ISSN: 2320-8090

Available online at http://www.journalijcst.com

International Journal of Current Science and Technology
Vol.5, Issue, 3, pp.353-356, March, 2017

RESEARCH ARTICLE

SCREENING OF SALT TOLERANT RHIZOBIA FROM GROUNDNUT IN TSUNAMI AFFECTED COASTAL REGION OF CUDDALORE DISTRICT OF TAMILNADU

N. Pandeeswari* and S. Kalaiarasu

Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608 002, Chidambaram, Tamil Nadu, India.

ARTICLE INFO

Article History:
Received 20th December, 2016
Received in revised form 8th January, 2017
Accepted 24th February, 2017
Published online 28th March, 2017

Key words:
Salt tolerant, NaCl, *B. japonicum*

ABSTRACT

Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil, and the area of land affected by it is increasing day by day. Now we focused the native rhizobia from Tsunami affected area of Cuddalore district. In this study, total thirty rhizobial isolates were tested different concentration of NaCl level. Among the thirty isolates such as GNBJ-4, GNBJ-8, GNBJ-9 and GNBJ-28 recorded the maximum of salt stress upto 3.0% of NaCl. Further this four isolates recorded the maximum of IAA, EPS production, number of nodules, nodule ARA activity and nodule N content at 3.0% NaCl concentration respectively. The four isolates of *B. japonicum* namely GNBJ-4, GNBJ-8, GNBJ-9 and GNBJ-28 were selected for further study of mutation efficiency

INTRODUCTION

Salinization is one of the most crucial factors threatening agricultural land throughout the world. Approximately one third of the 260 million ha of irrigated land worldwide is affected by salinization (El-Akhal *et al.,* 2013). Higher temperatures and altered rainfall distribution are alterations predicted for southern Europe (Connor *et al.,* 2012). These alterations can increase agricultural salinization by increasing the rates of surface water evaporation and thus the water demand for irrigation. In consequence, nutrient acquisition decreases, biological nitrogen fixation is reduced, and nutrient cycling is disrupted (St.Clair and Lynch, 2010).

Rhizobia are of considerable scientific, economic and ecological interest because of their ability to establish nitrogen-fixing nodules on leguminous hosts. This feature enables plant growth in soils with low nitrogen levels and good crop yields without high nitrogen fertilization, decreasing the contamination of water reservoirs by inorganic nitrogen compounds (Singh *et al.,* 2011). Microorganisms, like rhizobia, can also impart some degree of tolerance to plants towards abiotic stresses like drought and salinity (Yang *et al.,* 2009; Grover *et al,.* 2011).

**Corresponding author: N. Pandeeswari*

Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai
Nagar-608 002, Chidambaram, Tamil Nadu, India.

Salinity affects free-living rhizobia, since it imposes both ionic and osmotic stress, which can be extremely detrimental for microflora survival (Figueira 2000; Dominguez-Ferreras *et al.,* 2006). Salinity also considerably restrains the nodulation and symbiotic nitrogen fixation (Bolanos *et al.,* 2003; Domınguez-Ferreras *et al.,* 2006; Bianco and Defez, 2009; Brı´gido *et al.,* 2013). Therefore, it becomes imperative to find symbiotic partners that can fix nitrogen under stressful conditions. The selection and characterization of salt-tolerant rhizobia strains, able to fix nitrogen symbiotically under salt conditions, may constitute a strategy for improving legume symbiosis in adverse conditions and may constitute a better economic and sustainable alternative to chemical fertilization (Rejili *et al.,* 2012).

However, salt resistant strains can only be identified if the tolerance of rhizobia to salinity is investigated. Thus, the study to attempt the screening of salt tolerant rhizobia from ground in Tsunami affected coastal region of Cuddalore district.

