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Research Article

SCREENING AND CHARACTERIZATION OF STRESS TOLERANT RHIZOBIA FROM DIFFERENT SOILS OF TELANGANA

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Abstract- Present *in vitro* experiment was carried out to identify plant growth promoting rhizobial isolates for abiotic stress tolerance. Rhizospheric soils from normal, salt affected, drought affected were collected from different places of Telangana state. From all soil samples, twenty one *rhizobial* isolates were collected. Among the twenty one isolates, eleven isolates were showed different plant growth promoting properties. The results of different *in vitro* abiotic stress tolerance of isolates reveals that, only one isolate showed tolerance to temperature from 20°C-50°C (YR-21), three isolates were showed tolerance to temperature from 20°C - 45°C (RR-1, GR-5, GNR-8), one isolate was showed tolerance to temperature from 30°C- 45°C (MR-11), only one isolate were showed tolerance to temperature from 30°C - 40°C (ASR-17). Two isolates showed tolerance to water potential from - 0.05 Mpa to- 0.15 Mpa (RR-1, RR-3), three isolates were showed tolerance to water potential from - 0.05 Mpa to -0.30 Mpa (GNR-8, ASR-19, YR-21), only one isolate showed tolerance to water potential at - 0.05 Mpa (GNR-7).

Keywords- *Rhizobium,* PGPR, temperature, drought tolerance.

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Introduction

The rhizobia-legumes symbioses can benefit not only the host crop, but it may also have positive effects for subsequent crops [1]. Furthermore, rhizobia may also act as non-symbiotic PGPB as in the case of economically important non-legume crops such as rice or wheat, which are the best studied examples that benefit from rhizobia as endophytes [2], [3]. Values estimated for various legume crops and pasture species are often impressive, commonly falling in the range of 200 to 300 kg of N ha $^{-1}$ year^{-1} [4]. Yield increases of crops planted after harvesting of legumes are often equivalent to those expected from application of 30 to 80 kg of fertilizer N ha^{-1}. A fully functional symbiosis requires successful survival ability of *Rhizobium* even under adverse environmental conditions.

One of the major problems in rain fed agro-ecosystems is predominance of abiotic stresses like high temperature, salinity and drought where the applied bioinoculants survival and viability is a major issue in Indian conditions. Abiotic and biotic stresses are the limiting factors negatively affecting the crop growth and productivity worldwide. Plants responses to such factors are very complex which manifest in a range of developmental, molecular and physiological modifications that lead to either stress sensitivity or tolerance/resistance [5]. A given stress may also have more than one effect: e.g., salinity may act as a water stress, which affects the photosynthetic rate, or may affect nodule metabolism directly. Populations of *Rhizobium* and *Bradyrhizobium* spp., vary in their tolerance to environmental factors; consequently, screening for tolerant strains has been pursued. However, rhizobial populations are known to vary in their tolerance to major environmental factors [6].

The wild (naturally-growing) leguminous plants living in arid and semi arid regions are subject to severe environmental conditions. In addition, desertification causes disturbance of plant-microbe symbioses, which are a critical ecological factor in helping further plant growth in degraded ecosystems. Among several environmental conditions, which are limiting factor such as salinity, temperature extremes and pH stress are probably the most problematic. A competitive and persistent rhizobial strain is not expected to express its full capacity for nitrogen fixation as the limiting factors (e.g. salinity, unfavourable soil pH, temperature extremes, nutrient deficiency etc.) impose limitations on the vigour of the host legume. Inoculation of stress tolerant strains of rhizobia may enhance the nodulation and nitrogen fixation ability of plants under stress conditions.

Plant growth promoting rhizobacteria can improve plant growth and productivity by several mechanisms. Few strains from genera such as *Bacillus, Pseudomonas, Erwinia, Caulobacter, Serratia, Arthrobacter, Micrococcus, Flavobacterium, Chromobacterium, Agrobacterium, Hyphomycrobium, Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium and Allorhizobium* are well known PGPR. They aid in improving plant stress tolerance to drought, salinity and metal toxicity. The underlying mechanisms of plant growth promotion by PGPR have been comprehensively described in several articles [7, 8].

