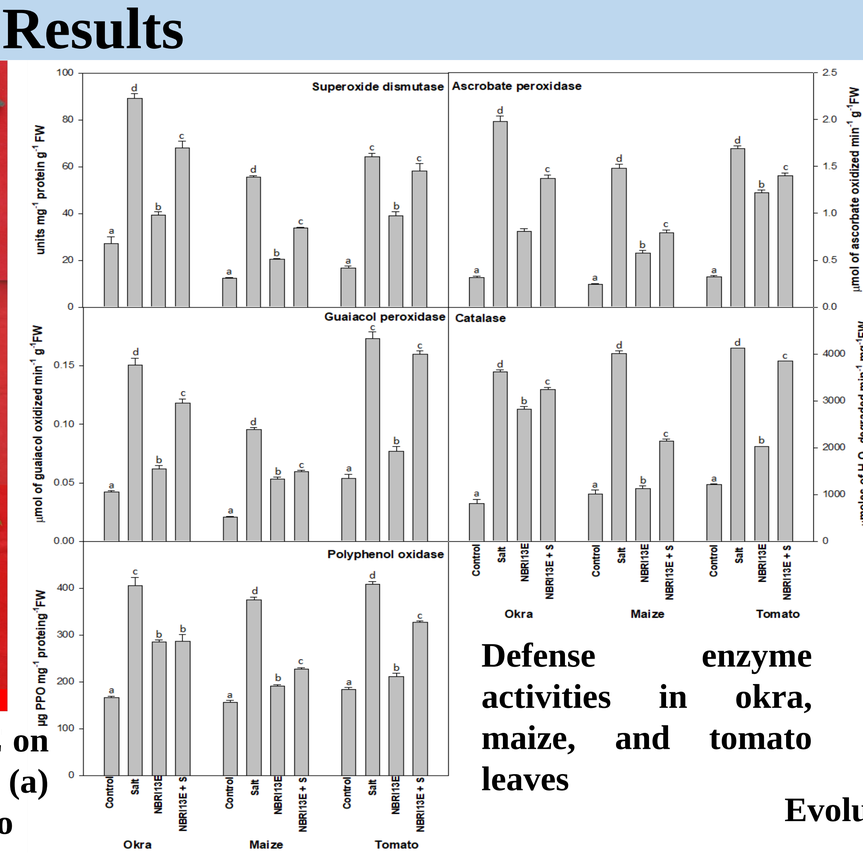
Plant growth promoting attributes of NBRI 13E under different salt (NaCl) stress condition

Plant growth promoting traits	0 M NaCl	0.5 M NaCl	1 M NaCl
Biofilm[1]	0.84±0.01^{b}	0.94±0.00^{c}	0.29±0.00^{a}
IAA[2]	105.47±1.90^{b}	101.27±1.63^{b}	22.21±0.16^{a}
P-Solubilization[3]	25.15±0.07^{c}	23.94±0.12^{b}	11.11±0.16^{a}
ACC deaminase[4]	0.71±0.02^{a}	0.88±0.00^{b}	0.76±0.02^{a}
EPS[5]	582.83±1.93^{b}	659.75±1.12^{c}	455.43±1.77^{a}
Alg[6]	388.48±1.15^{b}	586.72±1.15^{c}	378.08±1.25^{a}

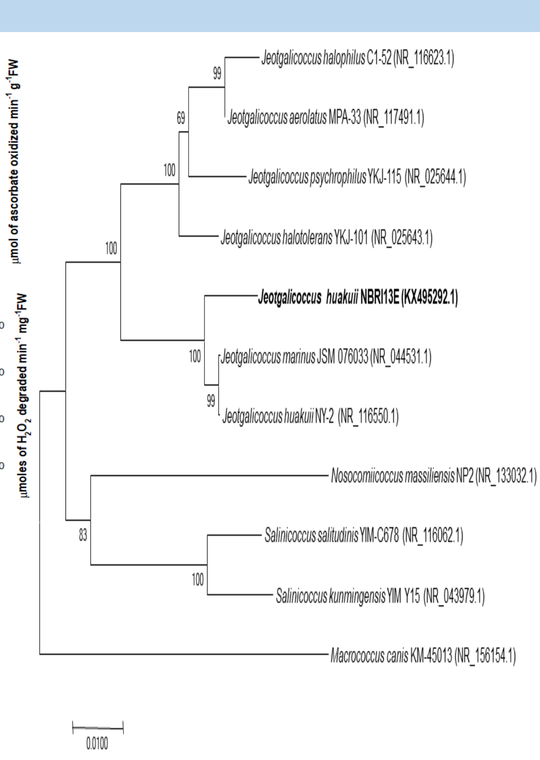


Effect of NBRI 13E Evaluation of NBRI 13E on growth promotion of (a) okra (b) maize (c) tomato

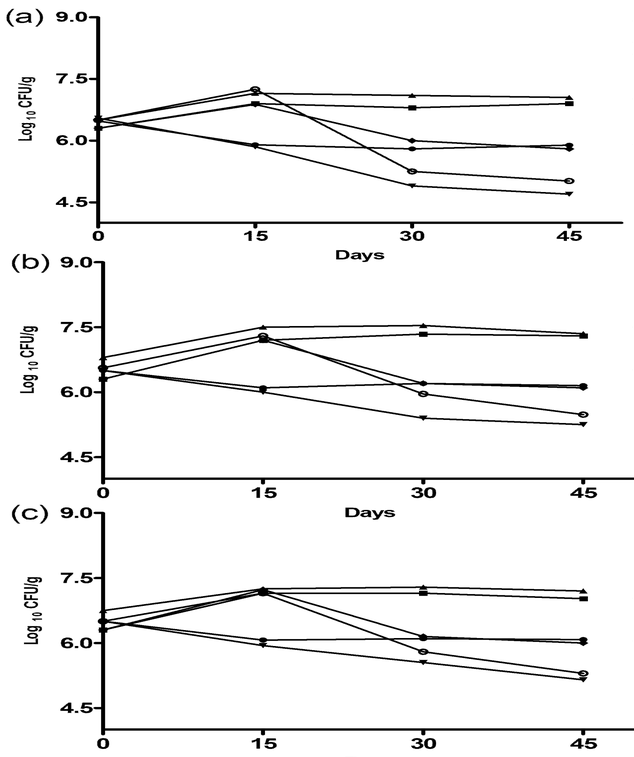
Evaluation of NBRI 13E on growth promotion of (a) okra (b) maize (c) tomato



Defense enzyme activities in okra, maize, and tomato leaves



Evolutionary relationships of NBRI 13E



Survival and competence in the rhizosphere of (a) okra, (b) maize, and (c) tomato by NBRI 13E

Effect of NBRI 13E on plant parameters of maize under field condition

Parameters	Control	NBRI 13E	50% NPK	100% NPK
Plant height (cm)	178.50±1.70^{a}	239.17±1.17^{d}	210.94±1.49^{b}	228.17±1.55^{c}
Plant fresh weight (g)	345.27±0.01^{a}	501.50±0.03^{c}	395.72±0.02^{ab}	404.28±0.01^{b}
Plant dry weight (g)	171.05±0.01^{a}	263.22±0.01^{c}	173.00±0.01^{a}	228.39±0.01^{b}
No. of cob per plant	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
Cob weight (g)	152.22±6.41^{a}	281.39±7.93^{d}	203.67±6.38^{b}	252.89±6.96^{c}
No. of rows per cob	14.33±0.33^{a}	17.44±0.22^{d}	15.67±0.33^{b}	16.56±0.22^{c}
No. of seeds per row	26.78±1.00^{a}	38.44±0.51^{d}	32.17±0.86^{b}	35.78±0.74^{c}
Total seeds per cob	387.67±21.83^{a}	671.22±13.95^{d}	505.67±21.06^{b}	594.44±19.35^{c}
Seed weight per cob	107.64±7.81^{a}	173.42±4.00^{d}	127.31±5.37^{b}	154.47±4.84^{c}
100 seed weight (g)	25.20±0.21^{a}	26.23±0.19^{b}	25.27±0.23^{a}	25.92±0.22^{b}
Total yield (t h^{-1})	5.07±0.08^{a}	8.27±0.08^{c}	5.47±0.08^{b}	8.13±0.08^{c}

Measurement of vegetative parameters of okra, maize and tomato plants

Okra (*Abelmoschus esculentus*)

Treatment	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh plant wt. (g)	Dry Plant wt. (g)
Control	15.76±0.26^{c}	10.23±0.27^{b}	3.00±0.00^{b}	2.32±0.08^{b}	0.62±0.02^{b}
Salt	13.13±0.18^{a}	9.00±0.26^{a}	2.00±0.00^{a}	2.03±0.02^{a}	0.55±0.02^{a}
NBRI 13E	16.87±0.28^{d}	10.50±0.17^{c}	3.00±0.00^{b}	3.28±0.02^{d}	0.72±0.02^{c}
NBRI 13E+S	14.77±0.15^{b}	9.57±0.01^{ab}	3.00±0.00^{b}	2.87±0.02^{c}	0.65±0.02^{b}

Maize (*Zea mays*)

Treatment	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh plant wt. (g)	Dry Plant wt. (g)
Control	42.07±1.67^{b}	24.13±0.55^{c}	3.67±0.33^{b}	5.58±0.04^{b}	0.69±0.02^{b}
Salt	30.73±0.52^{a}	17.27±0.49^{a}	2.67±0.33^{a}	3.86±0.03^{a}	0.51±0.03^{a}
NBRI 13E	54.73±0.87^{c}	24.27±0.30^{c}	4.33±0.33^{b}	6.42±0.03^{d}	0.72±0.02^{b}
NBRI 13E+S	43.83±0.84^{b}	21.57±0.44^{b}	4.00±0.00^{b}	5.90±0.03^{c}	0.70±0.02^{b}

Tomato (*Solanum lycopersicum*)

Treatment	Shoot length (cm)	Root length (cm)	No. of leaves	Fresh plant wt. (g)	Dry Plant wt. (g)
Control	27.53±0.13^{b}	9.53±0.22^{a}	12.00±0.58^{b}	11.25±0.03^{b}	4.44±0.02^{b}
Salt	18.50±0.15^{a}	9.00±0.21^{a}	8.67±0.33^{a}	7.70±0.02^{a}	2.30±0.02^{a}
NBRI 13E	28.57±0.23^{c}	11.13±0.22^{b}	12.33±0.33^{b}	11.65±0.03^{c}	4.58±0.02^{c}
NBRI 13E+S	28.33±0.29^{c}	12.10±0.21^{c}	12.33±0.33^{b}	11.74±0.03^{c}	4.49±0.02^{b}

Conclusions

- NBRI 13E imparted phytobeneficial effect by enhancing photosynthetic pigments and reduced proline accumulation and antioxidant enzymes in three different host plants
- NBRI 13E compromised the amount of chemical fertilizer to achieve enhanced maize yield as that of recommended dose under degraded soil

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Exploitation of agro-climatic environment for selection of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase producing salt tolerant indigenous plant growth promoting rhizobacteria

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Reg No. 3.16

Background

- Land degradation due to salinization has drastically affected the global crop production.
- PGPR has been proved to be one of the alternatives in mitigating the effect of several abiotic stresses in an eco-friendly manner.
- PGPR having ACC deaminase activity reduces the level of ethylene stress and thereby, enhances growth of plant under various stresses.
- The native PGPR has been observed to perform better in the corresponding soil environment along with the particular host than the exotic ones.

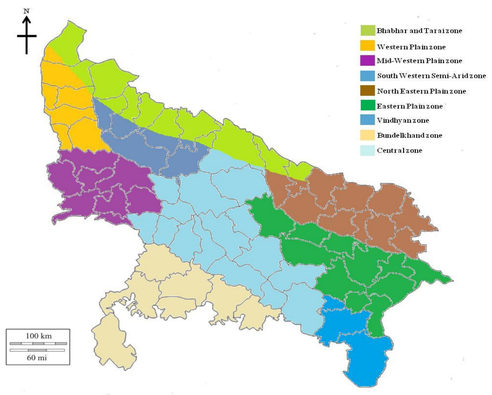
Objectives

- Isolation and characterization of ACC deaminase producing salt tolerant PGPR from nine agro-climatic zones of U.P, India.
- Evaluation of PGPR for salt stress amelioration using rice as the model plant

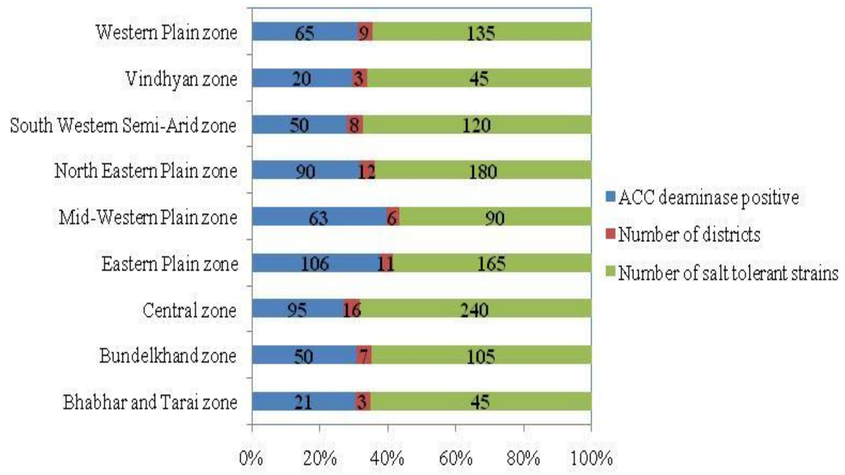
Materials and Methods

- Soil sampling from 09 different agroclimatic zones of Uttar Pradesh state and their physicochemical analysis.
- Isolation and characterization of salt (1 M NaCl) tolerant and ACC deaminase producing bacterial strains.
- *In vitro* seed germination test in rice and characterization on the basis of various PGP traits and abiotic stress tolerance.
- Evaluation of selected strains for their *in vitro* effect on rice biomass enhancement and ethylene stress amelioration (GC analysis).

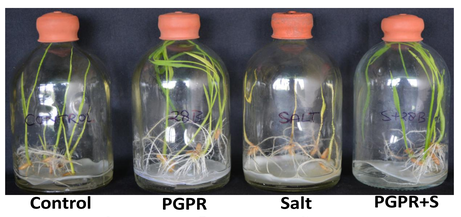
Results



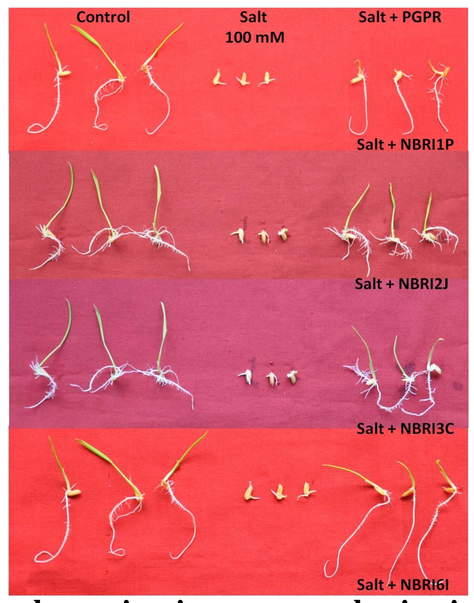
Geographic map representing soil sampling sites



Relative abundance of dominant bacterial phyla



Experimental setup for stress ethylene estimation



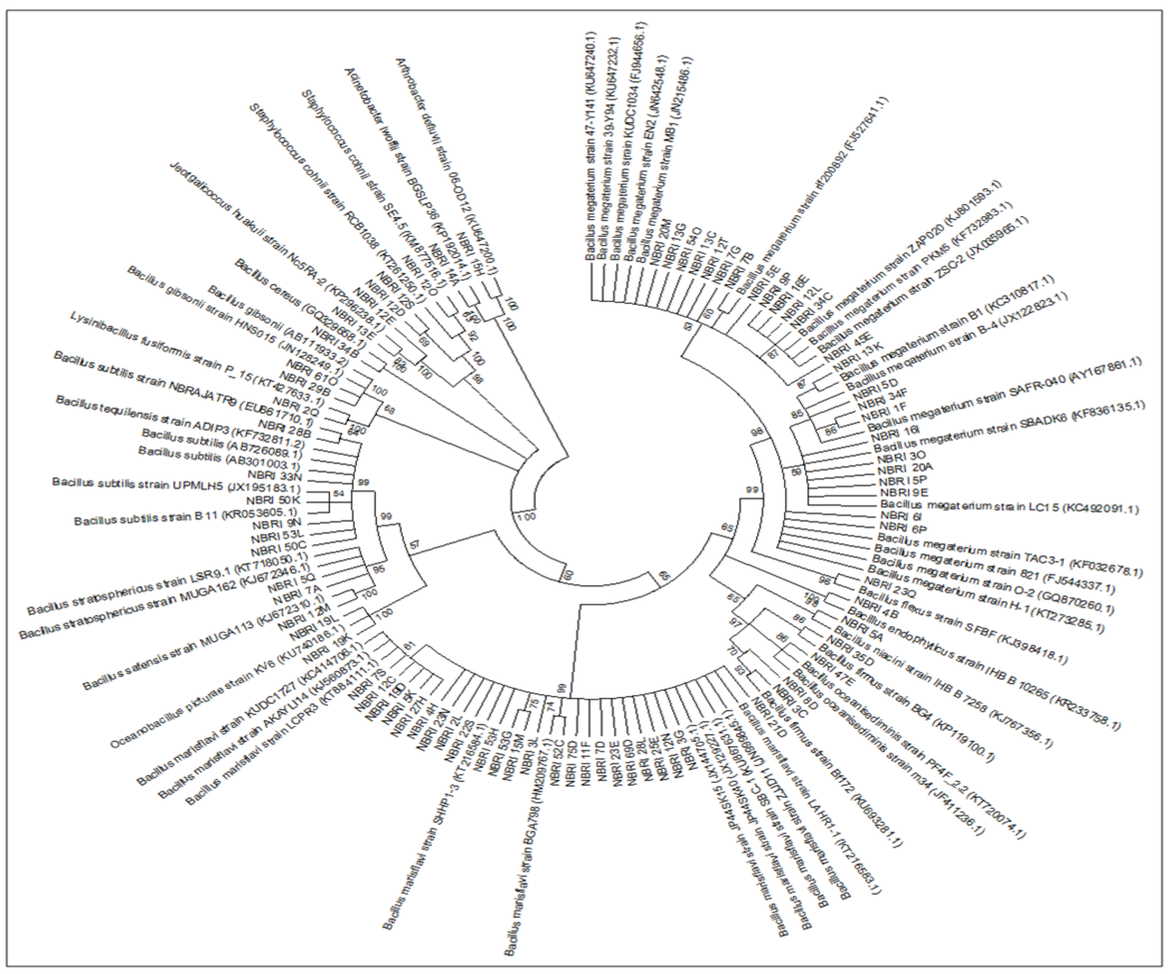
Seed germination assay under *in vitro* conditions