MATERIALS AND METHODS

Selection of Rhizobial culture

The selection of rhizobial cultures were obtained from thirty different location of Tsunami affected area in groundnut grown of Cuddalore district of Tamilnadu. The *Brdyrhizobium* isolates (GNBJ-1 to GNBJ-30) were identified based on their morphological, biochemical characters and their nitrogen fixing ability, nodulation capacity and Intrinsic Antibiotic Resistance (IAR). Further these isolates were tested salt tolerant efficiency.

Screening of Bradyrhizobium isolates for salt tolerance efficiency

Determination of Salt tolerance level of the root nodule isolates

The Yeast extract manitol broth was prepared at different levels of sodium chloride concentrations *viz.,* 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 per cent. 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 (Somasegaran and Hoben, 1994).

Effect of salt concentrations on the production of IAA and EPS by Rhizobial strains

The Yeast extract mannitol broth was prepared at different levels of sodium chloride concentrations *viz.,* 1.0, 1.5, 2.0 and 3.0 per cent. pH was adjusted to 7.0 and distributed in 100 ml quantities in to 250 ml Erlenmeyer flasks. The sterilized YEM broth without NaCl served as control. The flasks were inoculated with 1ml of standardized inoculum of salt tolerant mutants and their parents and reference strain. The flasks were incubated for 72 hrs at 30°C with intermittent shaking. Then the production of IAA and EPS by these isolates at different concentrations was estimated as described by Islam and Ghoulam (1981).

Effect of salt concentrations on the nodulation, nodule ARA activity and nodule N content

A pot culture experiment was conducted at Department of Microbiology, Faculty of Agriculture, Annamalai University under split plot design with five replications to study the effect of different concentrations of salt (NaCl) *viz.,* 1.0, 1.5, 2.0 and 3.0 % on the nodulation, nodule ARA activity and nodule N content of groundnut var. VRI-2 inoculated with wild parent and mutant isolates GNBJ-9, GNBJ-28, GNBJ-9M, GNBJ-28M and reference strain ATCC 55749.The soil without salt served as control. The nodulation, nodule ARA and nodule N content was estimated as described by Cordovilla *et al.,* (1999).

RESULTS AND DISCUSSION

Screening of Bradyrhizobium isolates for salt tolerance

The effect of salt towards on the growth of all the 30 isolates was studied. The salt tolerance potential of the thirty isolates were tested by growing them in Yeast extract-Mannitol (YEM) liquid medium prepared with salt 6000 at different concentrations (from 0%, 1.0%, 2.0%, 3.0%) (Table-1).

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 GNBJ-4, GNBJ-8, GNBJ-9 and GNBJ-28 were found to grow at 3.0% salt concentration. The isolate GNBJ-9 was able to grow up to OD 520=1.53 at 3.0% salt concentration. This similar results also reported by several researchers (Bernsteinl and Ogatag, 1966; Balasubraminiam and Sinhas,1976; Ali *et al.,* 2009). To our knowledge 10.8 % is the highest salt tolerance recorded for rhizobia (Zahran 1999; Bouhmouch *et al.,* 2005; Bianco and Defez, 2009; Brıgido *et al.,* 2012), which was displayed by 60 % of ALE isolates. Brıgido *et al.,* (2012) reported that isolates from Madeira, Beira Baixa, Ribatejo and Baixo Alentejo were less tolerant to 1.5 % NaCl than Algarve, Beira Alta and Beira Litoral isolates, showing an association between salt tolerance and provenance of the isolates.