Selection of effective, efficient and compatible stress tolerant rhizobial strains could help in ecological rehabilitation of degraded soils and increases soil fertility thereby improving the growth of associated plants of this Telangana region.

Materials and methods

Isolation of native rhizobia

The selection of *rhizobial* cultures were obtained from different locations, such as normal soils, salt affected drought soils grown legumes root nodules. The *rhizobial* isolates (R-1 to R-21) [Table-1] were identified based on their morphological, biochemical characters and fixing ability, nodulation capacity. Further these isolates were tested abiotic tolerant efficiency.

Table-1 *Number of hizobial isolates obtained from different districts along with their isolate number*

S. No	District	Rhizobial Isolates number	No. of isolates
1	Rangareddy	Redgram - 3 isolates (RR1 – RR 3), Greengram- 2 isolates (GR 4 – GR 5), Groundnut – 4 isolates (GNR 6- GNR 9)	9
2.	Mahabubnagar	Redgram -2 isolates (MR 10 – MR 11)	2
3	Wanaparthi	Redgram- 2 isolates (WR 12- WR 13), Groundnut -2 isolates (WR 14- WR 15)	4
4	Nagarkurnool	Soybean – 1 isolate (NSR 16)	1
5	Adilabad	Soybean-2 isolates (ASR 17 - ASR 19)	2
6	Yadagirigutta	Redgram- 1 isolate (YR 20), Soybean- 1 isolate (YR 21)	3

Determination of mineral solubilization, IAA production, antagonistic activity ACC Deaminase activity and EPS production

Phosphate solubilization activity was determined using Pikovskaya's agar medium containing 0.5 % (W/V) $Ca_3(PO_4)_2$ [9], Potassium solubilization determined using Aleksandrov medium containing 0.2 % potassium aluminum silicate [10], Zinc solubilization determined using Tris mineral salt medium containing 0.1 % ZnO [11], IAA production [12], Exo polysaccharide production at stress induced conditions were checked [13], bacterial utilization of ACC as sole nitrogen source was screened using qualitative assay [14]. Siderophore production was determined by the chrome azurol S plate assay [15], antagonistic activity was verified by following dual culture technique [16].

Screening for abiotic stress tolerance ability of *rhizobial* isolates

Screening of *rhizobial* isolates for temperature tolerance

0.1 ml of bacterial suspension (10^8 -10^9 cells ml^{-1}) was poured into the vials containing 5 ml TSB culture medium and culture in incubators at 20°C, 30°C, 40°C, 45°C and 50°C in three replicates for each. After 24 hrs of culture, their absorbance was measured at 600 nm.

Screening of *rhizobial* isolates for drought tolerance

Yeast mannitol broth with different water potentials (−0.05, −0.15, −0.30, −0.49, and −0.73 MPa) was prepared by adding appropriate concentrations of polyethylene glycol (PEG 6000) [17]. Log phase grown culture was inoculated in YEM broth supplemented with different concentration of polyethylene glycol (PEG 6000). Osmotic potential of broth media was measured by osmometer. Three replicates of each isolate with each concentration were prepared. Growth was estimated by measuring the optical density values at 600 nm using a spectrophotometer

Results and Discussion

Isolation of rhizobia

Two or three healthy pink nodules were collected from each plant and surface sterilized by using 0.1% $HgCl_2$ and 70% ethanol as described in Material and Methods. The nodules were crushed and streaked on YEMA medium plates containing congo red dye. The colonies from each nodule were purified by streaking 2-3 times on same media. In total twenty one rhizobial isolates were obtained from different districts [Table-1]. These isolates were further purified and maintained on YEMA slants for further studies.

