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SCREENING OF SALT TOLERANT RHIZOBIA FROM GROUNDNUT IN CUDDALORE DISTRICT OF TAMIL NADU, INDIA

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ABSTRACT

Salinity seriously constrains crop yield in irrigated agriculture throughout the world. Also, salinity is a serious threat to agriculture in arid and semi arid regions. Nearly 10 % of the world's land surface can be classified as endangered by salinity. Salinity in the soil and irrigation water is an environmental problem and a major constraint for crop production. Currently, 20 % of the world's cultivated land is affected by salinity, which results in the loss of 50 % of agricultural yield. At present, there are nearly 954 million hectares of saline soils on the earth's surface. All these salt affected soils are distributed throughout the world. The salinity response of legumes in general varies greatly depending on factors like climatic conditions, soil properties, salt tolerance and the stages of crop growth. Successful cultivation of legumes can be achieved by the selection and/or development of a salt-tolerant legume *Rhizobium* combination although high salinities are known to affect rhizobial activities. The aim of present study is the effect of strains of salt tolerant Rhizobia on IAA, EPS, nodule ARA activity, Nitrogen content, leghemoglobin content, siderophore production, IAR and salt concentration of Groundnut on coastal area of Cuddalore District of Tamil Nadu. The GNR CD-4 is the effect salt tolerance strain compared to other strains.

Keywords: *Rhizobium*, Groundnut, Sodium chloride, IAR, EPS, ARA

INTRODUCTION

Nitrogen is one of the major important nutrients essential for plants growth. The atmosphere contains about 10^{15} tonnes of N_2 gas and the nitrogen cycle involves the transformation of some $3x10^9$ tonnes of N_2 per year on the global basis (Delgoda, 1993). Biological nitrogen fixation occurs, generally at mild temperatures, by nitrogen fixing microorganisms, which are widely distributed in nature (Raymond *et al.,* 2004). Berrada and Fikri- Benbrahim (2014) recently reported that there are 98 species of legume nodulating bacteria belonging to 14 genera. We described about 238 species distributed among 18 genera. These 18 genera of root nodulating bacteria with some representative species of each genus.

The largest two genera are *Rhizobium and Bradyrhizobium* and we focus here on species those nodulate edible legumes. Among oilseeds, peanut is unique since it can be consumed directly as food stuff in India; peanut is mostly grown under rain-fed conditions, mostly by resource-poor farmers and hence, is most likely encountered by abiotic stresses like drought and salinity (Sarkar *et al.,* 2014; Bhauso *et al.,* 2014a, b). Salinity in the soil and irrigation water is an environmental problem and a major constraint for crop production. Currently, 20% of the world's cultivated land is affected by salinity, which results in the loss of 50% of agricultural yield. At present, there are nearly 954 million hectares of saline soils on the earth's surface. All these salt affected soils are distributed throughout the world. A large bulk of about 320 million hectares and of lands in South and South East Asia is under the grip of salinity (Aslam, 2006). In our objective was to screening of *Rhizobium* on IAA, EPS, ARA activity, Nitrogen content, Leghemoglobin content, siderophore production, IAR and Salt tolerance OD at 520nm for 72hr broth culture test a local 10 different location microbial strain for groundnut and select a efficient strain to enrich its productivity in soil with salt stress.

MATERIALS AND METHODS

Quantitative estimation of Indole acetic acid (IAA) and Exopolysacchrides (EPS) produced by *Rhizobium* isolates

The yeast extract mannitol broth in 100 ml quantities were prepared and supplemented with DL-Tryptophan, at a concentration of 100 mg/L after sterilization. This was followed by the addition of standard inoculum (1×10^7 cells/ml) of the isolates and incubated at 30°C under dark for a period of 7-12 days in order to prevent the photo inactivation of biologically active compounds. The solution was centrifuged at 7000 rpm for 30 minutes and the supernatant was reduced to 50 ml volume by flask evaporation under Vacuum and IAA extracted into ethylacetate and n-butanol by the procedure followed by Tien *et al.,* (1979)

Two ml of the inoculum was added to 100 ml of YEM liquid medium and incubated on Psychrotherm incubator shaker at 28°C for 72 hours. The cells are harvested by centrifugation at 4000 x g for 10 min and set aside.

Estimation of Nitrogenase activity by Acetylene Reduction assay (ARA) activity

One gram of root nodules were placed in 65 ml serum vials and closed with rubber stoppers. With the sterile disposable syringe, 6.3 ml of air from the serum vial was evacuated and 6.3 ml of acetylene gas was injected and these bottles were incubated at 28°C for one hour. At the time of assay, using a sterile disposable syringe, 0.5ml of gas sample was withdrawn after flushing twice and injected into gas chromatograph and tested for ethylene production. The factor 0.006 was arrived by injecting pure ethylene gas. The nitrogenase activity was expressed as a mole of ethylene produced per gram of nodules per hour (Hardy *et al.,* 1968).