Table-1 Screening of Bradyrhizobial isolates for Salt tolerance OD at 520nm for 72hr broth culture

S.No.	Isolates	Sodium chloride concentration (%)						
		0%	0.5%	1.0%	1.5%	2.0%	2.5%	3.0%
1	GNBJ-1	0.265	0.097	0.034	0.011	-	-	-
2	GNBJ -2	0.274	0.094	0.038	0.017	0.007	-	-
3	GNBJ -3	0.315	0.250	0.119	0.049	0.019	-	-
4	GNBJ -4	0.276	0.130	0.048	0.036	0.019	0.015	0.08
5	GNBJ-5	0.285	0.100	0.045	0.008	-	-	-
6	GNBJ-6	0.296	0.119	0.068	0.030	0.09	-	-
7	GNBJ-7	0.295	0.115	0.078	0.010	-	-	-
8	GNBJ-8	0.295	0.130	0.056	0.040	0.024	0.018	0.010
9	GNBJ-9	0.328	0.135	0.064	0.038	0.028	0.020	0.013
10	GNBJ-10	0.228	0.159	0.037	0.059	-	-	-
11	GNBJ-11	0.252	0.077	0.027	-	-	-	-
12	GNBJ-12	0.310	0.259	0.030	-	-	-	-
13	GNBJ-13	0.291	0.117	0.055	0.014	-	-	-
14	GNBJ-14	0.271	0.084	0.024	0.004	-	-	-
15	GNBJ-15	0.285	0.104	0.044	-	-	-	-
16	GNBJ-16	0.275	0.108	0.037	0.025	-	-	-
17	GNBJ-17	0.260	0.074	0.040	0.007	-	-	-
18	GNBJ-18	0.280	0.109	0.040	0.006	-	-	-
19	GNBJ-19	0.273	0.064	-	0.004	-	-	-
20	GNBJ-20	0.283	0.104	0.109	0.017	-	-	-
21	GNBJ-21	0.287	0.119	0.009	0.014	-	-	-
22	GNBJ-22	0.279	0.088	0.019	-	-	-	-
23	GNBJ-23	0.286	0.108	0.024	0.004	-	-	-
24	GNBJ-24	0.269	0.049	0.034	-	-	-	-
25	GNBJ-25	0.309	0.244	0.019	0.044	-	0.010	-
26	GNBJ-26	0.280	0.069	0.024	-	-	-	-
27	GNBJ-27	0.215	0.044			-	-	-
28	GNBJ-28	0.289	0.128	0.050	0.039	0.020	0.016	0.09
29	GNBJ-29	0.312	0.247		-	-	-	-
30	GNBJ-30	0.250	0.049		-	-	-	-

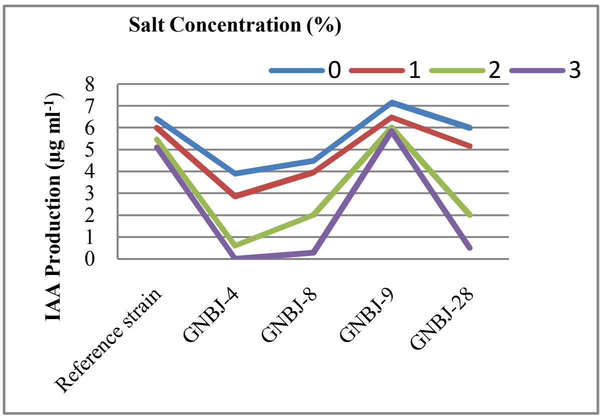
Table-2 Effect of salt concentration on the production of Indole acetic acid (IAA) and Exopolysaccharides (EPS) by Salt tolerant Bradyrhizobial strains

S.No.	Strains	IAA Production (µg ml^{-1})				EPS Production (µg ml^{-1})			
		Salt Concentration (%)				Salt Concentration (%)			
		0	1.0	2.0	3.0	0	1.0	2.0	3.0
1.	Reference strain	6.40	5.98	5.46	5.10	350.00	290.15	180.30	170.05
2.	GNBJ-4	3.90	2.85	0.60	0.00	220.00	100.00	075.00	-
3.	GNBJ-8	4.48	3.95	2.00	0.28	280.00	185.50	140.20	98.10
4.	GNBJ-9	7.15	6.47	6.00	5.85	380.00	315.00	295.10	270.00
5.	GNBJ-28	6.00	5.15	2.00	0.50	320.00	270.20	195.28	130.20
	SED	0.36	0.26	0.33	0.36	0.52	0.86	1.23	1.18
	CD	0.68	0.47	0.65	0.69	0.98	1.87	2.18	2.25