Soil sampling sites with their physico-chemical properties

Agro-climatic zones*	pH[a]	EC(µS/cm)[a]	TOC(%)[a]	MBC(ppm)[a]	BD(g/cm^3)[a]	Porosity[a]	WHC(%)[a]	S(ppm)[a]	P(mg/g)[a]	N(%)[a]
Bhabhar and Tarai zone	7.04–7.27	21.03–37.32	0.35–1.22	217.73–400.03	1.37–1.44	19.00–26.38	0.43–0.49	22.07–39.11	0.06–0.14	0.01–0.03
Bundelkhand zone	7.42–8.17	22.65–391.33	0.32–1.33	107.61–218.02	1.37–1.63	8.10–23.33	0.09–0.53	8.83–39.32	0.02–0.17	0.01–0.03
Central zone	7.39–8.5	21.96–879.09	0.30–1.30	34.21–423.57	1.33–1.54	10.46–27.60	0.28–0.53	19.47–47.18	0.03–0.27	0.02–0.03
Eastern Plain zone	6.55–8.37	25.21–388.77	0.23–0.68	36.26–290.04	1.36–1.56	8.87–25.48	0.38–0.53	14.66–69.65	0.02–0.32	0.02–0.03
Mid-Western Plain zone	7.23–8.94	37.05–778.53	0.64–1.29	72.23–291.74	1.37–1.52	12.80–24.29	0.34–0.46	12.38–83.48	0.04–0.30	0.01–0.03
North Eastern Plain zone	7.20–8.66	29.72–747.13	0.19–1.10	74.16–326.90	1.27–1.6	10.66–27.82	0.37–0.46	12.40–35.61	0.03–0.28	0.02–0.03
South Western Semi-Arid zone	7.81–8.28	211.26–452.94	0.59–0.95	180.75–292.16	1.39–1.52	17.60–27.25	0.37–0.41	18.71–154.54	0.12–0.33	0.02–0.03
Vindhyan zone	7.88–8.20	82.8–261.65	0.51–0.78	73.15–146.82	1.47–1.58	11.00–14.74	0.44–0.49	10.74–50.23	0.05–0.08	0.02–0.04
Western Plain zone	7.53–8.05	23.81–521.68	0.63–1.30	108.55–382	1.37–1.44	15.00–25.51	0.36–0.48	21.41–90.11	0.14–0.21	0.01–0.03

ACC deaminase activity of PGPR and its effect on ethylene stress amelioration in rice seedlings under salt stress

Isolates	Identification by 16S rRNA	ACC deaminase activity[a]	Biomass[b]		Ethylene emission[c]	
			No Salt stress	Salt stress	No Salt stress	Salt stress
Bhabhar and Tarai Zone						
NBRI 6I	*Bacillus megaterium*	9.86±0.019	0.32±0.01	0.23±0.02	6.44±0.25	4.26±0.61
Bundelkhand Zone						
NBRI 54O	*Bacillus megaterium*	4.25±0.007	0.33±0.01	0.31±0.02	5.46±0.48	4.46±0.21
NBRI 2Q	*Lysinibacillusfusiformis*	0.29±0.000	0.52±0.03	0.37±0.03	4.95±0.23	6.89±0.52
Central Zone						
NBRI 13E	*Jeotgalicoccushuakuii*	0.75±0.002	0.56±0.05	0.46±0.06	3.02±0.32	3.69±0.62
NBRI 9N	*Bacillus tequilensis*	12.91±0.055	0.53±0.02	0.42±0.03	7.79±1.08	5.62±0.63
Eastern Plain Zone						
NBRI 21D	*Bacillus marisflavi*	1.08±0.003	0.44±0.07	0.31±0.04	1.67±0.51	1.86±0.31
Mid-Western Plain Zone						
NBRI 16E	*Bacillus megaterium*	12.19±0.042	0.59±0.05	0.47±0.04	2.21±0.24	3.33±0.61
NBRI 16I	*Bacillus megaterium*	1.97±0.004	0.52±0.04	0.39±0.02	2.47±0.18	3.19±0.11
North Eastern Plain Zone						
NBRI 33N	*Bacillus subtilis*	3.07±0.007	0.33±0.01	0.25±0.01	2.02±0.21	2.21±0.25
NBRI 53L	*Bacillus subtilis*	9.63±0.027	0.52±0.12	0.39±0.03	2.44±0.73	2.08±0.29
South Western Semi-Arid Zone						
NBRI 28B	*Bacillus subtilis*	2.75±0.008	0.35±0.01	0.26±0.01	5.77±0.79	4.63±0.16
NBRI 20M	*Bacillus megaterium*	54.08±0.114	0.49±0.04	0.38±0.01	1.66±0.13	1.6±0.22
Vindhyan Zone						
NBRI 69D	*Bacillus marisflavi*	5.71±0.020	0.35±0.02	0.26±0.02	2.16±0.40	2.28±0.19
Western Plain Zone						
NBRI 12M	*Bacillus safensis*	3.80±0.008	0.36±0.01	0.3±0.02	2.57±0.05	2.19±0.48
NBRI 5K	*Bacillus marisflavi*	29.17±0.065	0.46±0.03	0.37±0.05	3.27±0.12	2.19±0.13
Control			0.3±0.02	0.13±0.01	2.84±0.53	6.19±0.31
LSD (P≤0.05)		0.028	0.021		0.074	



Molecular phylogenetic analysis

Conclusions

- Study for rhizospheric bacterial isolates exhibiting multiple PGP and different abiotic mitigating traits revealed *Bacillus* spp. to be the dominant genera among agro-climatic zones of U.P, India.
- Much of rhizobacterial performance differs with the agro-climatic zones suggesting their eco-adaptive features.

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Current status of fertility indicators associated with arsenic contaminated paddy soil.

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Introduction

- The presence of heavy metals (As) in rice raises serious concerns regarding the soil quality of paddy farming regions irrigated with groundwater.
- For agricultural soil manipulations, it is critical to investigate the functioning of soil microorganisms exposed to heavy metal contamination.
- To evaluate the physio- chemical properties of contaminated collected sample from West Bengal, India.
- To analyses the Carbon substrate utilization pattern and soil enzymatic activities by presence of As contaminated soil samples.

Materials and Methods

- Soil collection from cultivable and irrigated agricultural rice field of different districts of West Bengal, India.
- Soil physico-chemical, metal availability and soil enzyme analysis of soil sample were performed.
- Functional and molecular diversity of soil sample were performed on the basis of C-source utilization pattern and DGGE respectively.
- Statistical analysis was performed using SPSS 16.0

Results

Soil samples collection from different localities of five districts of West Bengal

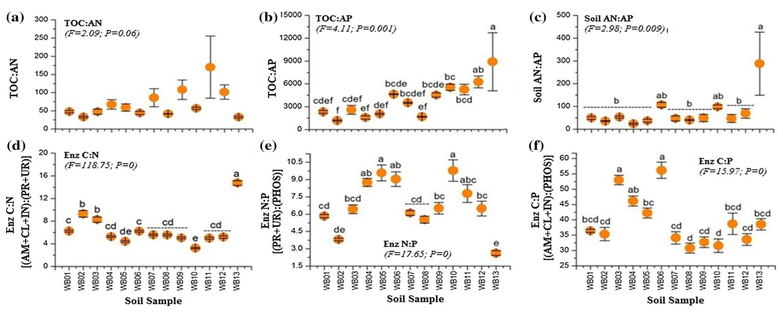
S. no.	Location/area	Coordinates	Composite sample
1	Babanpur, East Midnapur	22°11′00″N 87°59′00″E	WB1
			WB2
			WB3
2	South 24 Pargnas	22°27′34.7″N 88°28′01.0″E	WB4
			WB5
			WB6
3	Durgapur, Bardhaman	22°40′117″N88°58′293″E	WB7
4	Dogachia, North 24 Pargnas	23°00′09.3″N 88°47′06.9″E	WB8
			WB9
			WB10
5	Chinsurah, Hooghly	22°53′20.5″N 88°21′45.0″E	WB11
			WB12
			WB13

Heavy metal content of soil sample collected from thirteen different sites of West Bengal

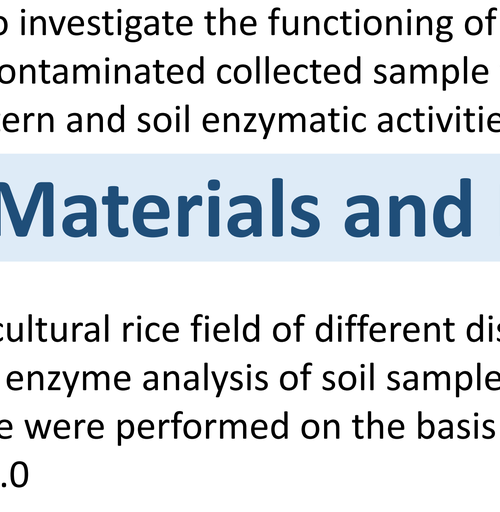
	As	Cr	Mn	Co	Cu	Se	Cd
WB1	11.53 ± 0.32^{d}	2.05 ± 0.24^{cd}	112.83 ± 3.49^{def}	4.67 ± 0.13^{ab}	8.68 ± 0.30^{c}	1.28 ± 0.05^{cd}	0.21 ± 0.01^{bc}
WB2	9.33 ± 0.73^{de}	0.86 ± 0.09^{e}	128.13 ± 14.83^{cde}	4.52 ± 0.09^{abc}	8.07 ± 0.28^{c}	1.05 ± 0.06^{d}	0.29 ± 0.01^{bc}
WB3	12.57 ± 0.89^{cd}	1.04 ± 0.03^{e}	134.10 ± 3.02^{cde}	4.31 ± 0.10^{abc}	16.59 ± 0.83^{b}	1.32 ± 0.06^{cd}	0.34 ± 0.03^{b}
WB4	12.28 ± 0.78^{cd}	0.92 ± 0.03^{e}	72.26 ± 4.81^{g}	4.05 ± 0.28^{bc}	19.99 ± 1.07^{a}	3.24 ± 0.33^{a}	1.27 ± 0.08^{a}
WB5	11.09 ± 0.56^{d}	0.92 ± 0.06^{e}	89.86 ± 5.56^{fg}	4.79 ± 0.30^{a}	21.08 ± 0.97^{a}	1.72 ± 0.10^{bc}	0.26 ± 0.01^{bc}
WB6	11.18 ± 0.58^{d}	1.40 ± 0.05^{cde}	71.74 ± 2.65^{g}	3.86 ± 0.12^{c}	22.30 ± 1.11^{a}	1.42 ± 0.10^{cd}	0.18 ± 0.01^{c}
WB7	6.18 ± 0.17^{e}	0.79 ± 0.02^{e}	30.25 ± 1.34^{h}	2.96 ± 0.07^{d}	14.94 ± 0.85^{b}	1.12 ± 0.07^{cd}	0.16 ± 0.01^{c}
WB8	18.79 ± 0.54^{b}	1.20 ± 0.02^{de}	107.63 ± 3.77^{efg}	1.11 ± 0.05^{e}	3.70 ± 0.10^{d}	2.18 ± 0.11^{b}	0.21 ± 0.01^{c}
WB9	15.31 ± 0.69^{c}	2.00 ± 0.14^{cd}	162.20 ± 8.45^{c}	0.95 ± 0.05^{e}	3.94 ± 0.17^{d}	1.54 ± 0.10^{cd}	0.18 ± 0.01^{c}
WB10	19.61 ± 0.72^{a}	2.41 ± 0.16^{c}	150.53 ± 5.86^{cd}	0.98 ± 0.05^{e}	5.60 ± 0.23^{cd}	1.53 ± 0.11^{cd}	0.17 ± 0.01^{c}
WB11	23.04 ± 0.96^{a}	3.79 ± 0.37^{b}	258.03 ± 10.59^{b}	1.49 ± 0.08^{e}	6.41 ± 0.35^{cd}	1.53 ± 0.07^{cd}	0.16 ± 0.01^{c}
WB12	22.48 ± 0.72^{ab}	4.97 ± 0.30^{a}	312.70 ± 8.48^{a}	2.70 ± 0.12^{d}	7.15 ± 0.39^{c}	1.61 ± 0.11^{bcd}	0.28 ± 0.01^{bc}
WB13	21.41 ± 0.92^{ab}	4.06 ± 0.25^{b}	245.83 ± 11.75^{b}	1.54 ± 0.07^{e}	6.83 ± 0.34^{cd}	1.57 ± 0.08^{cd}	0.17 ± 0.01^{c}

Mean ± SEM were compared by analysis of variance (ANOVA), followed by the Tukey's test. Statistically significant differences were then determined at p B 0.05, using the software Origin version 8

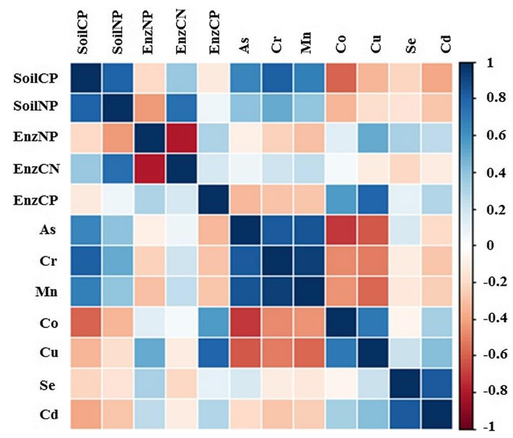
Stoichiometric ratios of nutrient and enzyme activity obtained for thirteen soil samples. Letters above the mean ± SE indicate significant differences at P B 0.05 using Tukey's post hoc tests.



Pearson's correlation matrix featuring relationships of heavy metals, soil nutrient, and activity of soil enzymes of soil samples collected from thirteen different sites of West Bengal. Significant correlations (p B 0.05) were determined using the R package version 3.2.4. The values used to determine Pearson's correlational matrix are means for their respective parameters



Pearson's correlation matrix featuring relationships of As, stoichiometric ratios of soil nutrient, and activity of soil enzymes and of soil samples collected from thirteen different sites of West Bengal. Significant correlations (p B 0.05) were determined using the R package version 3.2.4. The values used to determine Pearson's correlational matrix are means for their respective parameters



Physico-chemicales of soil samples collected from thirteen different sites of West Bengal

Physico-chemical properties of soil samples collected from thirteen different sites of West Bengal

	pH	EC	TOC (%)	Ext. SO4	Avail. N (ppm)	Total N (ppm)	Avail. P (ppm)	Total P (ppm)	Avail. K (ppm)	Total K (ppm)	% Loss on ignition
WB1	7.20 ± 0.06^{f}	61.40 ± 0.32^{e}	1.76 ± 0.01^{c}	0.64 ± 0.06^{h}	373.6 ± 18.68^{ab}	2300 ± 200^{bc}	7.72 ± 0.67^{bc}	106.77 ± 3.79^{e}	200 ± 10.00^{b}	9690 ± 380^{bcd}	7.23 ± 0.12^{f}
WB2	8.03 ± 0.03^{e}	110.43 ± 0.34^{a}	1.17 ± 0.01^{f}	1.98 ± 0.08^{bcd}	354 ± 18.68^{abc}	2800 ± 200^{ab}	9.97 ± 0.59^{a}	189.40 ± 7.59^{ad}	210 ± 50.00^{ab}	6500 ± 200de	5.87 ± 0.10^{g}
WB3	7.83 ± 0.03^{e}	59.20 ± 1.82^{e}	1.35 ± 0.02^{h}	1.24 ± 0.06^{f}	298.88 ± 49.42^{abc}	2300 ± 200^{bc}	5.75 ± 1.20^{bcd}	172.99 ± 8.61^{cd}	100 ± 20.00^{de}	6460 ± 1170de	6.18 ± 0.25^{g}
WB4	8.87 ± 0.03^{a}	75.47 ± 0.66^{c}	1.51 ± 0.00^{ef}	1.31 ± 0.13^{f}	242.84 ± 49.42^{bc}	3000 ± 400^{a}	10.04 ± 1.62^{a}	60.59 ± 1.95^{f}	80 ± 10.00^{e}	12,270 ± 90^{ab}	7.97 ± 0.11^{ef}
WB5	8.50 ± 0.06^{cd}	70.90 ± 0.17^{cd}	1.54 ± 0.01^{e}	1.17 ± 0.10^{fg}	280.2 ± 56.04^{abc}	1900 ± 100^{bc}	7.51 ± 0.23^{abc}	183.93 ± 0.93^{cd}	100 ± 0.00^{cde}	11,610 ± 1070^{b}	8.16 ± 0.20^{de}
WB6	8.50 ± 0.00^{cd}	65.13 ± 0.52^{de}	1.62 ± 0.01^{d}	0.73 ± 0.02^{gh}	373.2 ± 37.36^{ab}	2000 ± 100^{bc}	3.49 ± 0.01^{d}	231.72 ± 5.70^{a}	90 ± 10.00^{e}	10,960 ± 1700^{bc}	9.54 ± 0.05^{ab}
WB7	8.70 ± 0.06^{abc}	95.73 ± 0.90^{b}	1.53 ± 0.01^{ef}	2.17 ± 0.13^{abc}	205.48 ± 49.42^{bc}	2000 ± 100^{bc}	4.34 ± 0.05^{cd}	228.68 ± 7.19^{ab}	110 ± 10.00^{bc}	5280 ± 780^{e}	5.44 ± 0.20^{g}
WB8	8.73 ± 0.07^{ab}	89.03 ± 0.71^{b}	1.41 ± 0.01^{gh}	2.62 ± 0.07^{a}	336.24 ± 0.00^{abc}	1900 ± 100^{cd}	8.36 ± 0.21^{ab}	239.21 ± 13.99^{a}	130 ± 10.00^{bc}	7690 ± 1900^{cde}	6.10 ± 0.01^{g}
WB9	8.63 ± 0.03^{bc}	106.53 ± 2.99^{a}	1.76 ± 0.01^{c}	2.41 ± 0.13^{ab}	186.8 ± 49.42^{bc}	2100 ± 100^{bc}	3.92 ± 0.21^{d}	115.07 ± 7.21^{e}	190 ± 30.00^{abc}	15,490 ± 250^{a}	8.42 ± 0.04^{cde}
WB10	8.40 ± 0.00^{d}	71.73 ± 3.70^{cd}	2.00 ± 0.01^{a}	1.77 ± 0.06^{cde}	354.92 ± 18.68^{abc}	2400 ± 200^{abc}	3.65 ± 0.28^{d}	159.22 ± 1.13^{d}	220 ± 0.00^{ab}	11,550 ± 550^{b}	10.00 ± 0.11^{a}
WB11	8.37 ± 0.03^{d}	62.60 ± 1.15^{e}	1.9 ± 0.01^{b}	1.58 ± 0.13^{def}	168.12 ± 56.04^{c}	2200 ± 100^{bc}	3.72 ± 0.46^{d}	240.83 ± 4.76^{a}	130 ± 10.00^{bc}	11,360 ± 780^{b}	9.36 ± 0.27^{ab}
WB12	6.90 ± 0.06^{g}	32.17 ± 0.49^{f}	1.58 ± 0.01^{de}	1.44 ± 0.06^{ef}	168.12 ± 32.35^{c}	2300 ± 200^{bc}	2.59 ± 0.29^{d}	197.90 ± 4.82^{bc}	150 ± 20.00^{bc}	10,630 ± 950^{bc}	8.98 ± 0.09^{bc}
WB13	7.17 ± 0.03^{f}	38.80 ± 0.70^{f}	1.46 ± 0.01^{fg}	1.49 ± 0.04^{ef}	448.32 ± 32.35^{a}	1900 ± 200^{cd}	2.27 ± 0.78^{d}	62.62 ± 0.88^{f}	240 ± 10.00^{a}	10,130 ± 380^{bc}	8.92 ± 0.08^{bcd}

Mean ± SEM were compared by analysis of variance (ANOVA), followed by the Tukey's test. Statistically significant differences were then determined at $p \leq 0.05$, using the software Origin version 8