Characterization of rhizobia

About twenty one isolates showed small to medium, milky translucent, raised, mucoid colonies and formed non-spreading type of colonies. The colony morphology of isolates was examined on YEM agar plates. These pure cultures were checked for purity by streaking on different media like Hofer's alkaline agar, glucose peptone agar agar and lactose agar plates. None of the isolates showed any growth on these media plates. Among twenty one Gram negative *Rhizobium* bacterial isolates, all the isolates showed positive results for citrate utilization, oxidase test, catalase test. Nineteen isolates were positive for indole production. Nineteen isolates showed positive for methyl red test, thirteen isolates were positive for Voges-praskauer test. While all the isolates utilized mannitol, fifteen isolates utilized sucrose; ten isolates utilized lactose sugar and seventeen isolates utilized glucose sugar.

Plant growth promoting properties

Out of twenty one isolates, eleven isolates were positive for different plant growth promoting properties in *in vitro* conditions [Table-2].

Table-2 *Screening of rhizobial isolates for plant growth promoting properties*

Isolates	P solubilization (mm)	K Solubilization (mm)	Zn Solubilization (mm)	IAA Production (µg ml^{-1})	HCN Production	Antagonistic activity (mm)	ACC deaminase activity	EPS production
RR 1	11.66 ± 1.20	10.33 ± 0.88	8.33 ± 0.33	18.70 ± 0.14	-	-	++	++
RR 3	4.33 ± 0.33	7.33 ± 0.88	6.00 ±1.15	4.48 ± 0.25	-	-	-	+
GR 5	10.33 ± 0.88	6.00 ± 1.00	15.00 ±1.73	10.21 ± 0.00	-	-	+	+
GNR 7	2.33 ± 0.33	14.66 ± 0.88	14.66 ± 0.88	6.20 ± 0.57	-	-	-	-
GNR 8	8.33 ± 0.88	4.33 ± 0.88	3.00 ± 0.57	4.50 ± 0.25	-	-	+	+
MR 10	13.66 ± 0.33	13.00 ± 0.57	10.33 ± 0.88	10.36 ± 1.15	-	-	-	-
MR 11	5.33 ± 0.88	13.00 ± 1.00	3.33 ± 0.33	12.48 ± 0.25	-	-	-	-
WR 14	6.33 ± 0.33	2.83 ± 0.16	13.00 ±1.15	5.44 ± 0.92	+	13	++	++
ASR 17	7.33 ± 1.33	3.06 ± 0.06	3.33 ± 0.88	1.50 ± 0.25	-	-	+	+
ASR 19	18.00 ± 1.15	3.33 ± 0.88	2.66 ± 0.33	9.55 ±1.44	-	-	-	-
YR 21	3.33 ± 0.88	4.00 ±1.00	6.33 ± 0.33	8.50 ± 0.25	-	-	-	-
SE(m) ±	**0.859**	**0.812**	**0.893**	**0.670**				
CD	**2.535**	**2.397**	**2.637**	**1.979**				

\+ Weak production ++ Moderate production +++ Strong production − No production

**Values were mentioned in the mean with standard error

Mineral solubilization

Out of eleven isolates tested for phosphate solubilization activity, ASR-19 showed highest zone of solublization (18.00 ± 1.15 mm), followed by, MNR-10 (13.66 ± 0.33 mm), KTR-1 (13.66 ± 0.33 mm), GR-5 (10.33 ± 0.88 mm), GNR-8 (8.33 ± 0.88 mm). Among eleven isolates, GNR-7 showed highest potassium solubilization zone (14.66 ± 0.88 mm), followed by MR-11 (5.33 ± 0.88 mm), MNR-10 (13.00 ± 0.57 mm), RR-1 (10.33 ± 0.88 mm), RR-3 (7.33 ± 0.88 mm). Out of eleven isolates, GR-5 showed maximum zinc solubilization activity with solubilization zone (15.00 ± 1.73 mm), followed by GNR -7 (14.66 ± 0.88 mm), WR-14 (13.00 ±1.15 mm), MNR-10 (10.33 ± 0.88 mm) and RR-1 (8.33 ± 0.33mm). Eleven isolates were tested for production of indole acetic acid. The data revealed that RR-1 showed highest IAA production (18.70 ± 0.14 µg ml^{-1}),

followed by MR-11 (12.48 ± 0.25 µg ml^{-1}), MNR-10 (10.36 ± 1.15 µg ml^{-1}), GR-5 (10.21 ± 0.00 µg ml^{-1}), ASR-19 (9.55 ±1.44 µg ml^{-1}), YR-21 (8.50 ± 0.25 µg ml^{-1}).