Table-3 Effect of salt concentration on Nodulation, Nodule ARA activity and Nodule N content of Groundnut inoculated with salt tolerant Bradyrhizobial isolates

S.No.	Strains	Nodule number (Number of nodules plant^{-1})				Nodule ARA activity (n moles C_2H_4 h^{-1} g^{-1} nodule)				Nodule N content (%)			
		Salt Concentration (%)				Salt Concentration				Salt Concentration (%)			
		0	1.0	2.0	3.0	0	1.0	2.0	3.0	0	1.0	2.0	3.0
1.	Reference strain	38.00	29.00	20.30	16.10	220.00	195.20	180.00	165.00	6.20	5.25	4.15	3.00
2.	GNBJ-4	25.00	10.00	12.10	10.00	170.40	150.30	124.00	118.20	4.20	4.00	3.15	2.00
3.	GNBJ-8	31.00	24.10	15.00	12.10	192.00	165.00	148.20	135.00	5.10	4.75	3.65	2.40
4.	GNBJ-9	40.00	32.20	23.00	18.00	230.00	200.10	198.00	175.30	6.75	5.37	4.80	3.45
5.	GNBJ-28	36.00	27.40	17.30	14.00	200.10	185.00	167.10	155.00	5.85	5.10	4.00	2.88

The four isolates of *B. japonicum* namely GNBJ-4, GNBJ-8, GNBJ-9 and GNBJ-28 were selected for further study based on their efficiency and salt tolerance level.



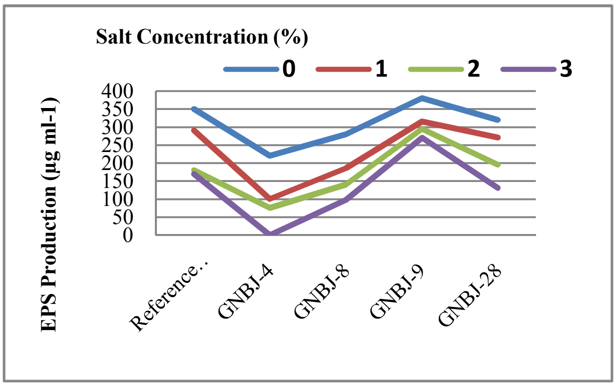


Fig-1 Effect of salt concentration on the production of Indole acetic acid (IAA) and Exopolysaccharides (EPS) by Salt tolerant Bradyrhizobial strains

Effect of salt concentrations on the production of IAA and EPS by salt tolerant Bradyrhizobia isolates

The effect of salt concentrations on the production of Indole acetic acid (IAA) and Exopolysacchride (EPS) by salt tolerant strains were studied and compared with reference strain (Table-2; Fig-1).

Salt concentrations reduced the different level of IAA and EPS productions were significantly. Among the four isolates were tested, the isolate GNBJ-9 produced desirable amount of IAA (5.85µg ml^{-1}) and EPS (270.00 µg ml^{-1}) at 3.0% of salt concentration. The isolates GNBJ-28, GNBJ-8 produced least amount of IAA and EPS i.e 0.50, 0.28 µg ml^{-1} of IAA, 130.20, 98.10 µg ml^{-1} of EPS. The isolate GNBJ-4 did not produced IAA, EPS, at 3.0% of salt concentration. This results conformity of the results of Islam and Ghoulam (1981); Lal and Khanna (1995)

Effect of salt concentrations on nodulation, nodule ARA activity and nodule nitrogen content of groundnut inoculated with salt tolerant Bradyrhizobial isolates

