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IN VITRO SELECTION FOR SALT TOLERANCE AMONG DIFFERENT STRAINS OF *BRADYRHIZOBIUM JAPONICUM*

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Abstract

An experiment was conducted to study the comparative salt tolerance of 12 different strains of *Bradyrhizobium japonicum* (NIFTAL and USDA series) and strains isolated from locally grown soybean nodules collected from 3 different field locations under different salinity levels of sodium chloride (0, 25, 50, 75 and 100 mM NaCl corresponding to 0, 0.15, 0.29, 0.44 and 0.59 % NaCl, respectively). The rhizobium strains isolated from nodules were found to be highly sensitive when compared to the strains of NIFTAL and USDA series since there was a growth reduction of more than 80 % at the level of 50 mM NaCl. A wide range of variation was observed in the growth response of NIFTAL and USDA series with comparatively less reduction at the level of 50 mM NaCl. The strains TAL-950, TAL-102, TAL-103, TAL-377 were found comparatively salt tolerant.

Introduction

Soil salinity is a worldwide problem since 23 % of the cultivated lands are severely affected by the problem related to high salinity (Massoud, 1981). There is huge phenomenol loss because of the limited crop productivity from the salt affected land proned areas as the growth of most crop plants is substantially reduced under the domain of saline conditions (Maas & Hoffman, 1977; Munns *et al*, 1995; Katerji *et al*, 2000). Among the various crops tested, legumes have generally been found to be more sensitive in saline soils. Soybean is one of the most important leguminous crop plants that has symbiotic association with *Bradyrhizobium japonicum*, a bacterium which displays a remarkable symbiotic interaction with roots of specific legume host that can profitably convert gaseous nitrogen (N_2) into ammonia, which can be used by the plant species. Through a successful symbiosis combination, the crop can develop phenomenol growth even without N fertilization, this definitely implies a significant cost reduction and a lower negative environmental impact affecting on the burgeoning population related to the use of fertilizers. Formation of adequate number of root nodules is essential for establishing growth of soybean plant depending on the symbiotic nitrogen fixation.

The timing and intensity of root nodulation are considered to be significantly affected by the rhizobial population in the soil system, and sufficient rhizobial population in the rhizosphere of seedlings is required for early infection and setting of root nodules (Iizuka *et al.*, 2002). It has been reported that most of these bacteria are very sensitive to soil water deficit (water stress) (Miller, 1996), and high salt concentration in the soil at the free living stage and during the symbiotic process (Lal & Khanna, 1994; Nogales *et al*, 2001). Experiment were carried out for the identification of more salt tolerant strains of *Bradyrhizobium japonicum* for affecting better rhizobium/host symbiosis for crop productivity.

Materials and Methods

Cultures of 12 different rhizobial strains (TAL-102, TAL-103, TAL-377, TAL-379, TAL-950, TAL-952, TAL-953, TAL-355, USDA-110, USDA-136, USDA-138 and USDA-142 obtained from NIFTAL and USDA), and those isolated from locally grown soybean nodules were grown on the yeast mannitol agar (YMA) medium (Vincent, 1970). Culture suspensions were subsequently made and incubated at 30°C for five days. One ml of these culture suspensions were serially diluted from 10^{-1} to 10^{-7} and 100 μl aliquots from each dilution were directly planted on cooled melted YMA medium containing different salinity levels (0, 25, 50, 75 and 100 mM NaCl) separately in series of sterilized Petri dishes. Before the agar gel solidified, the uniform distribution of rhizobial cells was ensured by manually rotating the dishes in all directions, systematically. Observations on the number of colonies formed were recorded after incubation at 30°C for one week. Average number of colonies were calculated for each salinity level, as number of viable cells/ml broth by using the following formula.

Colony forming unit (cfu) of rhizhobia = No. of colonies X dilution factor.

Results and Discussion

Sodium chloride salinity levels markedly decreased rhizobial populations and isolates from the locally grown soybean showed extreme sensitivity at 50 mM NaCl (> 80% reduction) and did not grow at all above the 50 mM NaCl (Table 1). The growth of all the 12 strains of NIFTAL and USDA series declined rapidly as the concentrations of sodium chloride increased from 0 – 75 mM and no growth was observed at 100 mM NaCl. There was substantially a marked difference in the size of colonies formed under control and saline conditions. The size of the colonies under the control treatment were comparatively larger than those formed under saline conditions.