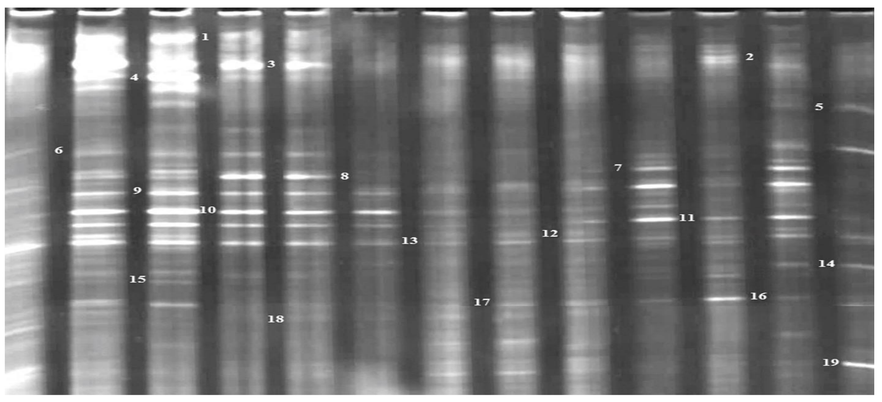
Mean ± SEM were compared by analysis of variance (ANOVA), followed by the Tukey's Statistically significant differences were then determined at p B 0.05, using the software Origin version 8

Activity of soil enzyme from samples collected from thirteen different sites of West Bengal

Sample	DHA	Phos	Protease	Urease	Amylase	Cellulase	Invertase
WB1	246.4 ± 3.5^{c}	5.3 ± 0.1^{bc}	6.9 ± 0.1a	24.2 ± 0.8^{d}	15.6 ± 0.2^{d}	42.5 ± 0.0^{ab}	135.7 ± 0.1^{c}
WB2	81.3 ± 3.1^{h}	5.2 ± 0.1^{c}	4.2 ± 0.1^{e}	15.5 ± 0.2^{f}	15.5 ± 0.3^{de}	24.0 ± 10.3^{de}	143.2 ± 0.2^{b}
WB3	107.2 ± 3.6^{g}	2.9 ± 0.1^{ef}	4.1 ± 0.1^{e}	14.8 ± 1.0^{f}	14.2 ± 0.2^{e}	38.5 ± 0.2^{bcd}	102.8 ± 0.2^{g}
WB4	108.6 ± 2.8^{fg}	3.2 ± 0.1^{ef}	2.5 ± 0.1^{ef}	25.3 ± 0.8^{d}	14.1 ± 0.2^{e}	35.7 ± 0.1^{cde}	96.3 ± 0.3^{i}
WB5	97.4 ± 2.3^{g}	4.0 ± 0.2^{de}	2.7 ± 0.1^{e}	35.1 ± 1.4^{b}	10.2 ± 0.3^{fg}	52.6 ± 0.2^{ab}	103.7 ± 0.3^{g}
WB6	203.9 ± 1.6^{d}	3.1 ± 0.1^{ef}	2.1 ± 0.0^{f}	25.9 ± 0.7^{d}	11.3 ± 0.5^{fg}	45.7 ± 0.2^{abc}	117.5 ± 0.2^{e}
WB7	645.7 ± 4.7^{a}	7.0 ± 0.4^{a}	5.8 ± 0.1^{b}	36.5 ± 1.0^{b}	19.3 ± 0.3^{c}	55.7 ± 0.1^{a}	160.8 ± 0.1^{a}
WB8	641.5 ± 3.2^{a}	6.4 ± 0.3^{b}	5.3 ± 0.1^{b}	30.1 ± 0.9^{c}	25.1 ± 0.2^{a}	45.9 ± 2.0^{abc}	126.1 ± 0.3^{d}
WB9	583.5 ± 1.4^{b}	5.2 ± 0.3^{c}	4.3 ± 0.1^{c}	29.2 ± 0.9^{c}	22.1 ± 0.2^{b}	40.0 ± 0.3^{abc}	106.5 ± 0.3^{f}
WB10	172.8 ± 1.4^{e}	5.1 ± 0.4^{cd}	3.4 ± 0.2^{d}	45.5 ± 1.0^{a}	19.0 ± 0.4^{c}	41.4 ± 0.4^{abc}	98.1 ± 0.3^{h}
WB11	122.3 ± 1.9^{f}	2.5 ± 0.2^{f}	2.0 ± 0.1^{f}	16.8 ± 0.6^{f}	9.9 ± 0.1^{g}	22.5 ± 0.1^{e}	61.2 ± 0.4 ^{l}
WB12	107.2 ± 2.6^{g}	3.5 ± 0.2^{ef}	1.9 ± 0.1^{f}	20.7 ± 0.9^{e}	10.3 ± 0.1^{fg}	29.3 ± 0.3^{de}	77.8 ± 0.2^{j}
WB13	81.4 ± 3.1^{h}	2.6 ± 0.1^{f}	1.9 ± 0.1^{f}	4.9 ± 0.2^{g}	9.2 ± 0.1^{g}	24.6 ± 0.2^{de}	66.8 ± 0.1 ^{k}

Mean ± SEM were compared by analysis of variance (ANOVA), followed by the Tukey's test for dehydrogenase, phosphatase ,protease, amylase, cellulase, and invertase, while Fisher's LSD for urease only. Statistically significant differences were then determined at p B 0.05, using the software Origin version 8.

DGGE pattern of the 16S rRNA gene from different sites WB1 to WB13.



Conclusions

- The current study draws important inference of negative impact on soil fertility indicators by heavy metal under As contaminated agricultural soil for paddy farming.
- Heavy metal availability led to the decrease in nutrient growth limiting factors (N and P) and activity of different soil enzyme.
- Different soil fertility indicators and nutrient growth limiting factor demonstrated that soil is getting degraded that warrants immediate attention of soil scientist, farmers and agronomists.

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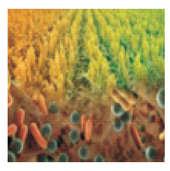


Comparative Proteomics Approach Reveal PEG Induced Drought Response in Rice Root

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Introduction

- **Comparative proteomic analysis plays a key role to identify numerous stress-responsive genes**
- **Rice (Oryza sativa L.) exhibit several adaptive and acclimatization strategies to combat environmental conditions such as drought.**
- **Drought response must be understood in order to unravel signaling pathways that will allow for improved adaptation.**
- **In Asia, drought, a major abiotic stress, is responsible for affecting about 20% of the total rice-growing area.**

Objectives

Proteome characterization of rice root proteins was carried out under PEG-simulated drought stress conditions in order to better understand the mechanism.

Materials and Methods

- **A drought tolerant variety Heena cultivated in northern Indian was selected.**
- **Rice were stressed for 7 days using 20% polyethylene glycol PEG-6000 in the nutrient solution and harvested on 1st , 3rd and 7th day.**
- **PD Quest analysis and MALDI-TOF MS-MS analysis was taken in account to rule out the 78 differentially expressed proteins spot among the 510 protein spots followed by 125 differentially regulated spots**

Results

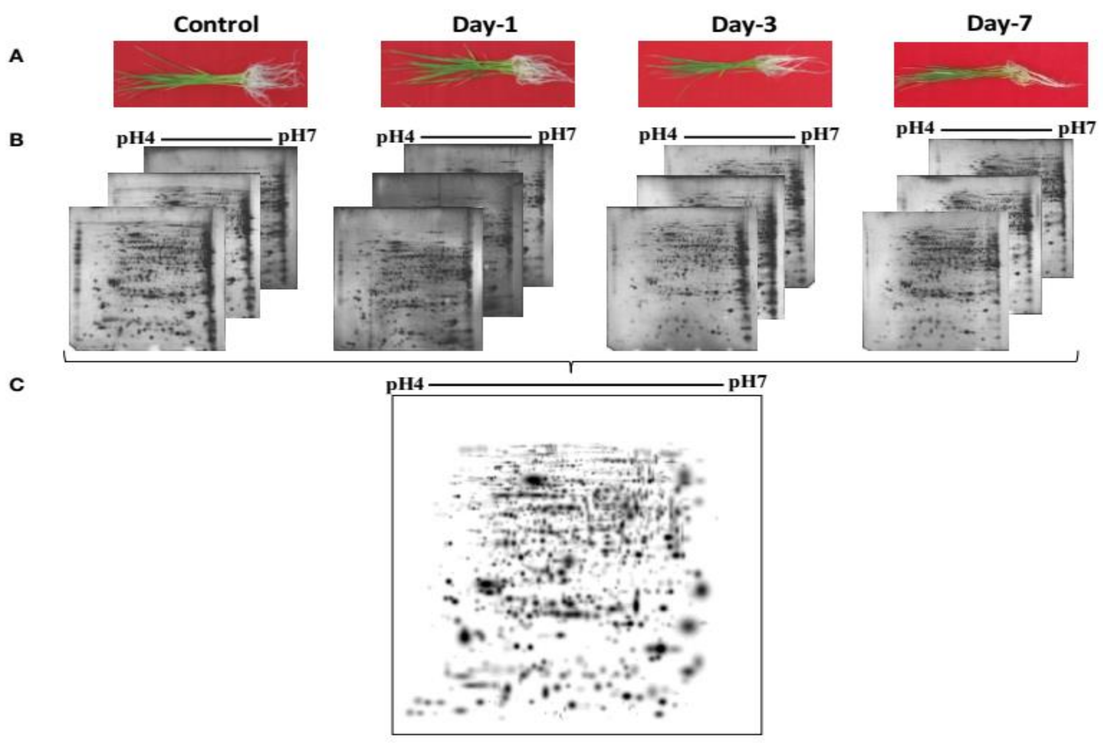

FIGURE 1 | 2-DE analysis of root proteome of rice. (A) Rice plant showing different stages of drought. (B) Proteins extraction from rice root tissue (C) Silver-stained gels for each stage were computationally combined using PDQuest software

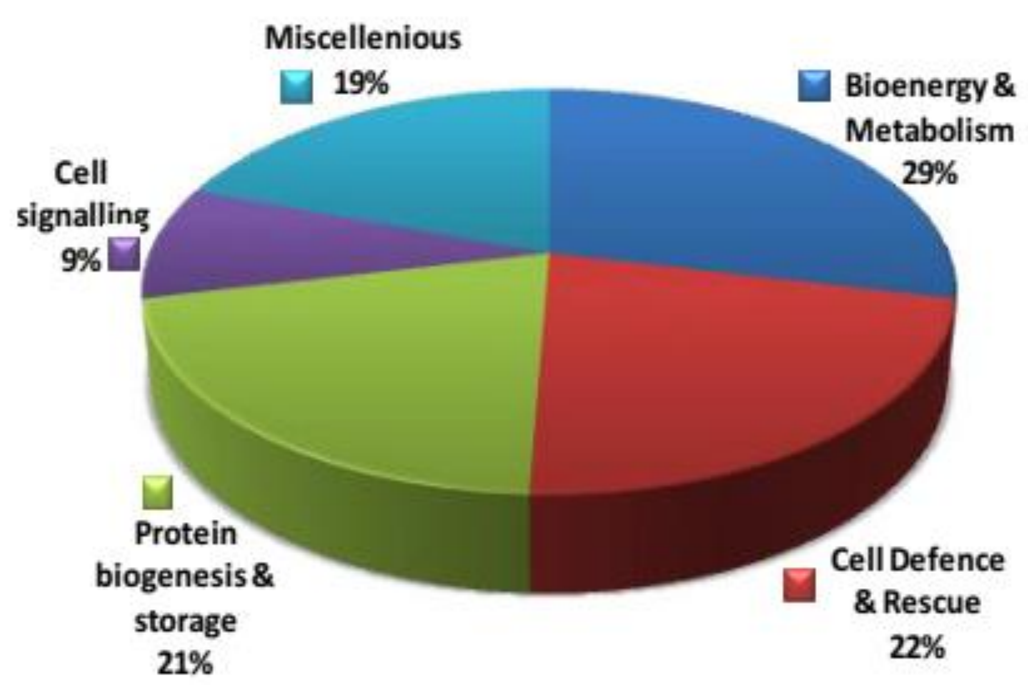

FIGURE 2 | Distribution of 78 identified differentially expressed rice root proteins in five functional classes based on their putative functions assigned them using protein database

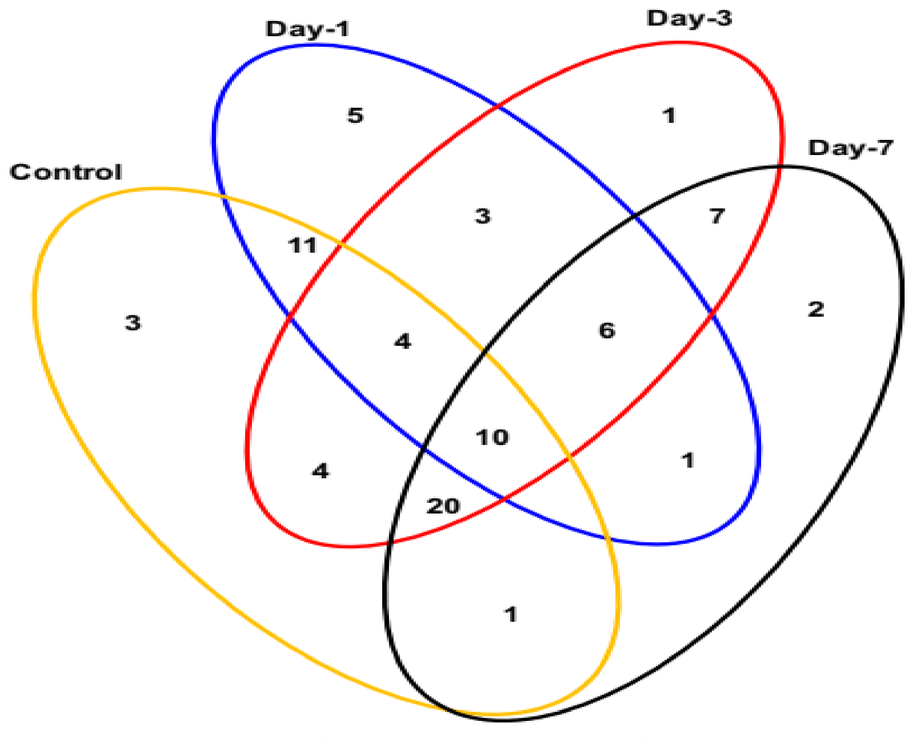

FIGURE 3 | Venn diagram showing the distribution of differentially expressed proteins in time-specific and overlapping manner during drought stress.

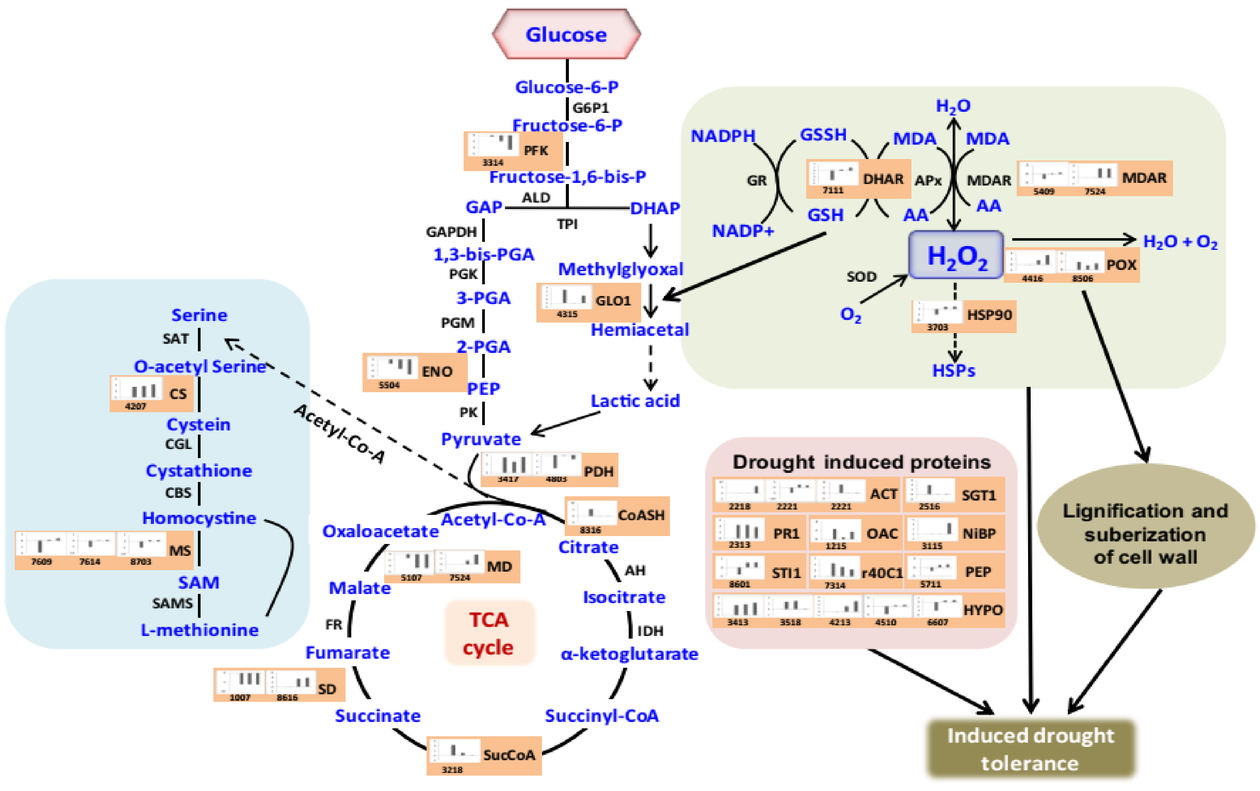

FIGURE 4 | Illustration of role of differentially regulated proteins involved in different pathways in rice for sustaining during drought stress.

Conclusions and Future Perspective

- **The root-specific comparative proteomes of rice identified a number of proteins that are putatively associated with stage specific drought tolerant.**
- **Of the 78 differentially expressed proteins, 10 were found to be differentially regulated in all the four stages during drought stress.**
- **Numerous additional drought-responsive proteins still need to be addressed with technological advancements, which may aid in better understanding of the drought response in rice.**

Effect of PGPR Co-inoculation on the growth of Maize plant (*Zea mays* L.) under drought stress.

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3.20

Introduction

The thin zone of soil lies just under the root system is referred to as the rhizosphere (Dobbelaere et al., 2003). Because of the accumulation of a wide range of plant exudates, such as amino acids and sugars, this part of the soil is dense in nutrients (Gray and Smith, 2005). Endophytic bacteria, rhizospheric bacteria, and phyllospheric bacteria are all types of plant-associated bacteria (PGPR). When it comes to plant pathogens, endophytic bacteria are described as bacteria that colonise the plant's interior tissue without producing apparent outward signs of illness or having a detrimental impact on their host (Schulz and Boyle, 2006). Plant-associated bacteria use a variety of mechanisms to promote plant development, which have been generally classified into two groups: direct and indirect. Direct growth promotion is the most common mechanism (Kloepper et al., 1989). Direct methods that stimulate plant development include: (i) nitrogen fixation by free-living endophytic bacteria, particularly diazotrophs. (ii) the provision of inaccessible nutrients such as phosphorus and other mineral nutrients, (iii) the production of growth and development regulators such as auxins, cytokinins, and gibberelines, and (iv) the breakdown of ethylene harmone in plants via 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. While indirect mechanism include : (i) Antibiotic production and lytic enzymes. (ii) Induced systemic response (ISR). (iii) HCN production (Glick et al., 2007).