Salt tolerant Bradyrhizobial isolates were subjected to identify the effect of salt concentration on nodulation, nodule ARA activity and nodule N content of inoculated groundnut plants in comparison with reference strain. The results were presented in Table-3.In general the increase in salt concentration from 0% to 3.0% proportionally decreased the nodulation, nodule ARA and nodule N content of groundnut plants. Among the isolates tested, GNBJ-9 performed better, and recorded the highest nodule number of 18.00 plant^{-1}, nodule ARA of 175.30 C_2H_4 h^{-1} g^{-1} and nodule nitrogen content of 3.45% in soils containing 3.0% of salt concentration. Other three isolates (GNBJ-28, GNBJ-8, GNBJ-4 and reference strain) were also performed better. Many researchers have reported that detrimental concentrations of NaCl on chickpea nodulation and N_2 fixation ranged between 8.6 mM (Elsiddig and Elsheikh 1992) and 100 mM (Soussi *et al.,* 1998). Similarly, according to Elsheikh and Wood (1990a) and Soussi *et al.,* (1998), nodulation of chickpea was reduced even by the lowest NaCl concentration (50 mM), while plant dry weight was affected only by 100 mM.

These results from our study confirmed earlier reports that chickpea rhizobia exhibited diversity in their salt tolerance (Zurayk *et al.,* 1998). It has been reported that growth of a number of rhizobia was inhibited by 100 mM NaCl (Zahran 2001). And also reported in several crops Hatice Ogutcu *et al.,* (2010) in chickpea, Rejili *et al.,* (2012) in Tunisa.

CONCLUSION

This study showed that there was considerable variability in the level of stress tolerance of rhizobial isolates obtained from groundnut plant in Tsunami affected area of Cuddalore district of Tamilnadu. Based upon the comparative assessment, we have screened thirty isolates from each salt tolerant study (GNBJ-1 to GNBJ-30), whereas four isolates recorded maximum NaCl concentration up to 3.0% level (GNBJ-4, GNBJ-8, GNBJ-9 and GNBJ-28). Further this four isolates recorded the maximum of IAA, EPS production, number of nodules, nodule ARA activity and nodule N content at 3.0% NaCl concentration respectively. The four isolates of *B. japonicum* namely GNBJ-4, GNBJ-8, GNBJ-9 and GNBJ-28 were selected for further study of mutation efficiency.

REFERENCES

Ali SF, Rawat LS, Meghvansi MK and Mahna SK, 2009. Selection of stress tolerant Rhizobial isolates of wild legumes growing in dry regions of Rajasthan, India. *ARPN Journal of Agricultural and Biological Science* 4 (1): 13-18.

Balasubraminiam V., and Sinhas,. K, 1976. Effects of salt stress on growth, nodulation and nitrogen fixation in cowpea and mung bean. *Physiologia Plantarum* 36, 197-200.

Bernsteinl and Ogatag, 1966. Effects of salinity on nodulation, nitrogen fixation and growth of

Bianco C and Defez R, 2009. *Medicago truncatula* improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. *J Exp Bot* 60:3097-3107.

Bianco C and Defez R, 2009. *Medicago truncatula* improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. *J Exp Bot* 60:3097-3107.

Bolanos L, El-Hamdaoui A and Bonilla I, 2003. Recovery of development and functionality of nodules and plant growth in salt stressed *Pisum sativum–Rhizobium leguminosarum* symbiosis by boron and calcium. *J Plant Physiol* 160:1493-1497.

Bouhmouch I, Souad-Mouhsine B, Brhada F and Aurag J, 2005. Influence of host cultivars and *Rhizobium* species on the growth and symbiotic performance of *Phaseolus vulgaris* under salt stress. *J Plant Physiol* 162:1103-1113.

Brı´gido C, Alexandre A and Oliveira S, 2012. Transcriptional analysis of major chaperone genes in salt-tolerant and salt-sensitive *Mesorhizobia*. *Microbiol Res* 167:623-629.