Biocontrol activity of *rhizobial* isolates

Among eleven isolates only one isolate showed weak (++) HCN production (WR-14). Antifungal activity of eleven isolates was checked against *Rhizoctonia solani* and under *in vitro* conditions using PDA media. One isolate (WR-14) showed highest inhibition zone (13 mm) with *Rhizoctonia solani*.

ACC deaminase activity

All the *Rhizobium* isolates were screened for ACC deaminase based on the enrichment method under drought stress conditions (-0.30Mpa) where ACC was used as the sole nitrogen source. Among eleven isolates, five isolates (45 %) positive for ACC deaminase production by utilization of ACC the sole nitrogen source. Among five isolates, three isolates were moderate (++) in ACCd production (RR-1, WR-14), two isolates were weak (+) for ACCd production (GR-5, GNR-8, ASR-17). [18] reported similar results i.e fifty *Mesorhizobium* isolates were obtained from nodules of chickpea plants on yeast extract mannitol agar medium. *Mesorhizobium* isolates were screened for ACC utilization and growth at different salt concentrations in YEMA medium. They had also observed that *Mesorhizobium* strains having ACC utilization ability caused an increase in the nodule number, nodule weight and shoot dry weight after plant growth for 50 and 80 days, both with and without NaCl

Exo polysaccharide production

All the *Rhizobium* isolates were screened for exo polysaccharide production based on the enrichment method under drought stress conditions (-0.30Mpa). Among the eleven isolates, six isolates (54 %) were positive for EPS production. Among six, two isolates showed moderate (++) EPS production (RR-1, WR-14) and four isolates showed weak (+) EPS production (RR-3, GR-5, GNR-8, ASR-17). [19] reported greater EPS production with greater acid tolerance for a non-isogenic set of strains of *Rhizobium. Rhizobium* spp. YAS34 was selected from the indigenous sunflower tap root micro flora for its ability to produce large amounts of gel-forming exo polysaccharides that play an active role in plant development in a non-symbiotic context [20], whereas EPS production by *Rhizobium* populations is usually known to be crucial for establishment of successful symbiosis with legumes.

Temperature tolerance of *rhizobial* isolates

Screening of *rhizobial* isolates for temperature tolerance. All the twenty one rhizobial isolates were tested for temperature tolerance at 30, 35, 40, 45 and 50°C temperature [Table-3] & [Fig-1]. Almost all the rhizobial isolates showed considerable growth at 30 and 35°C while at 40ºC. Only one isolate showed tolerance to temperature from 20ºC-50ºC (YR-21), three isolates were showed tolerance to temperature from 20ºC - 45ºC (RR-1, GR-5, GNR-8), one isolate was showed tolerance to temperature from 30ºC- 45ºC (MR-11),only one isolate were showed tolerance to temperature from 30ºC - 40ºC (ASR-17). High tolerance to heat shock is common in in chickpea rhizobia and other species also [21], [22]. [23] obtained several strains of *Rhizobium japonicum* tolerant up to 40°C. [24] studied growth of *Rhizobium* was at different temperatures by incubating bacterial cultures in YEM agar at 32ºC, 34ºC, 36ºC, 38ºC and 40ºC. [25] conducted greenhouse experiment at 28ºC and 38ºC to study the nitrogen fixing capacity of the soybean isolates showed that ten isolates had a symbiotic index of 80% effectiveness or greater compared to nitrogen fertilizer treatments at 28ºC. Some thermo tolerant isolates showed good nitrogen fixing performance at 38ºC. A large number of rhizobia from root nodules of *Prosopis juliflora* were screened for high-temperature tolerance by [26].