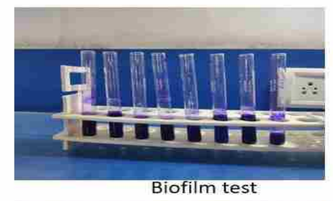
Objective

The objective of this research to isolate the PGPR from rhizosphere soil and evaluate the growth of Maize plant under drought stress and its assessment for proline content.

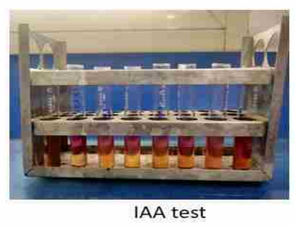
S.No.	Isolates	Ammonia test	HCN production	Phosphate solubilisation	Siderophore Production	IAA	Urease test	Cellulose test	Catalase test	Indole test	Biofilm test
1	PKR 1	+++	-	19mm	21mm		-	12mm	+++	+	+
2	PKR 2	+++	+	16mm	17mm		+	10mm	+	+	+
3	PKR 3	++	++	negative	15mm		+	18mm	++	+	+
4	PKR 4	+	++	14mm	18mm		+	-	+++	+	+
5	PKR 5	++	+	17mm	17mm		+	27mm	+++	+	+
6	PKR 6	++	++	20mm	16mm		+	-	+	+	+
7	PKR 7	+++	-	12mm	13mm		+	16mm	+	+	+
8	PKR 8	+++	++	14mm	17mm		+	-	++	+	+


IAA ring test


Antibiotic test


Biofilm test


Urease test

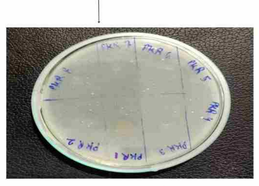

IAA test


Catalase test

Methodology

Isolation of PGPR from rhizospheric soil of maize plant from different region of Madhya Pradesh.

Biochemical characterization and screening test of Bacterial Isolates

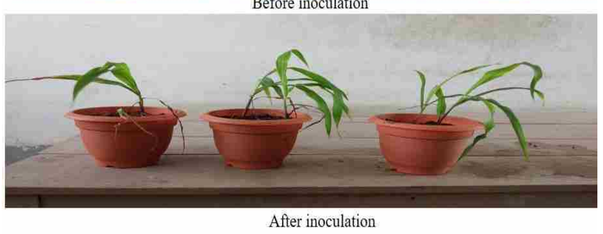

ACC test for bacterial isolates

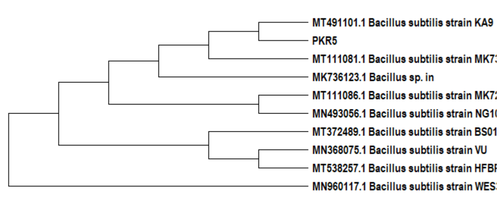
Based on ACC test bacterial isolates were characterized by 16srRNA Sequence analysis

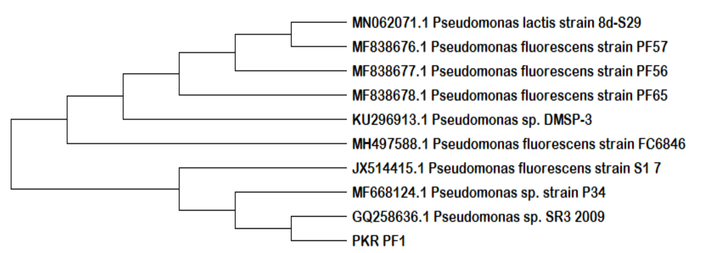

Plant with inoculation


Plant without inoculation


Before inoculation


After inoculation





Experiment no.	Root length (cm)	Shoot length (cm)
1. Normal plant	12	22
2. Plant with PKR1 Inoculation	19	33
3. Plant with PKR1 + PKR5 Inoculation	22	35

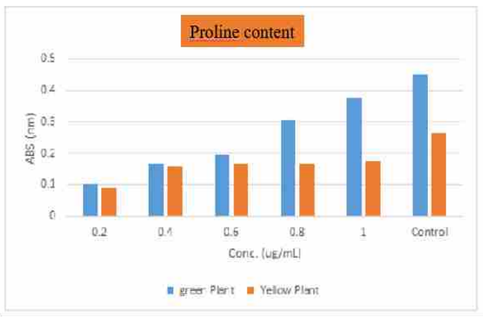
Conclusion:

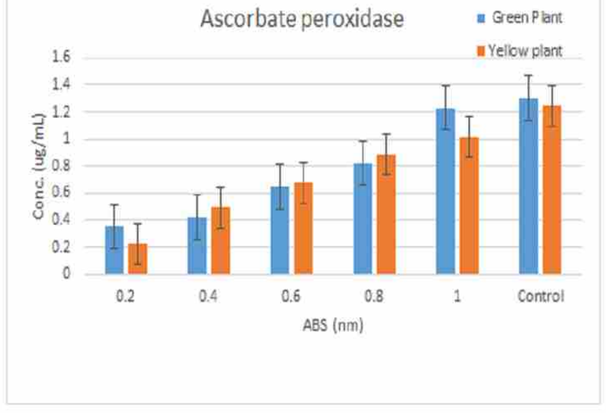
The co-inoculation of bacteria in plant under drought stress condition results in significant rise of proline, Ascorbate peroxidase and chlorophyll content. Also it is helping in elevating drought Stress in plants. Further studies should be conducted at genetic level to know the gene and proteins during before inoculation and after inoculation.

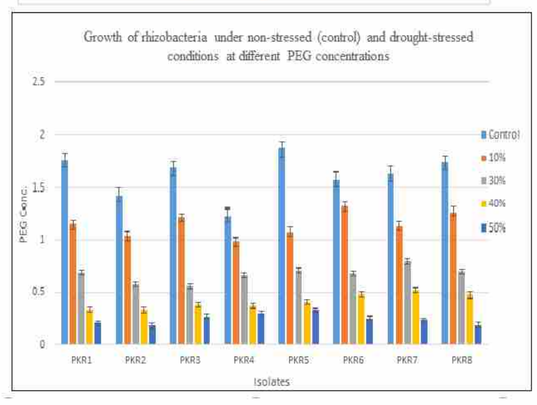
Results

Chlorophyll content

absorbance	645	652	663
green plant	0.167	0.238	0.197
drought plant	0.246	0.196	0.709







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6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment

(3-4, September 2021)

Characterization of Arsenic and Cadmium Resistant Bacterial Diversity from Industrial and Mining Affected Area of Chhattisgarh

Reg. No. 3.21

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INTRODUCTION

Chhattisgarh is one of the major coal producers state in India and the process of coal extraction, mostly opencast mining, and electrical generation by coal-fired power plants release a range of gaseous and solid chemicals and heavy metals like arsenic, cadmium, lead, nickel, manganese, silicon and aluminum into the atmosphere as a by-product of this process.

Goal And Objectives

Aim: These heavy metal resistant bacteria can be exploited for bioremediation of arsenic and cadmium contaminated soil and water because they have to the potential for minimized to toxicity. Keeping in view all the above mentioned points the present study was conducted with the following objectives:

Objectives:

- Collection of soil & water sample from industrial and mining affected areas of Chhattisgarh.
- Isolation, Screening, Biochemical and Molecular characterization of arsenic (As) and cadmium (Cd) tolerance bacteria isolates.
- Bioremediation assay of arsenic (As) and cadmium tolerance bacteria isolates.

METHODS

- In the present study, we isolated 108 arsenic and 58 resistant bacteria (both from soil and water) from 26 samples collected from 20 villages/city different industrial and mining sites of Chhattisgarh to explore the heavy metal bacterial diversity.
- Surface soil samples (0–15 cm) were collected from different industrial and mining areas of Chhattisgarh.

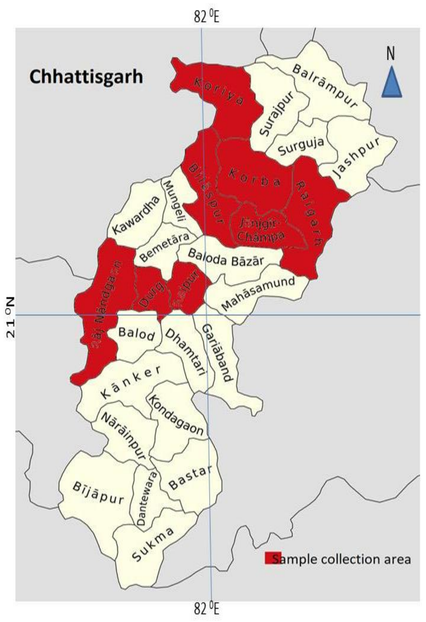


Fig. 1: Pictorial view of sample collection sites in Industrial and Mining affected area Chhattisgarh (India).

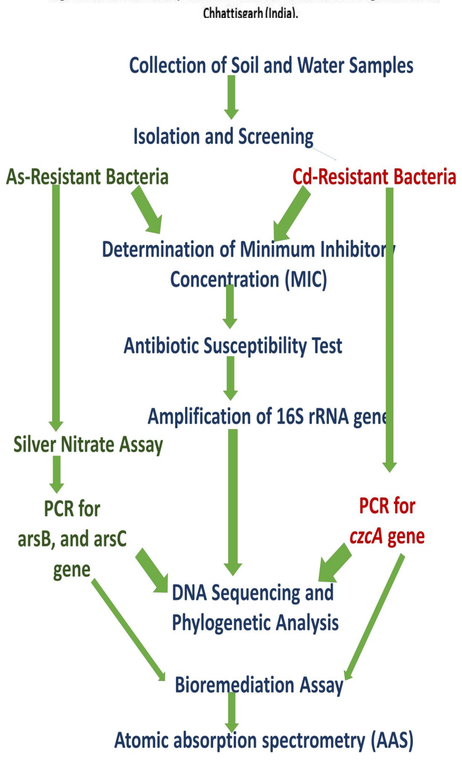
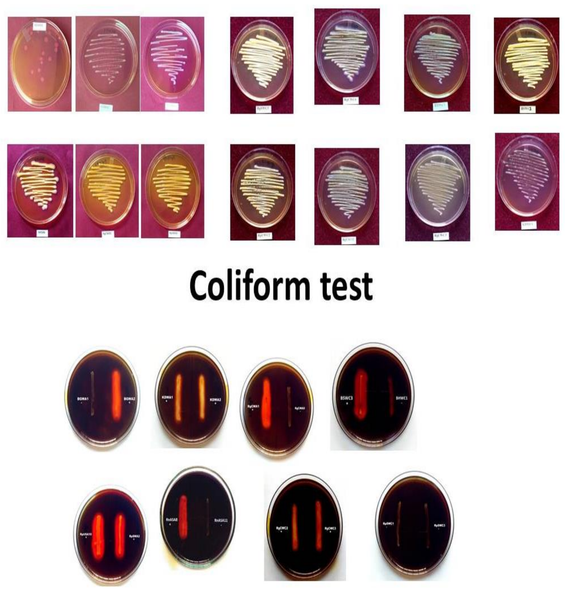
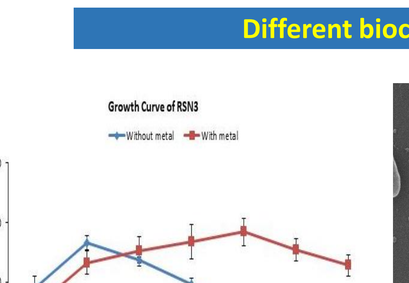


Fig. 2: Overall methodology adopted in the study

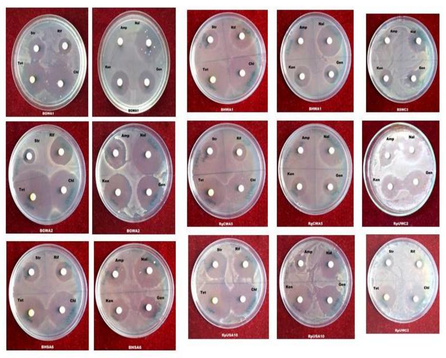
RESULTS & DISCUSSION



Coliform test

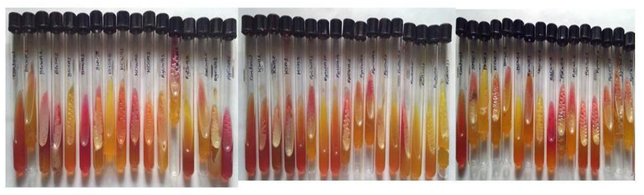


Amylase Production test

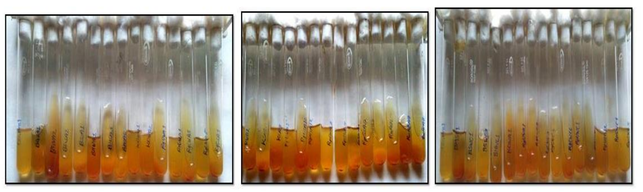


Antibiotic susceptibility test

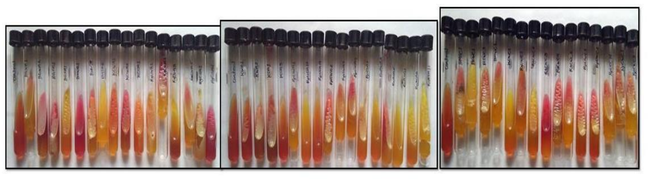
Catalase test



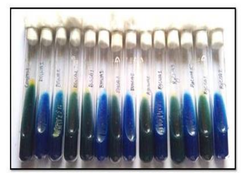
TSI test

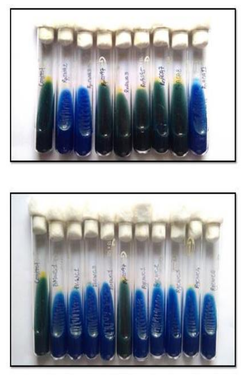


Gelatin Iron Test

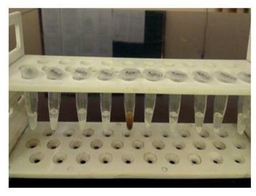


Urease test



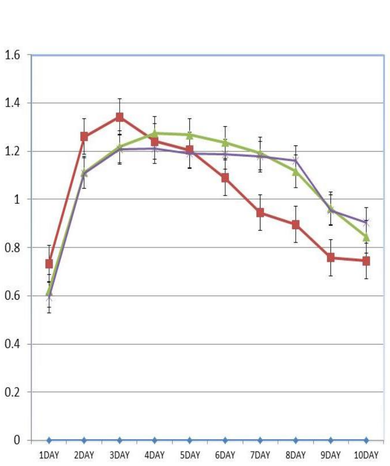


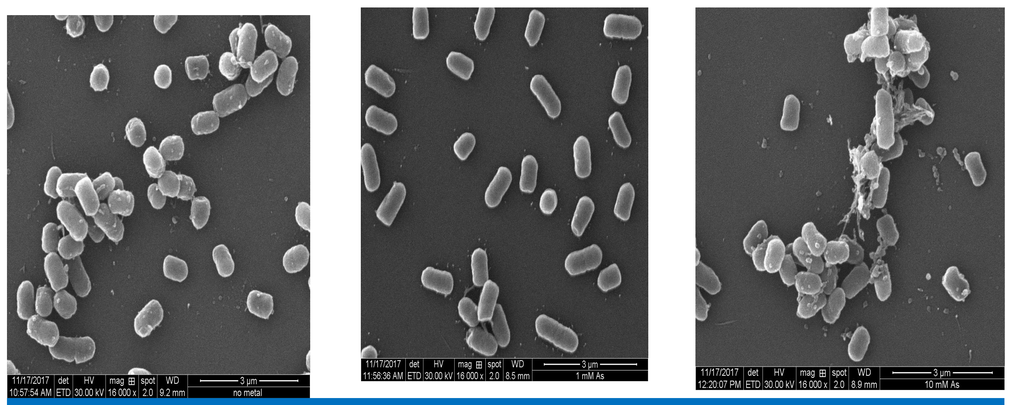
Citrate Utilization



Silver nitrate test

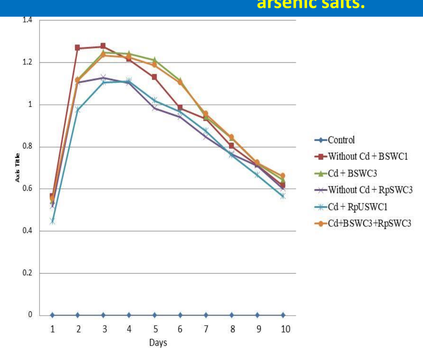
Different biochemical tests performed during this study



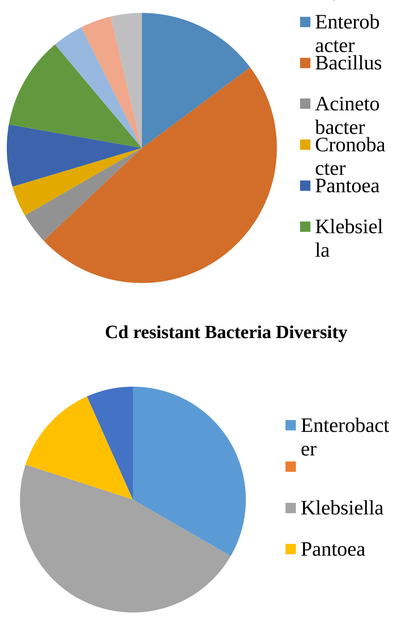


Scanning electron microscopic analysis (at 16,000X) of arsenic impact on RSN3. A) with no arsenic, B) 1mM Na_3AsO_4, C) with 10mM Na_3AsO_4

Growth of different arsenic resistant bacterial isolates in presence and absence of arsenic salts.



Growth of Cd resistant bacterial isolates in presence and absence of cadmium salt.



CONCLUSION

- Total 24 potential isolates out of 108 for their ability to tolerate a high level of arsenic and 15 bacterial isolates were able to grow in presence of 40 mM cadmium chloride
- Atomic absorption spectroscopy (AAS) of the sample obtained from bioremediation assay revealed that *Klebsiella pneumoniae* RnASA11 was able to reduce the arsenic concentration significantly in the presence of arsenate (44 %) and arsenite (38.8%) as compared to control. An absorption of 32.22% arsenic was observed by the *Enterobacter cloacae* RSN3 strain.
- The detoxification efficiency of these two isolates *S. liquefaciens* BSWC3 and *K. pneumoniae* RpSWC3 in combination indicates good potential for application in bioremediation of cadmium from polluted sites. BSWC3, RpSWC3 and Combination were significantly reduce of cadmium concentration i.e. 44.46%, 40% and 50.92%, respectively as compared to control.
- .Furthermore, results of scanning electron microscopy (SEM) for morphological variations revealed an initial increase in the cell size at 1 mM sodium arsenate; however, it was decreased at 10 mM concentration in comparison to control. This uptake and expulsion also confirmed the arsenite efflux system.

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6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment

(3-4, September 2021)

Comparative efficacy of soybean bradyrhizobial strains and AM fungi for moisture stress tolerance in soybean by modulating antioxidant enzymes, osmolytes and improving nodulation, nutrient uptake and plant fitness

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Introduction

- Soybean (*Glycine max* L. Merrill) is emerging as one of the fastest-growing oilseed crops in the world.
- Among the major abiotic stresses, the drought and moisture stress are the most important factors limiting the productivity of soybeans and hence needs attention.
- The potential application of microbes especially, bradyrhizobia and arbuscular mycorrhizal fungi (AMF) can help in nutrient mobilization and confer tolerance to plants by alleviating adverse effects of stresses (Igiehon and babalola 2021).
- There is need to develop moisture stress tolerant compatible soybean rhizobia which can enhance nitrogen fixation, physiological, productivity and plant fitness of soybeans plants under moisture stress conditions.
- In the present study novel strain *B. daqingense* strain has been found to be the superior strain in promoting nodulation, plant fitness and mitigating moisture stress in soybean and hence, can be utilized for large scale field evaluation trials.