Table 1. Effect of different levels of NaCl salinity on *Bradyrhizobium japonicum* strains isolated from locally grown soybean nodules.

Locations	Control	mM NaCl			
	(0.0)	25	50	75	100
		No. of cells/mlx10^6			
1	6.00	5.45	0.45	0.0	-
	(0.0)	(-9.17)	(-92.50)		
2	11.80	9.45	1.52	0.0	-
	(0.0)	(-19.92)	(-87.12)		
3	9.5	7.4	2.0	0.0	-
	(0.0)	(-22.10)	(-78.95)		

Figures shown in the parentheses give an estimate of % reduction over control.

Table 2. Average number of colonies of *Bradyrhizobium japonicum* strains (10^7 cells/ml) at different levels of NaCl salinity.

	Control	MM NaCl			
	(0.0)	25	50	75	100
TAL – 102	92.33	106.00	100.33	00.0	-
	(0.0)	+14.81)	(+8.66)	-	
TAL – 103	60.00	51.00	48.33	0.63	-
	(0.0)	(-15.0)	(-19.45)	(-99.91)	
TAL – 355	16.27	10.20	5.80	0.87	-
	(0.0)	(-37.31)	(-64.35)	(-94.65)	
TAL – 377	183.00	92.00	93.67	36.00	-
	(0.0)	(-49.73)	(-48.81)	(-80.00)	
TAL – 379	10.27	10.13	9.83	0.0	-
	(0.0)	(-1.36)	(-4.28)	-	
TAL – 950	62.0	61.00	48.67	35.0	-
	(0.0)	(-1.61)	(-21.50)	(-43.55)	
TAL – 952	11.37	13.00	8.47	0.0	-
	(0.0)	(+14.34)	(-25.51)	-	
TAL – 953	74.33	69.33	52.0	0.0	-
	(0.0)	(-6.73)	(-30.04)	-	
USDA –	30.0	40.0	30.00	0.0	-
110	(0.0)	(+33.33)	(0.0)	-	
USDA –	28.0	26.0	14.67	0.0	-
136	(0.0)	(-7.15)	(-47.61)	-	
USDA –	12.67	13.0	0.0	0.0	-
138	(0.0)	(+2.60)	-	-	
USDA –	19.33	15.00	7.33	0.0	-
142	(0.0)	(-22.40)	(-62.08)	-	

LSD for strains 2.16
LSD for treatments 1.25 @ 0.05

Figures shown in the parentheses give an estimate of % reduction over control.

Taking a less than 50 % reduction in the population as a criterion, these strains were tolerant up to 50 mM NaCl except TAL-355, USDA-136 and USDA-142, where a reduction of 64.35 47.61, 62.08 % respectively was observed as compared to control (Table 2). USDA-110 remained unchanged and TAL-102 even showed slight stimulation at 25 and 50 mM NaCl, but there seem to be a sharp cut-off point above these salt concentrations as both these strains failed to survive at 75 mM NaCl. Significant reduction in the number of colonies were observed at 75 mM NaCl, where the growth of all the strains were inhibited and only strains (TAL-950, TAL-103, TAL-377 and TAL-355) were four to grow. The same phenomenon of rhizobial growth reduction was observed by Miller (1996) and Bouchmouch *et al.*, (2001). There was a lag period of 4-6 days at the higher level of salinity (75 mM NaCl). These results are in agreement that at higher level of salts, there could be a substantial lag time before growth commences (Sheikh & Wood, 1989).

The results of the present study would suggest that the growth of *Bradyrhizobium japanicum* was very sensitive to salinity and its strain possibly can not grow in the presence of more than 75 mM NaCl. Saline stress in soil is usually associated with changes in the osmotic pressure due to the varying concentration of different salts, and pH, that may cause the harmful changes in the structure of Lipopolysacchride of bacteria. There might be a possibility of adapting different cellular mechanism under stress and there are reports of using various organic solutes like glutamate, aspartate and glycine betain, by which these bacteria osmotically protected themselves under the saline conditions (Bouchmouch *et al.*, 2001).

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