Drought tolerance of *rhizobial* isolates

The drought tolerance of all the twenty one rhizobial isolates was tested in YEM broth supplemented with different concentrations of PEG 6000 [Table-4] & [Fig-2].

Table-3 *Growth in terms of absorbance at 600 nm of rhizobial isolates at different temperatures*

Isolates	Temperature tolerance (*)				
	20ºC	30ºC	40ºC	45ºC	50ºC
RR 1	0.20	0.89	0.09	0.100	0
RR 3	0.00	0.92	0.00	0.000	0
GR 5	0.20	0.80	0.45	0.207	0
GNR 7	0.00	0.80	0.00	0.000	0
GNR 8	0.02	0.95	0.10	0.090	0
MR 10	0.02	0.89	0.00	0.000	0
MR 11	0.00	0.90	0.22	0.180	0
WR 14	0.00	1.00	1.00	0.000	0
ASR 17	0.00	0.89	0.25	0.000	0
ASR 19	0.00	1.00	0.00	0.000	0
YR 21	0.12	0.98	0.20	0.137	0.02
CD	**0.019**	**0.045**	**0.125**	**0.013**	
SE(m)	**0.006**	**0.015**	**0.042**	**0.004**	

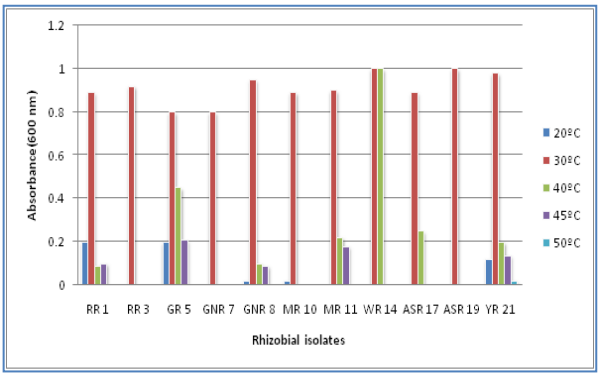

Fig- 1 Temperature tolerance by *rhizobial* isolates

The results of drought tolerance ability of *Rhizobium* isolates revealed that, two isolates showed tolerance to water potential from - 0.05 Mpa to- 0.15 Mpa (RR-1, RR-3), three isolates were showed tolerance to water potential from - 0.05 Mpa to-0.30 Mpa (GNR-8, ASR-19, YR-21), only one isolate showed tolerance to water potential at - 0.05 Mpa (GNR-7). The growth and persistence of rhizobia and bradyrhizobia in soils are negatively impacted by drought conditions [27]. [28] studied 30 isolates using YEM broth supplemented with PEG and he reported that the concentration of PEG increased, the growth of rhizobial isolates was found to decrease. [29] grouped *Sinorhizobium* spp. in two clusters: sensitive and tolerant based on their growth rate in YEM broth containing different concentrations of PEG. [30] reported that *Rhizobium* spp. NBRI2505 *sesbania,* when subjected to drought stress, tolerated YEB containing 45% polyethylene glycol 6000 for up to 5 days of incubation at 30°C.