Brıgido C, Nascimento FX and Duan J, 2013. Expression of an exogenous1-aminocyclopropane-1-carboxylate deaminase gene Salt tolerance of rhizobial populations in *Mesorhizobium* spp. reduces the negative effects of salt stress in chickpea. *FEMS Microbiol Lett* 349:46-53.

Connor JD, Schwabe K, King D, Knapp K, 2012. Irrigated agriculture and climate change: the influence of water supply variability and salinity on adaptation. *Ecol Econ* 77:149-157.

Cordovilla MDP, Ligero F and Lluch C, 1999. Effect of salinity on growth, nodulation and nitrogen assimilation in nodules of faba bean (*Vicia faba* L.). Applied Soil Ecology 11: 1-7.

Domınguez-Ferreras A, Perez-Arnedo R, and Becker A, 2006. Transcriptome profiling reveals the importance of plasmid pSymB for osmoadaptation of *Sinorhizobium meliloti*. J Bacteriol 188:7617-7625.

El-Akhal MR, Rincon A and Coba de la Pena T, 2013. Effects of salt stress and rhizobial inoculation on growth and nitrogen fixation of three peanut cultivars. Plant Biol 15:415-421.

Elanchezhian R, Rajalakshmi, S and Jayakumar V, 2009. Salt tolerance characteristics of *rhizobium* species associated with *Vigna marina.* Indian Journal of Agricultural Sciences 79 (12): 980–85.

Elsiddig A and Elsheikh E, 1992. Effect of salinity on growth and nitrogen yield of inoculated and N fertilized chickpea (*Cicer arietinum*). Archives of Biotechnology 1: 17-28.

Figueira E (2000) Aspectos da tolerancia salinaem *Pisum sativum* L.: influencia da nutricao azotada. http://ria.ua.pt/handle/10773/947.

Grover M, Ali SZ and Sandhya V, 2011. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World J Microbiol Biotechnol* 27:1231-1240.

Hatice Ogutcu, Caner Kasimoglu and Erdal Elkoca, 2010. Effects of *Rhizobium* strains isolated from wild chickpeas on the growth and symbiotic performance of chickpea as (*Cicer arietinum* L.) under salt stress. *Turk J Agric For* 34: 361-371.

Islam R and Ghoulam W, 1981. Screening of several strains of faba bean *Rhizobium* for tolerance to salinity. FABIS-Newsletter (ICARDA) 3: 34.

Lal B and Khanna S, 1995. Selection of salt tolerant *Rhizobium* isolates of *Acacia nilotica*. *World Journal of Microbiology and Biotechnology* 10: 637-39.

Rejili M, Mahdhi M and Fterich A, 2012. Symbiotic nitrogen fixation of wild legumes in Tunisia: soil fertility dynamics, field nodulation and nodules effectiveness. *Agric Ecosyst Environ* 157:60-69.

Singh JS, Pandey VC and Singh DP, 2011. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric Ecosyst Environ* 140:339-353.

Somasegaran P and Hoben HJ, 1994. Handbook for Rhizobia methods in legume-*Rhizobium* technology. Springer, New York

Soussi M, Ocana A and Lluch C, 1998. Effects of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (*Cicer arietinum* L.). *Journal of Experimental Botany* 49: 1329-1337.

St.Clair SB and Lynch JP, 2010. The opening of Pandora's box: climate change impacts on soil fertility and crop nutrition in developing countries. *Plant Soil* 335:101-115.

Yang J, Kloepper JW and Ryu CM, 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant* Sci 14:1-4.

Zahran HH, 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol Mol Biol Rev* 63:968–989

Zurayk R, Adlan M, Baalbaki R and Saxena MC, 1998. Interactive effects of salinity and biological nitrogen fixation on chickpea *(Cicer arietinum* L.) growth. *Journal of Agronomy and Crop Science* 180: 249-258.