Siderophore production

Salicylate type siderophore was measured as follows. To 20 ml of supernatant, 20 ml of ethyl acetate (pH 2) was added and extraction was done twice. Hathway reagent (1 ml of 0.1 M ferric chloride in 0.1 N HCl added to 100 ml of distilled water and to this 1 ml of 0.1 M Potassium ferric cyanide was added) was prepared (Reeves *et al*., 1983). 5 ml of the assay solution was added to 5 ml of Hathway reagent and absorbance was determined 560 nm with sodium salicylate as standard.

Determining the Intrinsic Antibiotic Resistance (IAR) and Salt tolerance level of root nodules isolates

Stock solution of the antibiotics was prepared immediately before use in sterile distilled water. Appropriate quantities of the antibiotic stock solution were added to YEMA at 48°c, mixed thoroughly and then poured on petri dishes. Isolates were grown in YEM broth for 48hrs. A portion of each culture was diluted in sterile water to prepare for solutions so that 0.1 ml of each isolates inoculated on a petri dish. The plates were incubated at 28°C for 7 days. The growths of the antibiotic plates were compared with that on Tryptone Yeast extract agar control plates.

The Yeast extract manitol broth was prepared at different levels of sodium chloride concentrations *viz.,* 0.0 %, 0.5%, 1.0 %, 1.5%, 2.0 %, 2.5% and 3.0 %. pH adjusted to 7.2 and distributed in 100 ml quantities into 250 ml Erlenmeyer flasks and sterilized. Yeast extract mannitol broth without NaCl served as control. The flasks were inoculated with 1 ml of standardized inoculum ($1x10^7$ cells) of *Rhiobium* isolates. The flasks were incubated for 72 h at 30°C with intermittent shaking. The growth measured as absorbance at 520 nm in Spectronic-20 spectrophotometer.

RESULT AND DISCUSSION

Screening Of Groundnut Rhizobial Isolates For Their Efficiency

The groundnut root nodules isolates GNR CD-1 to GNR CD-10 were screened for their efficiency based on IAA and EPS production, nodule ARA activity, leghemoglobin content, nodule N content, siderophore production, antibiotic resistance and salt tolerance.

IAA and EPS

All the isolates produced IAA on tryptophan supplemented yeast extract mannitol broth and the quantity ranged from 0.82 to7.82µg ml^{-1} of the culture medium. Among the 10 isolates, 3 isolates *viz*., GNR CD-3, GNR CD-4 and GNR CD-8 were best producers. The strain GNR CD-4 produced maximum IAA of 7.82µg ml^{-1}.

The isolates produced Exopolysaccharides (EPS) in varying quantities ranged from 35.05 to 318.35 µg ml^{-1} in culture broth. Three isolates *viz.,* GNR CD-3, GNR CD-4 and GNR CD-8 produced > 254.00 µg ml^{-1} and they were selected for further study. These isolates were also efficient in IAA production. The isolate GNR CD-4 produced maximum of 318.35 µg ml^{-1} Table – 1.

Table:1 Screening of Groundnut Rhizobial isolates for the production of Indole Acetic Acid (IAA) and EPS

S.No	Isolates	IAA (µg ml^{-1})	EPS (µg ml^{-1})
1	GNR CD-1	2.40	35.05
2	GNR CD -2	1.82	78.25
3	GNR CD -3	6.97	265.25
4	GNR CD -4	7.82	318.35
5	GNR CD -5	0.82	56.50
6	GNR CD -6	0.97	40.05
7	GNR CD -7	2.67	68.25
8	GNR CD -8	4.52	254.05
9	GNR CD -9	2.57	158.05
10	GNR CD -10	3.22	185.65

Ara Activity And N Content

All the 10 isolates were also screened for their ARA activity and N content. The ARA activates showed by the isolates were ranged from 98.00 to 215.50 n moles C_2H_4 formed $h^{-1}g^{-1}$..The isolate GNR CD-4 produced maximum of 215.50 n moles C_2H_4 formed h^{-1} g^{-1} of ARA. Total nitrogen content ranged from 3.20 to 7.25 %. The isolate GNR CD-4 had maximum nitrogen content of 7.25 % Table: 2

Table: 2 Screening of Groundnut Rhizobia on nodule ARA activity (ARA n moles C_2 H_4 h^{-1} g^{-1}nodule) and Nitrogen content (%)

S.No	Isolates	ARA (n moles C_2 H_4 h^{-1} g^{-1}nodule)	Total Nitrogen content (%)
1	GNR CD-1	100.85	3.20
2	GNR CD-2	98.10	4.05
3	GNR CD-3	198.20	6.90
4	GNR CD-4	215.50	7.25
5	GNR CD-5	127.10	4.05
6	GNR CD-6	136.85	5.25
7	GNR CD-7	120.60	5.95
8	GNR CD-8	175.10	6.60
9	GNR CD-9	110.10	5.30
10	GNR CD-10	138.50	4.00

Siderophore Production And Leghemoglobin (Nodule Mg G^{-1})

The siderophore production ranged from 1.70 to 5.25 mg l^{-1} of the culture medium. Among the 10 isolates, the isolate GNR CD-4 produced maximum siderophore of 5.25 mg l^{-1}. Leghemoglobin content ranged from 0.230 to 2.105 nodule mg g^{-1} at 15 Days, 0.375 to 2.230 nodule mg g^{-1} at 30 Days and 0.220 to 2.115 at 45 Days nodule mg g^{-1}. The leghemoglobin content was found to be relatively high in strain GNR CD-4. Table : 3.