Methodology

Experimental Details:

- **Design:** Completely randomized design
- **Treatments:** 12 (6×2): 6 inoculations and 2 stress conditions (stress and normal)-three rhizobia i.e., *B. daqingense* (KX230052), *B. liaoningense* (MTCC-10753) from PK-472, *B. japonicum* (MTCC-10751) from JS 93-05 soybean cultivars; one each AMF and commercial local rhizobial strain and a uninoculated control tested in 6 replications.
- Stress: imposed at R5 stage by withholding water for 10 days
- **Container & Variety:** Black colour gusseted polyethylene bags-12 kg capacity; Cultivar- JS 95-60

Inoculation :

- **Rhizobial inoculation:** Culture with 1 OD growth prepared in 0.85% saline applied @ 1 ml per seed on the surface
- **AMF inoculation:** 120 spores/m² applied by layering method on seed surface in pots during sowing.

Parameters:

-Nodulation stage: Nodule mass, leghaemoglobin, total N in nodules

-At wilting stage: Chlorophyll content, RWC, Proline content and ROS in leaves

At harvest: N & P content in seed and straw.

Statistical Analysis: Data analysis was carried out using one way and two ANOVA analysis using Costat software.

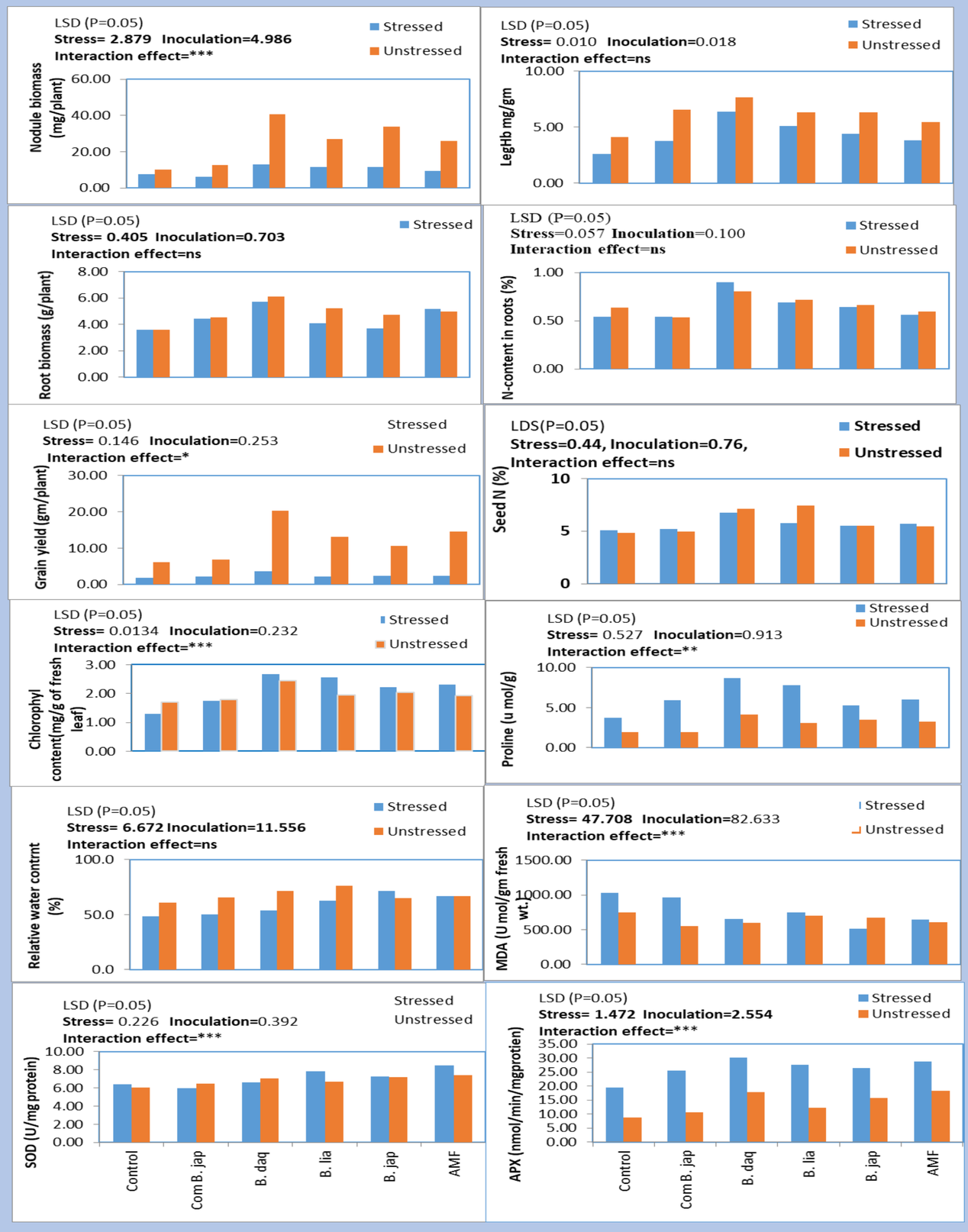

Fig:1 Nodulation, physiological and stress tolerance parameters assessed in soybean inoculated with different bacterial cultures under microcosm conditions. Data are average of 3 replications; LSD, least significance difference at 5% level of significance LSD (P0.05) of ANOVA.

Results and Discussion

- Amongst all, irrespective of stress conditions, inoculation of *B. daqingense* and *B. liaoningense* had higher nodulation (leghemoglobin, nodule biomass), root biomass and N content in root than the rest of inoculants.
- Inoculation of *B. daqingense* and *B. liaoningense* has also increased physiological parameters such as chlorophyll content, proline, relative water content (RWC), lipid peroxidase (MDA), superoxide dismutase (SOD) & Ascorbate peroxidase (APX) and hence *B. daqingense* and *B. liaoningense* found to be the superior *strains* followed by AMF.
- The improved nodulation, growth, physiological parameters and plant fitness due to inoculation of superior strain i.e., *B. daqingense* in soybean signifies its role in moisture stress mitigation and hence can be utilized as potential bio-inoculant for application in soybean after field validation.

Take home message

The novel strain *B. daqingense* has been found to be the most efficacious strain sustaining the nodulation, growth and mitigating the moisture stress in soybean by improving the plant tolerance and fitness against the moisture stress.

Reference

- Igiehon, O.N., Babalola, O.O. *Rhizobium* and Mycorrhizal Fungal Species Improved Soybean Yield Under Drought Stress Conditions. *Curr Microbiol* 78, 1615–1627 (2021).

Acknowledgements

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PGPR for abiotic stress management in Soybean

Code 3.25

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Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most important leguminous plants, contains 40 % protein and 20 % oil and is called as "Gold from soil". (Han et al, 1991). The utmost abiotic stresses are drought, heavy metals, waterlogging, heat, salinity, and suboptimal root zone compaction have vital implications for the adaptability and productivity of crop plants throughout the world. It was reported that yield loss in crops is due to abiotic stresses are more than 50 %. (Boyer 1982). To develop soybean plants with increase tolerance to stress, a basic understanding of the physiological, gene regulatory and biochemical networks is essential. Direct mechanisms involve various things like the production of phytohormones example-indole-3-acetic acid (IAA), ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) degradation, nutrient status of plants are improved, nitrogen fixation, disease-resistance mechanisms. This environmentally-sound, bacterial population is effective in increasing productivity of crop and manage disease under stress conditions. Throughout the world *Bradyrhizobium* is popularly used as an bio-inoculant which is using is slow growing root nodule symbiont, in soybean fields Plant growth-promoting rhizobacteria (PGPR) is one of the best option and emerging technologies used to resolve this problem naturally. The present study aimed to isolate/screen drought tolerant *Bradyrhizobia/PGPR Pseudomonas)* and to characterize their drought tolerant efficiency. Studies reported that stresses in plants can be regulated by the ACC deaminase activity of PGPB.

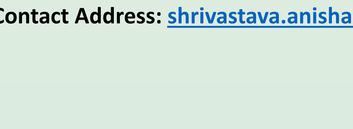
"Isolation and characterization of rhizobacteria for developing inoculants to mitigate abiotic stress in soybean". Was carried out with these objective**:-**

To isolate *Bradyrhizobium* and *Pseudomonas* from nodules/rhizospher soils of various Soybean germplasms.

To select promising *Bradyrhizobium* and *Pseudomonas* isolate(s) to mitigate abiotic stress in soybean.

Table 1.1 Biochemical test- methods and result

Catalase test
➢**Prepare the smear of strain**
➢**on a clean and dry glass slide**
➢**add few drops of 3% H_2O_2 to the slide.**
➢ **Production of gas bubbles (within 5-10 sec)**



& *Pseudomonas*Biochecal test (Catalase test) of *Bradyrhizobium*

urease test
Prepare urea broth
add pH indicator phenol red
Inoculate with loopfull of strain
Incubate for 24-48 hrs at 30ºC

Biochemical test (urease test) of *Bradyrhizobium* & *Pseudomonas*

Amylase test
Streak isolates in Starch Agar Medium and incubated at 30ºC for 24hrs
Iodine (0.1N) solution is flooded to the plates

Biochemical test (Amylase test) of *Bradyrhizobium* & *Pseudomonas flourescence* isolates of Soybean

Oxidase test
on YEM agar plate inoculate isolates incubate foe 24 hrs at 30ºC
add few drops of p-aminodimethylaniline oxalate oberved for the production of color

Biochemical test (Oxidase test) of *Bradyrhizobium* & *Pseudomonas flourescence* isolates of Soybean

S.No.	Name of Soybean Germplasm	Name of Isolates	Biochemical Characterization			
			Catalase test	Urease test	Oxidase test	Amylase test
1.	NRC-138	S-Brh1	+	+	-	++
2.	JS-9752	S-Brh 2	+	+	+	-
3.	SKFPS-11	S-Brh 3	+++	+	+	-
4.	DS3-108	S-Brh 4	++	+	+	-
5.	NRCSL-1	S-Brh 5	++	+	+	-
6.	NRC-136	S-Brh 6	+++	+	+	-
7.	MACS-1493	S-Brh 7	+	+	-	+
8.	RKS-18	S-Brh 8	+++	+	+	-
9.	NRC-130	S-Brh 9	+	+	+	-
10.	RSC-1103	S-Rh 10	+	+	+	++
11.	NRC-147	S-Brh11	++	+	+	-
12.	NRC-137	S-Brh12	+	+	-	-
13.	BAUS-103	S-Brh13	++	+	+	-
14.	PS1611	S-Brh14	+	+	+	++
15.	RSC-1107	S-Brh15	+++	+	+	++

S.No.	Name of Soybean Germplasm	Name of Isolates	Biochemical Characterization			
			Catalase test	Urease test	Oxidase test	Amylase test
1.	NRC-138	S-Brh1	+	+	-	++
2.	JS-9752	S-Brh 2	+	+	+	-
3.	SKFPS-11	S-Brh 3	+++	+	+	-
4.	DS3-108	S-Brh 4	++	+	+	-
5.	NRCSL-1	S-Brh 5	++	+	+	-
6.	NRC-136	S-Brh 6	+++	+	+	-
7.	MACS-1493	S-Brh 7	+	+	-	+
8.	RKS-18	S-Brh 8	+++	+	+	-
9.	NRC-130	S-Brh 9	+	+	+	-
10.	RSC-1103	S-Rh 10	+	+	+	++
11.	NRC-147	S-Brh11	++	+	+	-
12.	NRC-137	S-Brh12	+	+	-	-
13.	BAUS-103	S-Brh13	++	+	+	-
14.	PS1611	S-Brh14	+	+	+	++
15.	RSC-1107	S-Brh15	+++	+	+	++

Table 1.3 Screened isolates

S. No.	Name of Soybean Germplasm	Promising stress tolerant strain	Bacteria
1.	RSC – 1107	S-Ps-1	Pseudomonas
2.	BAUS – 103	S-Ps-2	Pseudomonas
3.	NRC – 137	S-Ps-3	Pseudomonas
4.	RKS – 18	S-Ps-5	Pseudomonas
5.	JS – 9752	S-Brh 2	Bradyrhizobium
6.	SKFPS – 11	S-Brh 3	Bradyrhizobium
7.	NRCSL – 1	S-Brh5	Bradyrhizobium
8.	NRC – 136	S-Brh 6	Bradyrhizobium
9.	RKS – 18	S-Brh 8	Bradyrhizobium

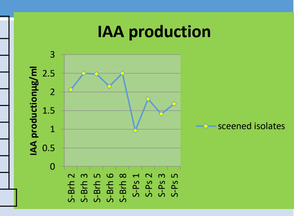
Table 1.4 Testing IAA

Method

➢Grown culture in(YEM) and KING'S B broth medium
➢ Take loopful of fully culture
➢Add in broth supplemented with 500 µg/ml L-tryptophan and without L-tryptophan as control
➢Incubated for 72 hours at 30ºc
➢After incubation Bacterial cells were removed by centrifugation at 4,000 rpm for 20 min at 4ºc supernatant was mixed with Salkowski's reagent in ratio1:2 in *Bradyrhizobium*
➢And 2 drops ortho phosphoric acid in *Pseudomonas*
➢Incubated at room temperature for 30 min

result

S. No.	Name of Soybean Germplasm	Name of Isolates	IAA production µg/ml
1	RSC - 1107	S-Ps 1	0.96
2	BAUS - 103	S-Ps 2	1.81
3	NRC - 137	S-Ps 3	1.41
4	RKS - 18	S-Ps 5	1.68
5	JS - 9752	S-Brh 2	2.06
6	SKFPS - 11	S-Brh 3	2.50
7	NRCSL - 1	S-Brh 5	2.48
8	NRC - 136	S-Brh 6	2.15
9	RKS - 18	S-Brh 8	2.50
CD 5%			0.23



Testing for IAA production by stress tolerant screened *Bradyrhizobium* and *Pseudomonas* isolates of Soybean

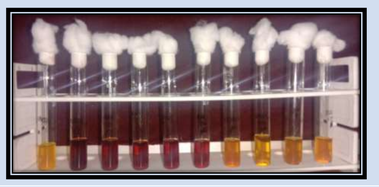


Table 1.2 Screening of isolates on basis of Stress (temperature, pH and Salt) tolerant tests

Methodology

pH
➢**Tested at different 4.0, 4.5, 5.0, 5.5, and 6.5 pH in YEMA and KING'S B medium by adjusting the pH.**
➢ **All the plates were incubated at 30°C for 72 hours**

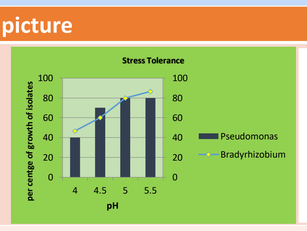
Temperature
inoculated in 5ml of YEM broth and KING'S B broth tube and incubated in rotatary shaker incubater at 35°C, 45°C, 50°C and 55°C for 72 hours 2000rpm.

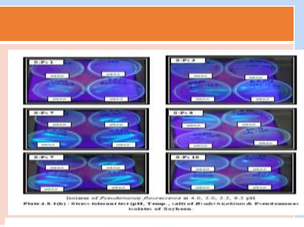
Salt
Grow in different concentration of salt was tested by streaking them on YEMA and KING'S B medium containing 2.0%, 4.0%, and 6.0% (wt/v) NaCl.

Result

S.No.	Name of Soybean Germplasm	Name of Isolates	Stress Tolerance (pH)				
			4	4.5	5	5.5	6.5
1.	NRC-138	S-Brh1	+	+	+	+	+
2.	JS-9752	S-Brh 2	+	+	+	+	+
3.	SKFPS-11	S-Brh 3	-	+	+	+	+
4.	DS3-108	S-Brh 4	-	+	+	+	+
5.	NRCSL-1	S-Brh 5	-	+	+	+	+
6.	NRC-136	S-Brh 6	-	+	+	+	+
7.	MACS-1493	S-Brh 7	-	+	+	+	+
8.	RKS-18	S-Brh 8	-	+	+	+	+
9.	NRC-130	S-Brh 9	+	+	+	+	+
10.	RSC-1103	S-Brh 10	-	-	-	-	+
11.	NRC-147	S-Brh11	-	-	+	+	+
12.	NRC-137	S-Brh12	+	+	+	+	+
13.	BAUS-103	S-Brh13	+	+	+	+	+
14.	PS1611	S-Brh14	-	-	-	-	+
15.	RSC-1107	S-Brh15	+	+	+	+	+

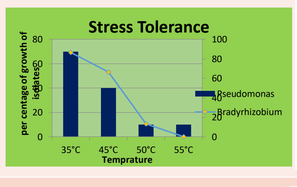
S.No.	Name of Soybean Germplasm	Name of Isolates	Stress Tolerance (PH)				
			4	4.5	5	5.5	6.5
1.	RSC-1107	S-Ps 1	-	-	+	+	+
2.	BAUS-103	S-Ps 2	+	+	+	+	+
3.	NRC-132	S-Ps 3	-	-	-	-	+
4.	MACS-1493	S-Ps 4	+	+	+	+	+
5.	RKS-18	S-Ps 5	+	+	+	+	+
6.	RSC-1103	S-Ps 6	-	+	+	+	+
7.	NRC-147	S-Ps 7	-	+	+	+	+
8.	NRC-137	S-Ps 8	+	+	+	+	+
9.	PS1611	S-Ps 9	-	+	+	+	+
10.	PS1613	S-Ps 10	-	+	+	+	+

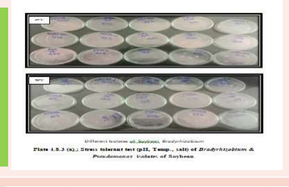


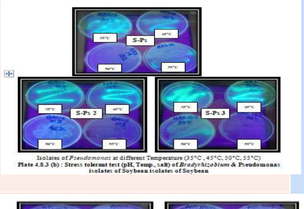


S.No.	Name of Soybean Germplasm	Name of Isolates	Stress Tolerance (temperature in °C)			
			35	45	50	55
1.	NRC-138	S-Brh1	++	+	-	-
2.	JS-9752	S-Brh 2	+	+	-	-
3.	SKFPS-11	S-Brh 3	++	++	+	-
4.	DS3-108	S-Brh 4	+	+	-	-
5.	NRCSL-1	S-Brh 5	++	++	+	-
6.	NRC-136	S-Brh 6	+	+	-	-
7.	MACS-1493	S-Brh 7	+	-	-	-
8.	RKS-18	S-Brh 8	++	++	+	-
9.	NRC-130	S-Brh 9	++	+	-	-
10.	RSC-1103	S-Brh 10	+	-	-	-
11.	NRC-147	S-Brh11	-	-	-	-
12.	NRC-137	S-Brh12	+	-	-	-
13.	BAUS-103	S-Brh13	++	+	+	-
14.	PS1611	S-Brh14	+	-	-	-
15.	RSC-1107	S-Brh15	+	-	-	-