Table-4 *Growth in terms of absorbance at 600 nm of rhizobial isolates supplemented with different concentration of polyethylene glycol (PEG 6000)*

Isolates	Drought stress (*)			
	-0.05 Mpa	-0.15 Mpa	-0.30 Mpa	-0.73 Mpa
RR 1	0.20	0.02	0.00	0.00
RR 3	0.26	0.03	0.00	0.00
GR 5	0.00	0.00	0.00	0.00
GNR 7	0.35	0.00	0.00	0.00
GNR 8	0.28	0.02	0.01	0.00
MR 10	0.00	0.00	0.00	0.00
MR 11	0.00	0.00	0.00	0.00
WR 14	0.00	0.00	0.00	0.00
ASR 17	0.00	0.00	0.00	0.00
ASR 19	0.38	0.30	0.01	0.00
YR 21	0.40	0.20	0.04	0.00
CD	**0.033**	**0.009**	**0.003**	
SE(m)	**0.011**	**0.003**	**0.001**	

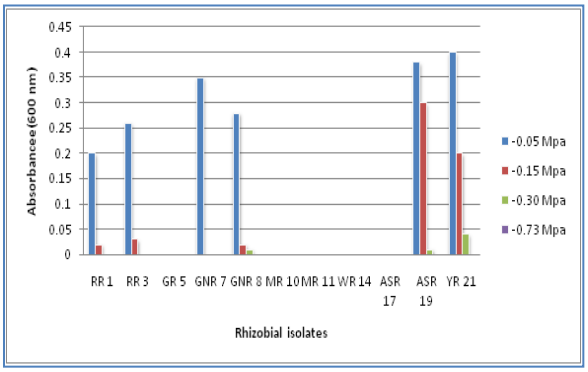

Fig- 2 Drought tolerance by *rhizobial* isolates

For plant growth promoting properties, *Rhizobium* isolate RR-1 showed good nodulation and moderate P-solubilization in addition to highest IAA production, ACC deaminase & EPS production and considerable other PGP properties. MR-10 showed efficient nodulation, moderate p-solubilization, k- solubilization, zn solubilization and considerable other PGP properties. WR-14 showed efficient nodulation, Zn-solubilization and ACC deaminase, EPS production. GR-5 showed highest Zn-solubilization and moderate in ACC deaminase & EPS production.

For biocontrol properties such as HCN production, Siderophore production and antifungal activity with *Rhizoctonia solani,* the *Rhizobium* isoalate WR-14 showed moderate HCN production and antifungal activity with *Rhizoctonia solani*

For plant growth promoting properties and biocontrol, *Rhizobium* isolate WR-14 was efficient as it showed efficient nodulation, Zn-solubilization and ACC deaminase & EPS production and moderate biocontrol activity, HCN production and antifungal activity with *Rhizoctonia solani.*

The *Rhizobium* isoalate MR-10 found efficient in terms of plant growth promoting properties and abiotic stress as it exhibited efficient nodulation, moderate P-solubilization, K-solubilization, Zn-solubilization and other PGP properties. It was able to tolerate temperature stress and drought stress (- 0.15 MPa - 0.3 MPa).

None of the isolates had shown positive for PGP properties, biocontrol activity and tolerance to abiotic stress conditions. However, *Rhizobium* isolates MR-10 and WR-14 mentioned earlier could be a used in combination to meet the requirement of PGPP, Biocontrol and Abiotic stress conditions. Hence these isolates might be potential as bioinoculants in different arid climatic regions of Telangana.

Conclusion

It can be summarized that PGPR isolates from the present study, *Rhizobium* isolates MR-10 and WR-14 mentioned earlier could be a used in combination to meet the requirement of PGPP, Biocontrol and Abiotic stress conditions. have good potential to be used as bioinoculants with further confirmation under *in vivo* conditions.

Application of research: The scope of the present research work as on isolation, screening and identification of PGP rhizobia for stress tolerance under in vitro conditions. The different rhizobia were studied for their stress tolerance properties. It is intended that the potential PGPR isolates would be studied under in vivo conditions with suitable crops for establishing their use as bioinoculants.

Research Category: Stress tolerant rhizobia

Abbreviations:

HCN: Hydrogencyanic acid
PGP: plant growth promoting properties
ACC: 1-amino cyclopropane cyclohexane
YEMA: yeast extract mannitol agar
EPS: exo polysaccharides
ASR: Adilabad soybean rhizobium
RR: *Rangareddy Rhizobium*
YR: *Yadagirigutta Rhizobium*

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