Table: 3 Screening of Groundnut Rhizobia on Siderophore production and Leghemoglobin (Nodule mg g^{-1})

S. No.	Isolates	Hydroxymate in mg l^{-1}	Leghemoglobin (Nodule mg g^{-1})		
			15 days	30 days	45 days
1	GNR CD-1	2.79	0.645	0.805	0.295
2	GNR CD -2	3.85	0.569	0.720	0.425
3	GNR CD -3	4.90	1.425	1.965	1.480
4	GNR CD -4	5.25	2.105	2.230	2.115
5	GNR CD-5	3.10	0.240	0.685	0.575
6	GNR CD 6	3.75	0.525	0.685	0.505
7	GNR CD-7	3.00	0.425	0.510	0.435
8	GNR CD-8	4.90	1.330	1.540	1.820
9	GNR CD-9	2.00	0.230	0.375	0.220
10	GNR CD -10	1.70	0.270	0.430	0.320

Intrinsic Resistance (IAR)

The intrinsic antibiotic resistance of the ten isolates was determined against two antibiotics viz., Tetracycline and Ampicillin.

The antibiotic resistance of the ten isolates to various antibiotics tested varied considerably. The isolates GNR CD-5 and GNR CD-9 were found to be sensitive to all the two antibiotics at different concentrations tested, the isolates GNR CD-4 possessed highest level of antibiotic tolerance i.e., upto 300ppm for tetracycline, 250ppm for Ampicillin and on the other strain hand found to be highly sensitive to Ampicillin. Table: 4

Table: 4 Screening of Groundnut *Rhizobium* for Intrinsic Antibiotic Resistance (IAR)

S.NO	Strains	Maximum tolerance level of different antibiotics (µg ml^{-1})	
		Tetracycline	Ampicillin
1	GNR CD-1	-	75
2	GNR CD-2	50	125
3	GNR CD-3	200	250
4	GNR CD-4	300	250
5	GNR CD-5	-	-
6	GNR CD-6	50	-
7	GNR CD-7	25	75
8	GNR CD-8	200	175
9	GNR CD-9	-	-
10	GNR CD-10	125	-

Salt Tolerance

The effect of salt towards on the growth of all the 10 isolates was studied. The salt tolerance potential of the ten isolates were tested by growing them in Yeast extract-Mannitol (YEM) liquid medium prepared with salt at different concentrations (from 0.0 %, 0.5%, 1.0 %, 1.5%, 2.0 %, 2.5% and cfc2f3.0 %) (Table-5).

All the isolates grow well in YEM liquid broth without NaCl as the concentration increased, the growth get decreased. Among the isolates tested, the isolates GNR CD-3, GNR CD-4 and GNR CD-8 were found to grow at 3.0 % salt concentration. The isolate GNR CD-4 was able to grow up to OD 520=0.023 at 3.0 % salt concentration.

Table-5: Screening of Rhizobial isolates for Salt tolerance OD at 520nm for 72hr broth culture

S. No	Iso-lates	Sodium chloride concentration (%)						
		0 %	0.5 %	1.0 %	1.5 %	2.0 %	2.5 %	3.0 %
1	GNR CD-1	0.275	0.010	0.044	0.021	-	-	-
2	GNR CD -2	0.284	0.104	0.048	0.027	0.017	-	-
3	GNR CD -3	0.305	0.140	0.066	0.059	0.034	0.028	0.020
4	GNR CD -4	0.338	0.145	0.074	0.048	0.038	0.030	0.023
5	GNR CD -5	0.410	0.269	0.040	-	-	-	-
6	GNR CD -6	0.301	0.127	0.066	0.024	-	-	-
7	GNR CD -7	0.281	0.094	0.034	0.014	-	-	-
8	GNR CD -8	0.285	0.118	0.047	0.035	0.036	0.029	0.022
9	GNR CD -9	0.270	0.084	0.050	0.017	-	-	-
10	GNR CD -10	0.290	0.119	0.050	0.016	-	-	-

CONCLUSION

Based on the result of the study it could be concluded that the strain GNR CD-4 can be efficient compared to other strain for groundnut grown in saline areas. GNR CD-4 are increasing root nodule and nitrogen fixation in saline areas. So GNR CD-4 can be used as a potential biofertilizer for groundnut grown in saline areas.

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