S.No.	Name of Soybean Germplasm	Name of Isolates	Stress Tolerance (temperature in °C)			
			35	45	50	55
1.	RSC-1107	S-Ps 1	+	+	-	-
2.	BAUS-103	S-Ps 2	++	+	-	-
3.	NRC-132	S-Ps 3	++	+	-	-
4.	MACS-1493	S-Ps 4	+	-	-	-
5.	RKS-18	S-Ps 5	++	++	+	+
6.	RSC-1103	S-Ps 6	-	-	-	-
7.	NRC-147	S-Ps 7	+	-	-	-
8.	NRC-137	S-Ps 8	+	-	-	-
9.	PS1611	S-Ps 9	+	-	-	-
10.	PS1613	S-Ps 10	-	-	-	-

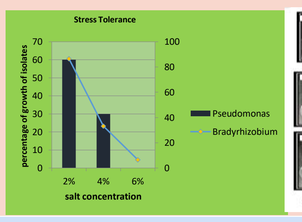


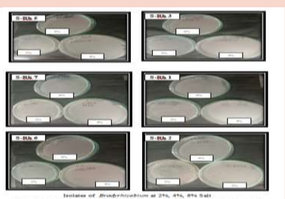




S.No.	Name of Soybean Germplasm	Name of Isolates	Stress Tolerance (salt)		
			2%	4%	6%
1.	NRC-138	S-Brh1	+	-	-
2.	JS-9752	S-Brh 2	+	-	-
3.	SKFPS-11	S-Brh 3	+	+	-
4.	DS3-108	S-Brh 4	+	-	-
5.	NRCSL-1	S-Brh 5	+	+	-
6.	NRC-136	S-Brh 6	+	+	-
7.	MACS-1493	S-Brh 7	+	-	-
8.	RKS-18	S-Brh 8	+	+	+
9.	NRC-130	S-Brh 9	+	-	-
10.	RSC-1103	S-Rh 10	+	-	-
11.	NRC-147	S-Brh11	+	+	-
12.	NRC-137	S-Brh12	+	-	-
13.	BAUS-103	S-Brh13	+	-	-
14.	PS1611	S-Brh14	-	-	-
15.	RSC-1107	S-Brh15	+	-	-

S.No.	Name of Soybean Germplasm	Name of Isolates	Stress Tolerance (salt)		
			2%	4%	6%
1.	NRC-138	S-Brh1	+	-	-
2.	JS-9752	S-Brh 2	+	-	-
3.	SKFPS-11	S-Brh 3	+	+	-
4.	DS3-108	S-Brh 4	+	-	-
5.	NRCSL-1	S-Brh 5	+	+	-
6.	NRC-136	S-Brh 6	+	+	-
7.	MACS-1493	S-Brh 7	+	-	-
8.	RKS-18	S-Brh 8	+	+	+
9.	NRC-130	S-Brh 9	+	-	-
10.	RSC-1103	S-Rh 10	+	-	-
11.	NRC-147	S-Brh11	+	+	-
12.	NRC-137	S-Brh12	+	-	-
13.	BAUS-103	S-Brh13	+	-	-
14.	PS1611	S-Brh14	-	+	-
15.	RSC-1107	S-Brh15	+	+	+





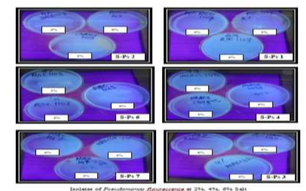


Table 1.5 PEG tolerance

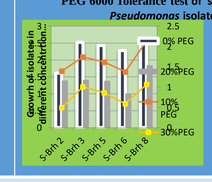
➢ **Loopful culture of each isolates were inoculated in 5ml YEM broth and KING'S B broth containing different level viz., 0%, 10%, 20%, and 30% of (PEG) 6000.**

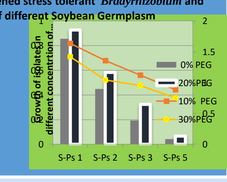
➢**incubated in rotator shaker incubator 120rpm for 72 hours at 30ºC.**

➢**After incubation bacterial growth was measured spectrophotometrically at OD 420 nm.**

S. No.	Name of Soybean Germplasm	Name of Isolates	PEG (6000) Tolerance Test			
			0%	10%	20%	30%
1	RSC - 1107	S-Ps 1	0.856	0.449	0.193	0.042
2	BAUS - 103	S-Ps 2	1.637	1.355	1.120	0.884
3	NRC - 137	S-Ps 3	0.926	0.584	0.323	0.067
4	RKS - 18	S-Ps 5	1.420	1.041	0.951	0.752
5	JS - 9752	S-Brh 2	1.616	1.397	1.271	0.494
6	SKFPS - 11	S-Brh 3	2.520	1.74	1.42	1.01
7	NRCSL - 1	S-Brh 5	2.43	1.616	1.39	0.856
8	NRC - 136	S-Brh 6	2.28	1.38	1.007	0.592
9	RKS - 18 P	S-Brh 8	2.65	2.14	1.85	1.07
CD at 5%			1.42	1.03	0.86	0.54

PEG 6000 Tolerance test of screened stress tolerant *Bradyrhizobium* and *Pseudomonas* isolates of different Soybean Germplasm





Result

➢In biochemical test, all the *Bradyrhizobium* and *Pseudomonas fluorescence* isolates showed positive result for the urease test and catalase test. Regarding Oxidase test, out of 15 *Bradyrhizobium* isolates, 3 isolates showed negative result whereas out of 10 isolates *Pseudomonas fluorescence* all except S-Ps 1, showed the positive results.
➢In Amylase test, out of 15 isolates *Bradyrhizobium* showed positive result only for 5 isolates (S-Brh14, S-Brh15, S-Rh 10, S-Brh 7, S-Brh1) and out of 10 *Pseudomonas fluorescence* isolates, 6 isolates (S-Ps 10, S-Ps 3, S-Ps 4, S-Ps 5, S-Ps9, S-Ps 2) showed positive result.
➢Efficacy of *Bradyrhizobium* and *Pseudomonas fluorescence* isolates against stress is carried out for acidity, temperature and salt tolerance tests full details r there in table
➢ On the basis of stress tolerant efficiencies of all rhizobacteria isolated tested as above, it was observed that a total of 9 isolates (i.e., S-Brh 3, S-Brh 2, S-Brh 8, S-Brh5, S-Brh 3 isolates of *Bradyrhizobium* and S-Ps-2, S-Ps-3, S-Ps-5, S-Ps-1isolates of *Pseudomonas*) were tolerant to pH, temperature and salt.
➢All S-Ps 2, S-Brh 2, S-Brh 8, S-Brh 5, S-Brh 6 stress tolerant isolates showed positive result in Indole acetic acid (IAA) production µg/ml . It was observed that isolate, S-Brh 8 and S-Brh 3 produced significantly maximum Indole acetic acid (IAA) followed by S-Brh 5, S-Brh 2, S-Brh 6, S-Ps 2.
➢The effect of drought towards the growth of all the 9 isolates were studied using YEM and KING'S B broth supplemented with PEG. Isolates of *Pseudomons* S-Pse 5, S-Pse 1 and Isolates of *Bradyrhizobium*, S-BRh 2, S-BRh 3 gave the best result in 20% PEG(6000) at OD 570 nm. This study aimed to screen drought tolerant isolates to enhance the Soybean productivity under drought conditions.

Acknowledgement

Keeping in view of work done related to isolation and characterization of Soybean rhizobacteria, the best native stress tolerant isolates of *Bradyrhizobium* and *Pseudomonas fluorescence* are S-BRh 8, S-Rh 3, S-Pse 5. However, the result is indicative and required further experimentation to arrive at a more consistent result. Further, these isolates are to be tested in field condition to assess whether inoculation effects enhance the Soybean yield under water stress.

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6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment
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DROUGHT STRESS ALLEVIATION BY ACC DEAMINASE PRODUCING RHIZOBACTERIA ISOLATED FROM SOYBEAN RHIZOSPHERE

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1 Introduction

Plants are constantly subjected towards a wide range of climatic perturbations which leads to different types of abiotic stress conditions. Among all these modulators, drought is considered as a major abiotic factor that limits crop yield and productivity [1]. However, these strategies have few limitations in terms of time requirement and ethical issue [2]. To embark on this, application of drought-tolerant bioinoculants or plant growth promoting rhizobacteria (PGPR) is an alternative strategy for sustainable agriculture under drought [3].

The aim of the present study was designed to:

1. Isolate, screening and identify ACC Deaminase producing bacterial strains with multifarious Plant growth Promoting (PGP) traits from soybean rhizosphere.
2. Testing drought tolerance potential of these bacterial isolates by growing them in different concentration of PEG 6000 (5-25%).
3. Assessing plant growth promotion in soybean by bio-priming soybean seeds with PGPR isolates under normal and drought stress conditions.

2 Methods

Collection of samples: Rhizospheric soil samples of disease resistant variety of soybean (JS-20-34) were collected from JNKVV, Bhopal Road, and Sagar (M.P.), India (23° 49°44” N latitude and 78° 42°46” E longitude)

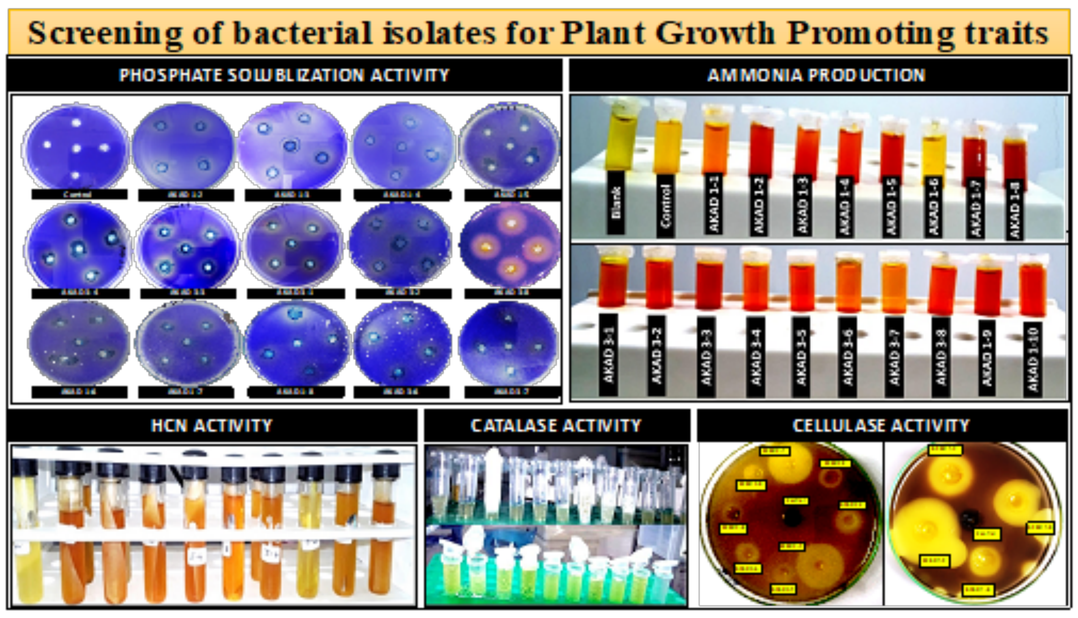
Isolation, screening and drought stress tolerance of bacterial isolates: Rhizobacterial strains were screened for ACCD, PSB, Auxin, ammonia, HCN, catalase and cellulase production, and biofilm formation. Drought tolerance potential of these bacterial isolates were tested by growing them in different concentration of PEG 6000 (5-25%).

Identification of rhizobacteria strains: All the bacterial isolates were identified morphologically, biochemically and molecularly up to species level by 16S rRNA gene sequencing and submitted to GenBank for accession number.

Effect of selected isolates on plant growth promotion: Soybean seedlings growing under drought and well watered condition, were harvested and analyzed for different morphological parameters like shoot/root length, fresh and dry weight. Biochemical analysis of the soybean seedlings was done by using standard protocol [4-7].

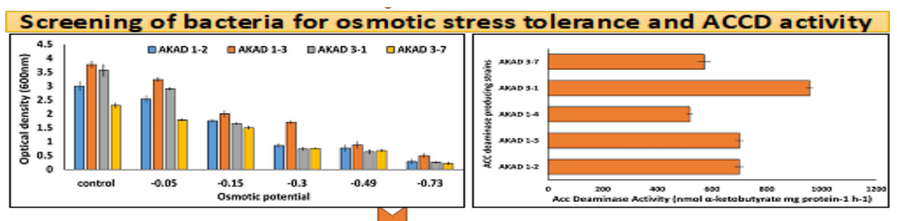
SEM analysis: The surface colonisation by PGPR strains was examined with scanning electron microscopy (SEM) of root tissue. After harvesting, root samples were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7) for 24 h at 4 °C [5].

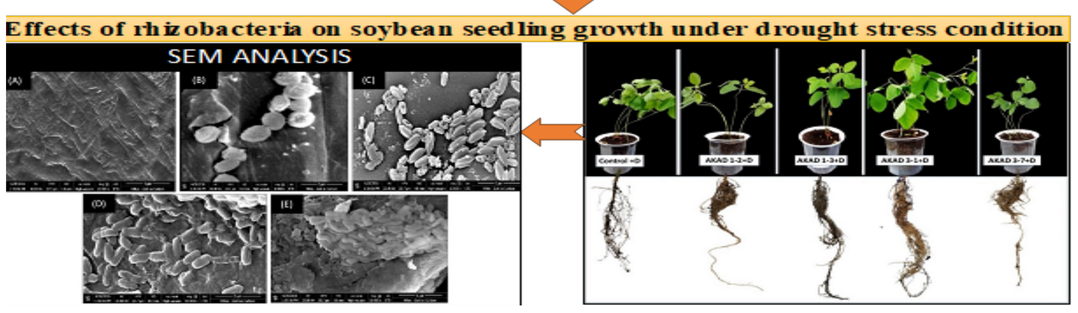
3 Results

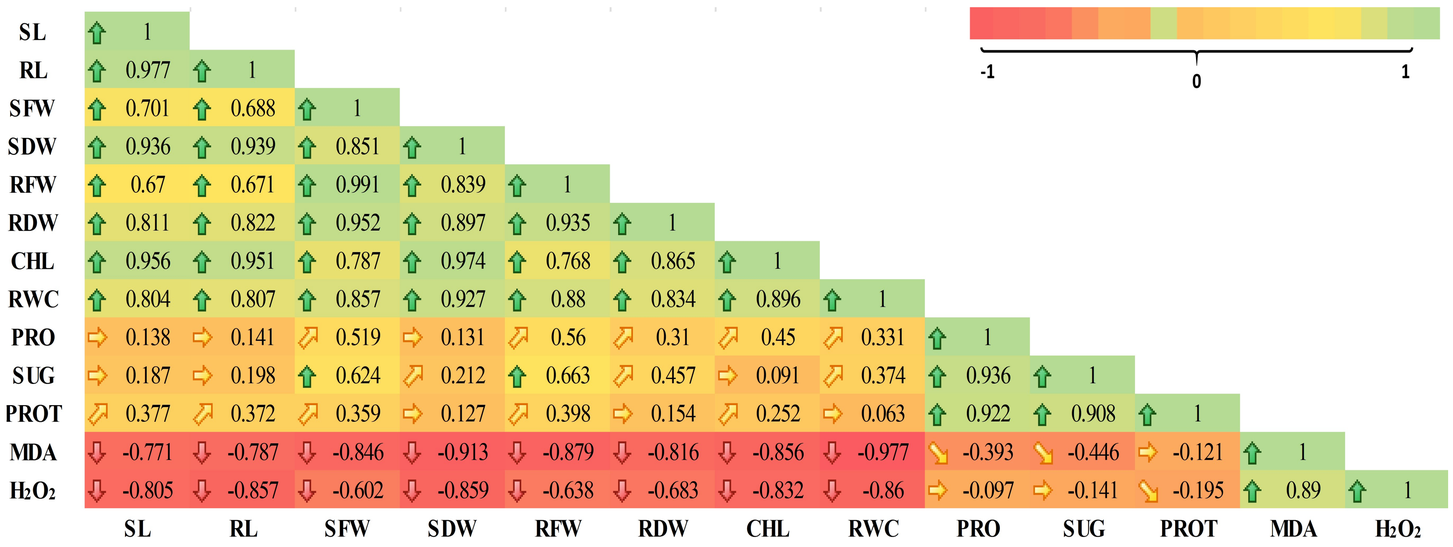


SN.	Morphological parameter							Biochemical Parameter					Oxidative stress	
Treat	SL (cm)	RL (cm)	SFW (g)	SDW (g)	RFW (g)	RDW (g)	No of nodules	RWC (%)	Chlor (mg/g Fwt)	Proline (mg.g^{-1}.Fwt)	TSS (mg.g^{-1}.Fwt)	Protein (µg.g^{-1}.Fwt)	MDA (nmol.g^{-1}.Fwt)	H_2O_2 (mM. g^{1}.Fwt)
T1	30.41b	23.23b	2.58f	0.57d	1.87f	0.22c	12.81b	85.3de	2.19c	16.4a	1.40a	116.6a	19.41a	3.7g
T2	40.48e	35.06f	3.23h	0.78h	2.35h	0.57g	21.1gh	87.63f	2.99f	27.4c	1.54c	127.7b	13.9c	2.71d
T3	46.5h	38.41gh	3.45i	0.83i	2.48i	0.64h	22.48h	90.6g	3.16g	25.12b	1.58b	135.2d	11.53b	2.34b
T4	49.06i	40.2i	3.74j	0.95j	2.56j	0.78i	25.83j	93.2h	3.95h	28.1c	1.76c	149.7f	9.4a	2.16a
T6	35.52d	32.5e	2.85g	0.68f	2.14g	0.53f	20.3f	87.3ef	2.68e	23.18b	1.43a	125.8b	14.16c	2.4c
T7	23.5a	17.89a	1.52a	0.26a	0.897a	0.09a	5.8a	57.2a	1.7a	31.81d	2.2d	132.3d	62.10b	6.21h
T8	36.23d	30.25d	1.82c	0.55c	1.223c	0.23c	16.6d	77.3c	2.3d	37.52e	2.64f	158.0g	32.79d	3.09f
T9	41.85f	34.23f	1.94d	0.63e	1.33d	0.28d	18.25e	78.1c	2.6e	47.86f	2.79g	180.2h	27.2f	2.8e
T10	44.95g	37.02g	2.21e	0.76g	1.48e	0.35e	24.6i	85.16d	3.20g	53.79g	3.85h	195.8i	24.2e	2.6d
T11	31.52c	27.65c	1.67b	0.54b	1.08b	0.123b	14.85c	71.6b	1.85b	36.43e	2.5e	147.5e	34.08h	3.2f

Note: Values represent the mean of three replicates ± SD (n=6). Letters represent significant differences between treatments within a column according to Duncan’s multiple range test (p< 0.05)







4 Conclusions

- From this study we concluded that ACC deaminase producing rhizobacterial strains *Pantoea agglomerans* strain AKAD 1-2, *Bacillus subtilis* strain AKAD 1-3, *Bacillus cereus* strain AKAD 3-1, *Bacillus licheniformis* strain AKAD 3-7 can cope up with the adverse effect of drought stress by producing of osmolytes like proline, proteins, and sugars.
- Intensive root colonization potential of these rhizobacterial isolates were further visualized by using SEM which shows biofilm forming potential of these isolates under drought stress condition.

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Screening and characterization of plant growth promoting endophytes associated with the roots of wheat plant

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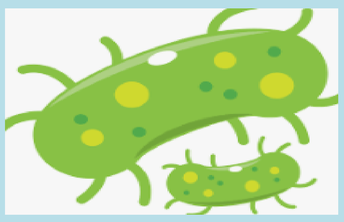
Introduction

- Wheat is India's prime staple food crop for human and one of significant grown cereal crop in the world in terms of both production as well as consumption (Rosenblueth *et al.*, 2018).
- Salinity is a major problem affecting crop production all over the world that inhibits plant development by inducing water stress, specific ion effects, and nutrient imbalance resulting in lessened crop growth and ultimately the harvestable yield.
- It is estimated that about 1 billion hectares i.e. 6.5% of the total global land mass is under salinity stress (FAO 2015; Singh 2018). In India alone, 10 million hectares land is affected by this stress (Sharma and Chaudhari 2012; Tewari and Arora, 2016).
- An endophyte is an endosymbiont, often a bacterium or fungus, that lives within a plant.
- Endophytic bacteria are the plant beneficial bacteria that thrive inside plants and can improve plant growth under normal and challenging conditions. They benefit host plants directly by improving plant nutrient uptake and by modulating growth and stress related phytohormones. Indirectly, bacterial endophytes can improve plant health by targeting pests and pathogens with antibiotics, hydrolytic enzymes, nutrition limitation, and by priming plant defenses.



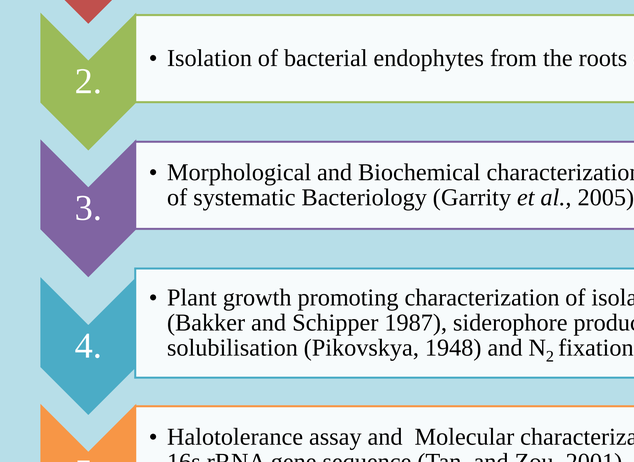
Objectives

1. Isolation of bacterial endophytes from roots of wheat plant.
2. Identification of endophytes and their morphological, biochemical, and PGP characterization
3. In vitro studies to assess the impact of screened endophytes on the growth of host plant

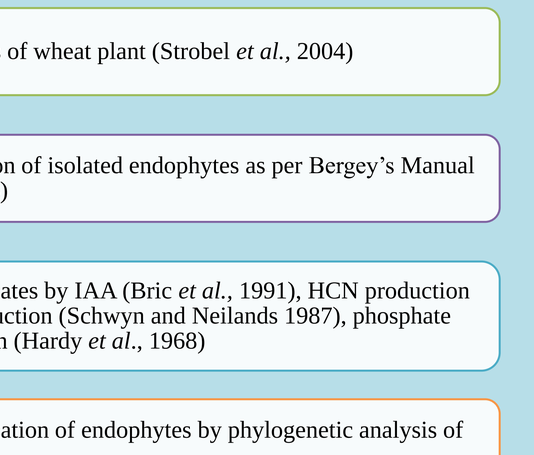
Work Plan

1. Collections of wheat plants from saline soils from Northern India
2. Isolation of bacterial endophytes from the roots of wheat plant (Strobel *et al.*, 2004)
3. Morphological and Biochemical characterization of isolated endophytes as per Bergey's Manual of systematic Bacteriology (Garrity *et al.*, 2005)
4. Plant growth promoting characterization of isolates by IAA (Bric *et al.*, 1991), HCN production (Bakker and Schipper 1987), siderophore production (Schwyn and Neilands 1987), phosphate solubilisation (Pikovskya, 1948) and N_2 fixation (Hardy *et al*., 1968)
5. Halotolerance assay and Molecular characterization of endophytes by phylogenetic analysis of 16s rRNA gene sequence (Tan and Zou, 2001)
6. Pot trials to check the impact of endophytes for their respective host plant

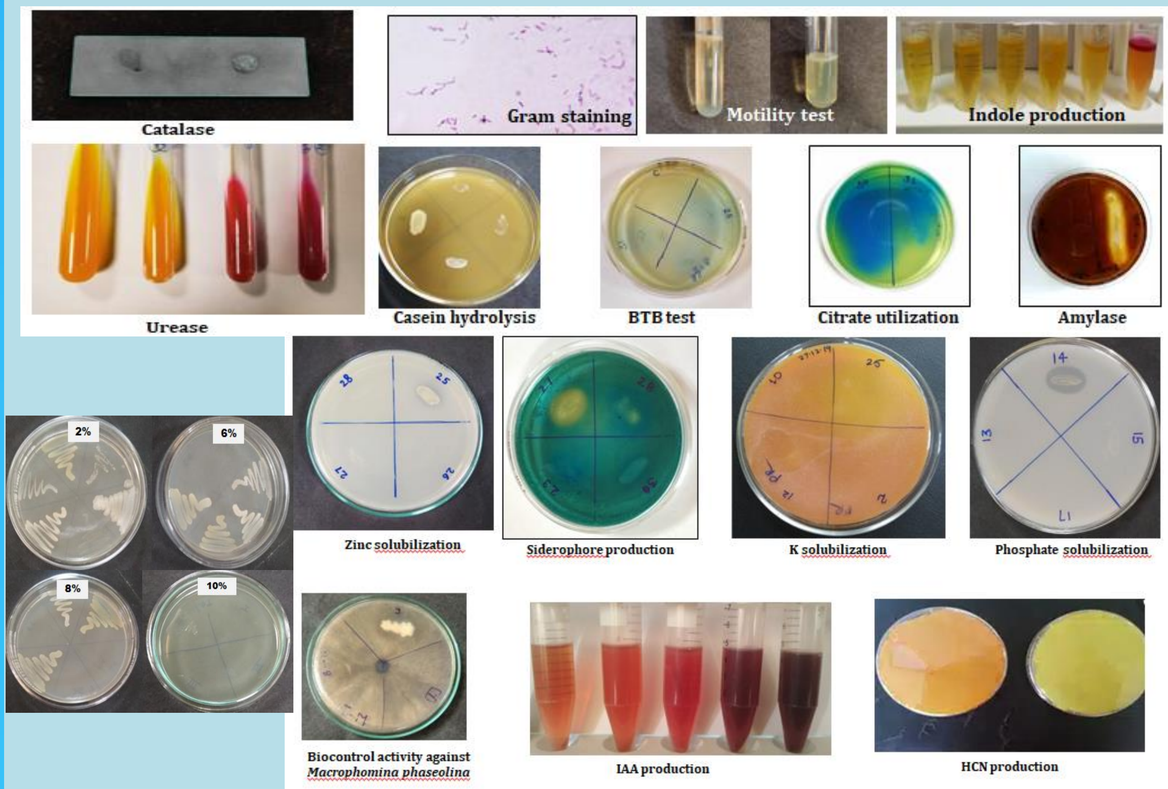
Results

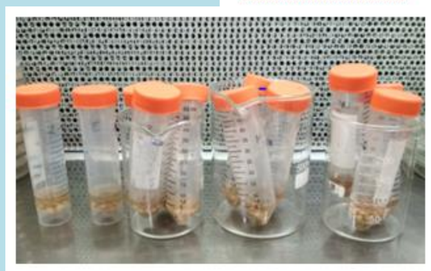


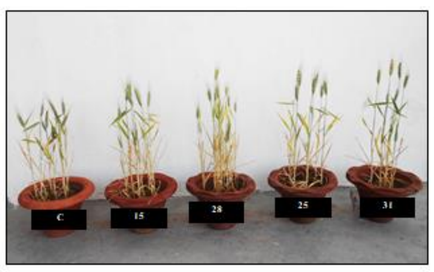
Isolated bacterial cultures



Results







(Pot-study)

Conclusion

- In the study, salt-tolerant nitrogen fixing endophytes were isolated from wheat roots. Amongst all the forty two isolates, only four isolates were selected on the basis of their plant growth promoting properties and salt tolerance.
- Results of the work showed that most of the selected isolates were positive for salt tolerance, however data of pot study illustrates that isolate PD25 was found to be the most potent bacterial endophyte to show potential effects as plant growth promoter.
- Thereby it can be concluded that application of endophytes in form of bioformulations can be a potential development strategy in boosting crop productivity in saline soils in the future.

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Special thanks to my guide Prof. Naveen Kumar Arora for supporting me throughout the study.

Characterization of exopolysaccharides extracted from salt-tolerant *Pseudomonas entomophila* PE3 and its role in promoting growth and yield of sunflower under salinity stress

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Introduction

- Salinity has been a hassling issue in agro-ecosystem and about 1 billion hectares of lands are degraded due to salinization including almost 100 countries and even Polar regions (Arora et al. 2020)
- Salinity effects physiology, biochemical, molecular and developmental processes of plants
- Application of salt-tolerant plant growth promoting rhizobacteria (ST-PGPR) and their associated metabolites can be a sustainable solution to enhance the growth and salt-resilience in plants (Fatima and Arora 2021)

Methods

(a) Isolated and selected potent ST-PGPR (PE3) from saline soil on the basis of salt-tolerance assay

(b) Identified PE3 through 16S rRNA sequencing and MALDI-TOF MS biotyping

(c) Checked PGP and salt-tolerance traits of PE3 at various salt levels (2-8%) (Fatima et al. 2020)

(d) Extracted and purified EPS and checked its composition and salt-tolerance traits (Mody et al. (1989)

(e) FTIR analysis of EPS was conducted to detect the functional groups

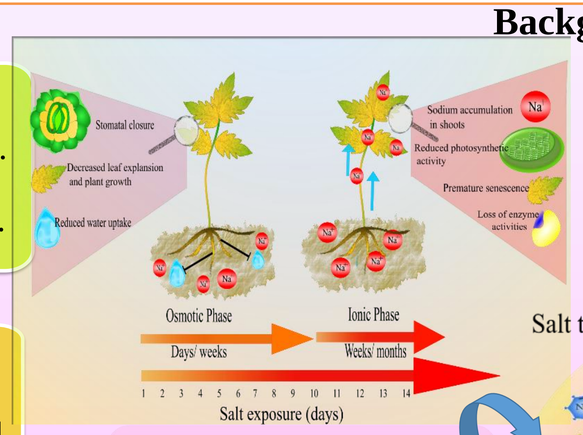
(f) Changes in EPS composition and properties at different salt-levels were checked

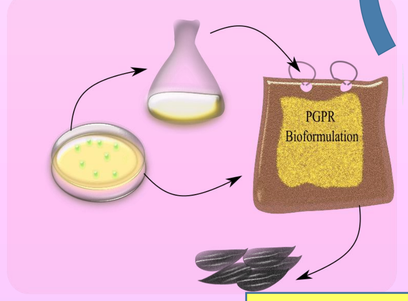
(g) Bioformulation using PE3 and different EPS conc. were developed (Nandakumar et al. 2001)

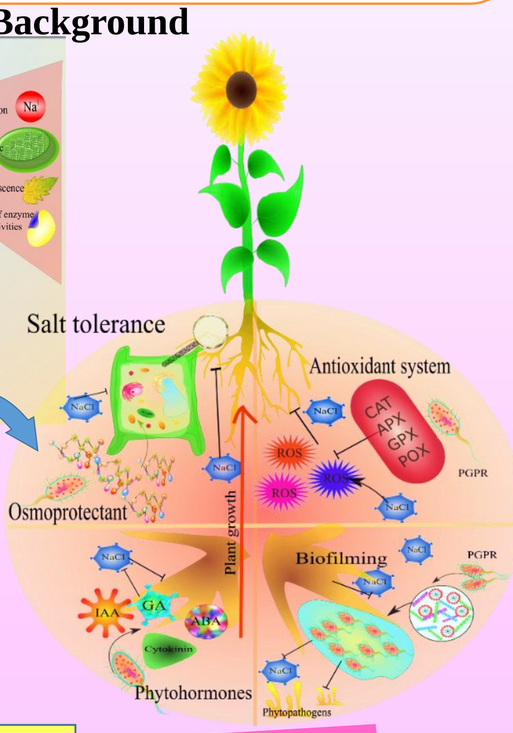
(h) Sunflower seeds were primed and sowed in saline field

(i) Effect of treatments on plant growth and salt-tolerance traits were checked and statistically analyzed

Background

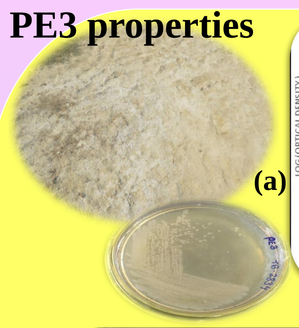




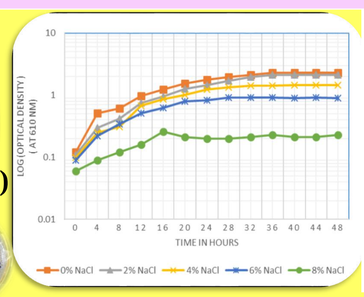


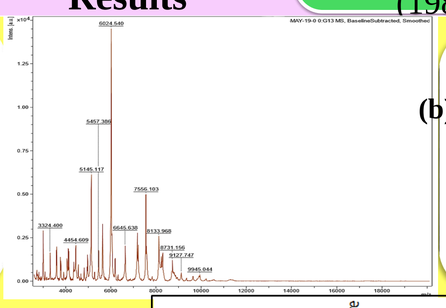
Results

PE3 properties



(a)





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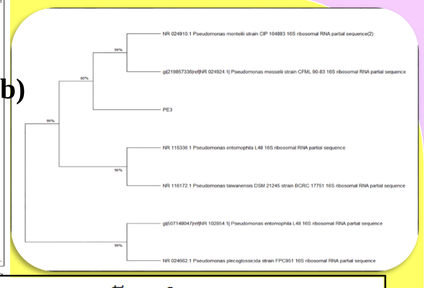
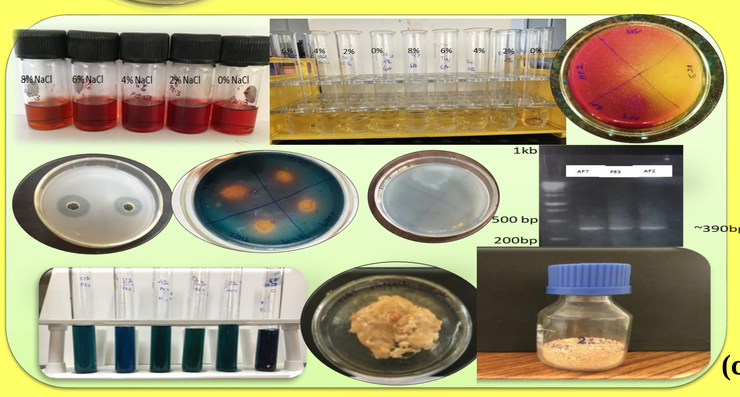
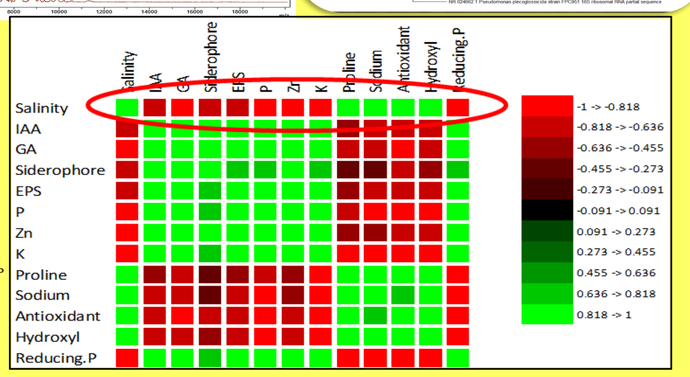
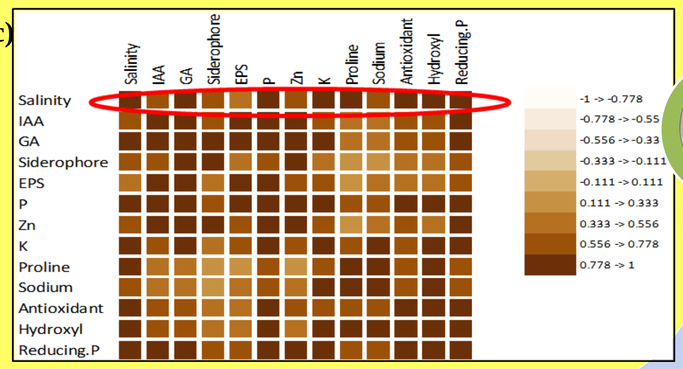


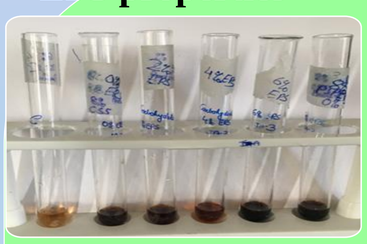


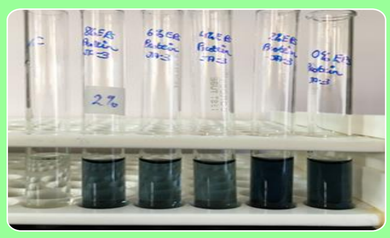
(c)

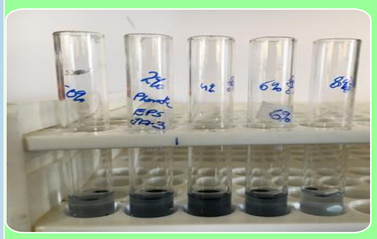


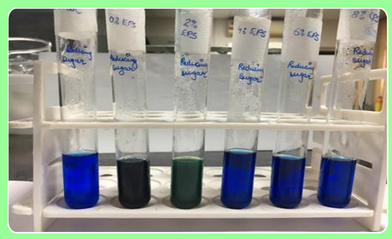


EPS properties

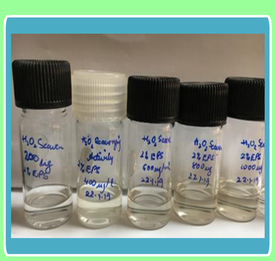


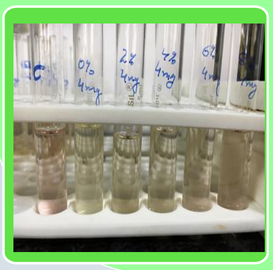


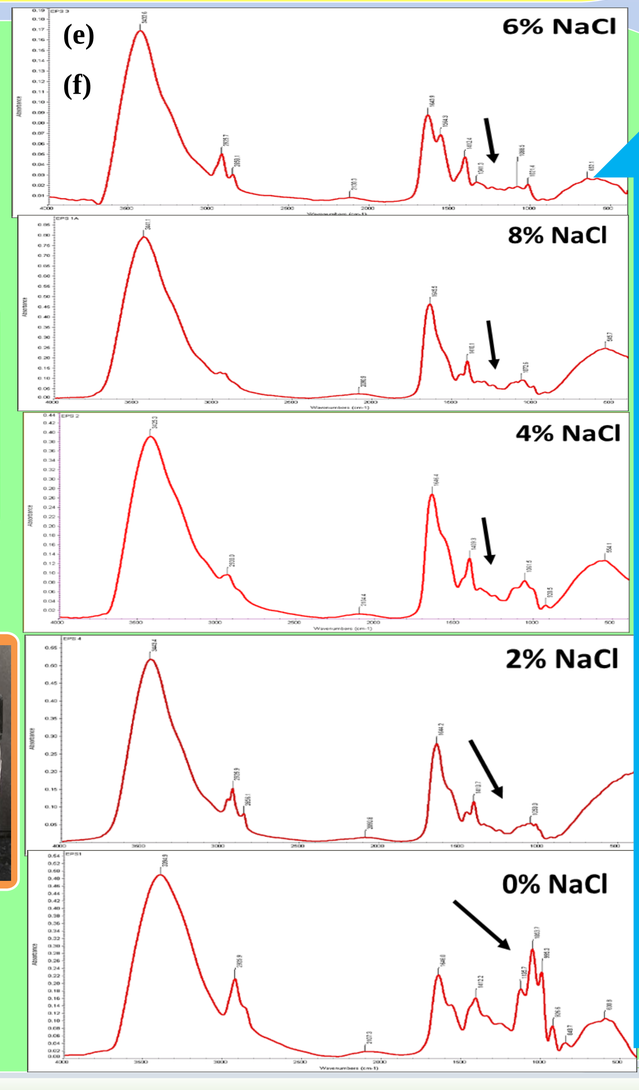




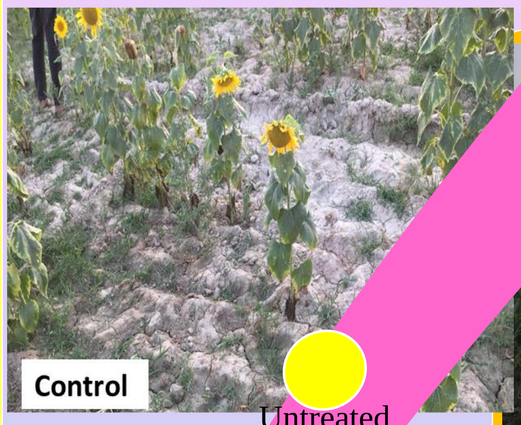
(d)



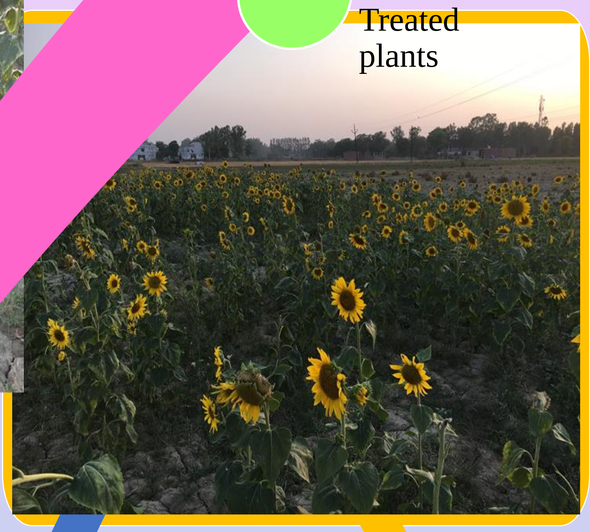




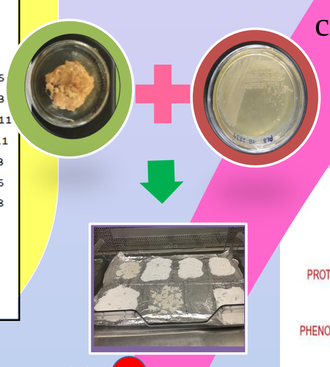
• T1-0.1% EPS
• T2-1.0% EPS
• T3-2% EPS
• T4-5% EPS
• T5-2% EPS+PE3
• T6-PE3
• T7-Untreated Control



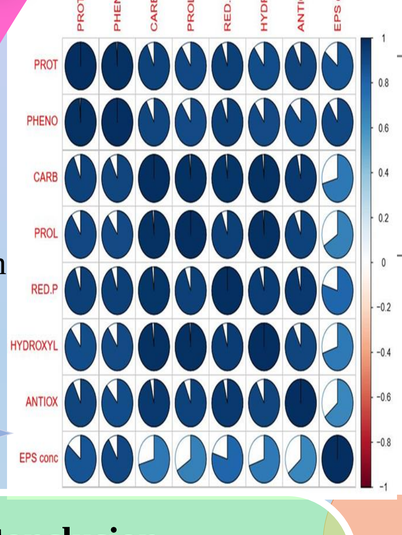
(h) Treated plants

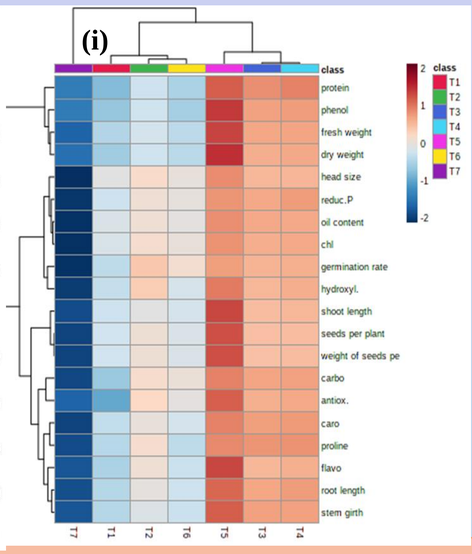


Untreated control plants



(g) Developed Bioformulation





Conclusion

- PE3 was found to be a potent salt-tolerant PGP strain
- PGP activities of PE3 were dominant upto 4% NaCl and at higher salinity salt-tolerance traits were prominent
- EPS production was the most significant response of PE3 to salinity
- PE3-extracted EPS showed salt-tolerance attributes which were maximum at 2% NaCl
- Salinity changed the properties of EPS proving its role as "stress molecule"
- Application of PE3 and EPS combination induced highest growth, yield and stress resilience in plants

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Potential of *Pseudomonas* sp. in plant growth promotion and micronutrient biofortification of wheat under drought stress

3.30

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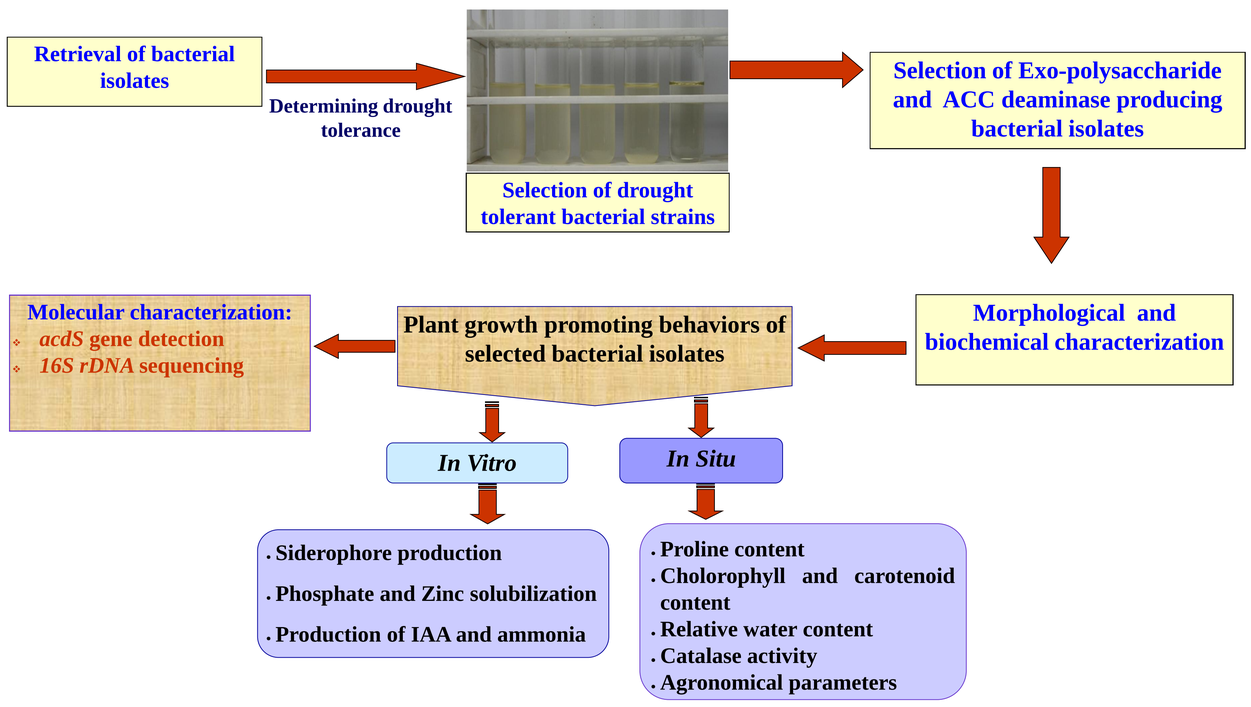
Introduction

- Water is most precious component for all forms of life. It maintains cellular turgor to sustain the turgidity of cells.
- Drought seems to be most deleterious abiotic stress. It minimizes crop productivity and yield by altering plant water relationships and nutrient content in soil.
- Chemical fertilizers are being used to enhance the crop productivity which often causes negative impacts on ecosystem
- Plant growth-promoting rhizobacteria (PGPR) are root associated microorganisms that are able to induce plant growth directly or indirectly through various mechanisms
- Some bacterial isolates can grow under drought stress conditions and can confer drought tolerance in plants and enhances crop yield

Objective

Isolation, identification and functional characterization of rhizospheric bacteria in order to develop a drought tolerant plant growth promoting biofertilizer

Methodology



Results and Discussion

1. Twenty bacterial isolates were selected on the basis of growth in tryptic soy broth supplemented with 10% poly -ethylene glycol and 18% NaCl

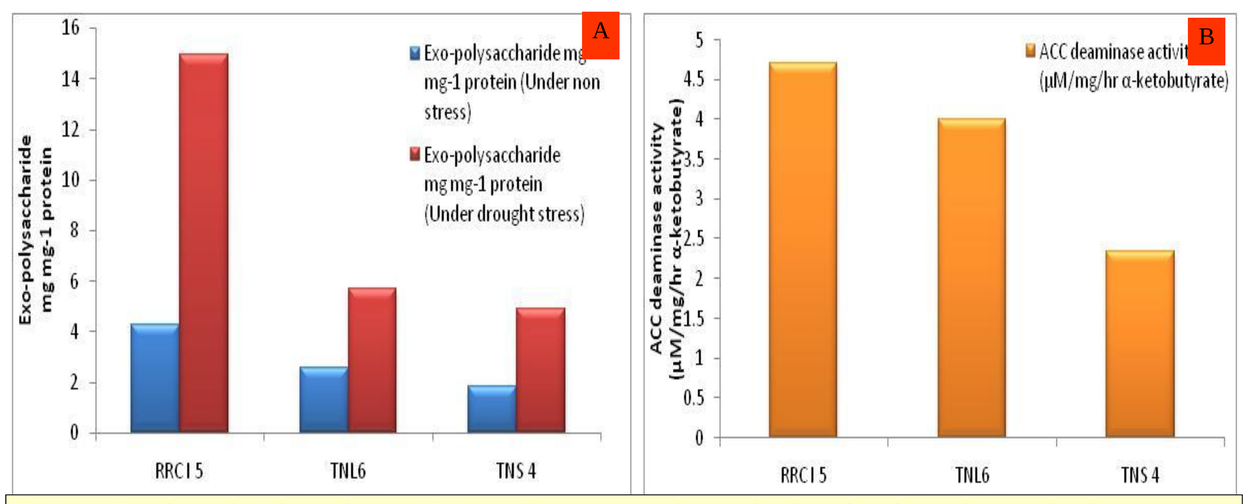


Fig 1: (A) Exoplysaccharide production (B) ACC deaminase production by drought tolerant bacterial isolates

2. On the basis of ACC deaminase and exo-polysaccharide production potential three bacterial isolates i.e. RRC I 5, TNL 6 and TNS 4 were selected for further study

3. Biochemical characterization

Table 1: Biochemical characteristics of selected potential bacterial isolates

S.N.	Bacterial Isolates	Catalase	Gelatinase	Caseinase	Amylase	Lipase	Cellulase	Pectinase	Laccase	Urease
1.	RRC I 5	+	-	-	-	-	-	-	-	+
2.	TNL6	+	+	+	+	-	-	-	-	+
3.	TNS 4	-	+	+	+	+	-	-	-	-

Table 2: Biochemical characteristics of selected potential bacterial isolates on Hi- IMVIC Kit

S. N.	Bacterial Isolates	Indole	Methyl Red	Voges Proskauer's	Citrate	Glucose	Adonitol	Arabinose	Lactose	Sorbitol	Mannitol	Sucrose
1.	RRC I 5	+	+	+	+	-	-	+	+	+	+	-
2.	TNL6	-	-	-	+	+	+	+	+	+	+	+
3.	TNS 4	+	-	-	-	+	+	+	+	+	+	+

4. *In vitro* plant growth promoting properties of bacterial isolates

Table 3: *In Vitro* plant growth promoting properties of selected bacterial isolates

S.N.	Bacterial isolates	Siderophore production	Zinc solubilization		Phosphate solubilization	Ammonia production	IAA production
			1% ZnO	1% ZnCO3			
1.	RRC I 5	+++	++	++	++	+	+++
2.	TNL 6	+++	+	+	+	-	-
3.	TNS 4	+++	++	++	++	+	+

5. *In situ* plant growth promoting response of bacteria on wheat growth under drought stress

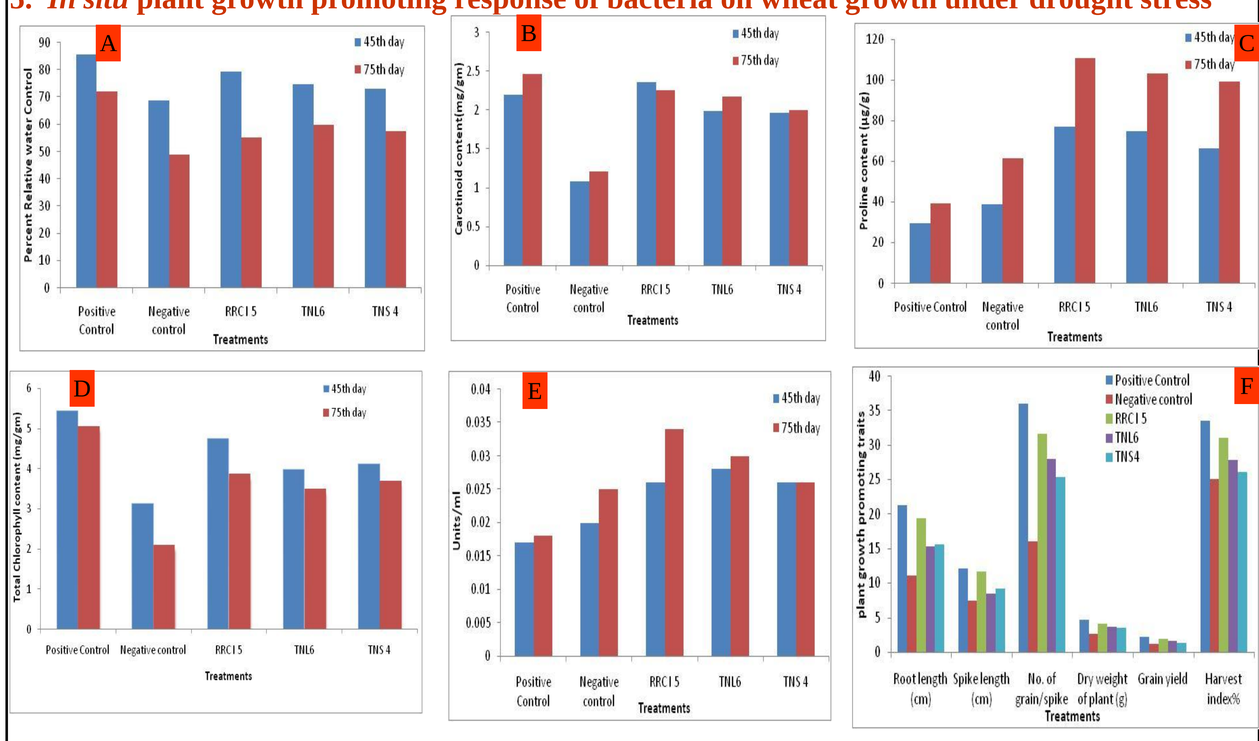


Fig: 2: Effect of bacterial isolates on biochemical, Physiological and yield attributes of wheat plants, A) Total chlorophyll content in leaves, B) Carotenoid content in leaves, C) Percent relative water content in leaves, D) proline content in leaves, E) Catalase activity in leaves, F) Agronomical parameters (Root length, spike length, No. of grain per spike, Dry weight of plants, grain yield, % harvest index) of wheat plant

. Among all bacterial inoculation, RRC I 5 showed highest level of chlorophyll, carotenoid, % relative water, proline content and catalase activity in leaves and enhancement in plant growth in terms of root length, shoot length, spike length, no. of grain per spike and % harvest index. Therefore, RRC I 5 bacterial isolate was sequenced through 16S rDNA sequencing.

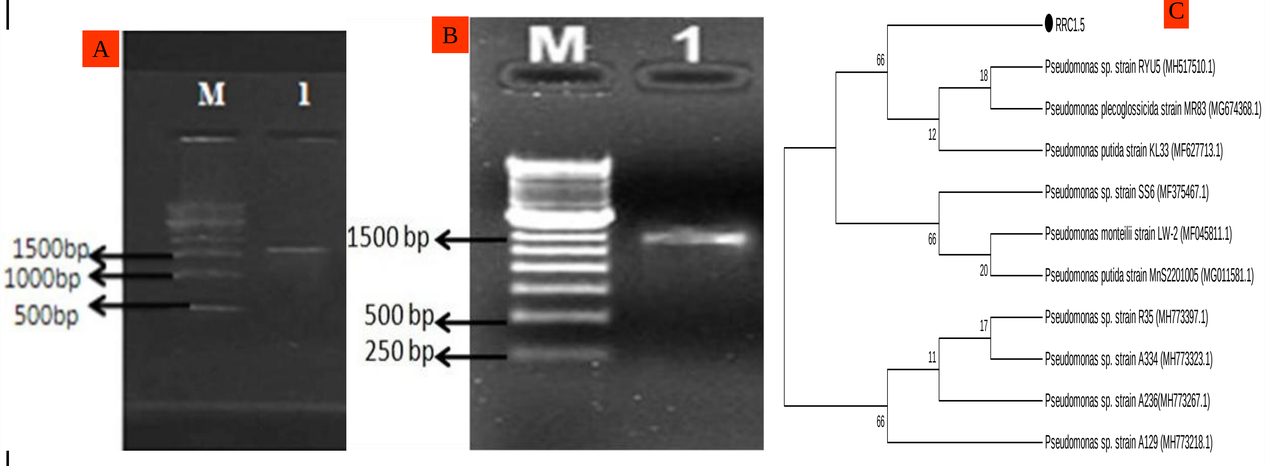


Fig: 3: A) Amplified product of *acdS* gene in RRC I 5, B) Amplified product of *16 S rDNA* of RRC I 5, C) UPGMA dendrogram of RRC I 5 isolate

On the basis *16S rDNA* sequencing RRC I 5 showed maximum homology with *Pseudomonas* sp.

16S rDNA sequences of RRC I 5 was submitted in NCBI data base under accession number MF433050

Conclusion

- Drought tolerant *Pseudomonas* sp. (RRC I 5) exhibited multiple plant growth promoting traits under *In vitro*. It was found to confer drought tolerance as well as improved plant growth and productivity of wheat. Thus, this bacterium could be used as alternative of chemical fertilizers as bio-inoculant to enhance the crop yield under drought stress for sustainable agriculture.

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