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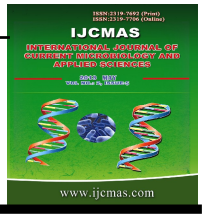

Original Research Article

Performance of Bradyrhizobial isolates under drought conditions

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Keywords

Legume plants; Rhizobium; Drought tolerance; Effect of PEG; Production of EPS.

A B S T R A C T

A favorable environment is vital to legume-*Rhizobium* interaction. Severe environmental conditions are limiting factors to the growth and activity of N2 fixing microorganisms. The legumes grown under arid and semi arid lands require drought tolerant rhizobia to form effective symbiosis. In the present study 30 soybean root nodule isolates of *Bradyrhizobium japonicum* from SAT regions of Tamil Nadu were screened for their drought tolerance potential. Among the 30 isolates, 4 isolates *viz.*, SBJ-2, SBJ-10, SBJ-14 and SBJ-23 were selected as potential drought tolerant isolates. The effect of PEG concentration on IAA, EPS production, nodulation, nodule ARA, nodule N content also studied. SBJ-14 performed better in all the parameters tested.

Introduction

Nitrogen is one of the major important nutrients essential for plant growth. Leguminous plants can obtain nitrogen from air by working symbiotically with special bacteria (*Rhizobium* or *Bradyrhizobium* species) in their root nodules. Severe environmental conditions are limiting factors to the growth and activity of N_2 fixing organisms. Zahran (1999) reported the behavior of some nitrogen fixing systems under severe environmental conditions such as salt stress, drought stress, acidity, alkalinity, nutrient deficiency, fertilizer, heavy metals and pesticides.

Bradyrhizobium japonicum is slow growing root nodule symbiont, which is widely used as an inoculant in soybean fields throughout the world. Generally, soybean inoculated with *Bradyrhizobium japonicum* forms highly effective nodules and frequently increased soybean yields, especially in fields where soybeans are cultivated for the first time (Caldwell and Vest, 1970).

The crop growing environment in the Semi-Arid Tropics(SAT) is highly variable due to erratic spacing and timing of season rainfall. The legumes grown

under semi-arid lands require drought tolerant rhizobia for effective symbiosis (Singh *et al*., 1999). The present study aimed to isolate drought tolerant *Bradyrhizobia* and to characterize their performance under drought conditions.

Materials and Methods

Determination of drought tolerance level of the isolates

The soybean root nodules isolates obtained from 30 different locations of SAT of Tamil Nadu were designated as SBJ-1 to SBJ-30 the isolates were authenticated as *Bradyrhizobium* sp by conducting several confirmative tests. The effect of drought on the growth of *Bradyrhizobium* isolates were studied using polyethylence glycol (PEG) 6000 at different concentration ranging from 0 to 30% in Yeast Extract Mannitol (YEM) broth. The bacterial growth was measured spectrophotometrically at OD 420 nm (Abdel-salam *et al*., 2010).

Effect of PEG concentration on the production of IAA, EPS by drought tolerant isolates.

Among the 30 isolates tested, the best four drought tolerant isolates (SBJ-2, SBJ-10, SBJ-14 and SBJ-23) were selected for further studies. YEM broth was prepared at different level of PEG concentrations *viz*., 0 to 30% and inoculated with drought tolerant isolates. The production of Indole acetic acid (IAA) and Exopolysaccharides (EPS) were estimated by Tien *et al.*, (1979) and Sutherland and Wilkinson, (1971) methods vice-versa.

Effect of PEG concentrations on nodulation, nodule ARA and nodule N content

A pot culture experiment was conducted under split plot design with five replications of ADT-1 soybean inoculated with drought tolerant isolates and the results were compared with reference strain *Bradyrhizobium japonicum* ATCC10324. Nodule production efficiency of the isolates to produce nodules was studied by modified Leonard jar experiment described by Somasegaran and Hoben, (1985). Nodule ARA activity was estimated by Hardy *et al*. (1968) and Nodule N content was done by Humphries, (1956).

Results and Discussion

This study aimed to screen drought tolerant bradyrhizobial isolates and their performance under drought conditions. The effect of drought towards the growth of all the 30 isolates were studied using YEM broth supplemented with PEG. All the isolates grew well in YEM broth without PEG. As the concentration of PEG increased, the growth gets decreased. The isolates SBJ-2, SBJ-10, SBJ-14 and SBJ-23 were found to grow at 30% PEG 6000 at OD 420 nm. The isolate SBJ-14 was able to grow up to 1.53 OD 420 nm at 30% PEG 6000 (Table. 1). These results are conformity with the results of Abdel-Salam *et al*., (2010).

Egamberdiyeva *et al*., (2004) noticed the significant effects on growth, nodule number and yield of soybean after inoculation wit *Bradyrhizobium* species.

A favorable rhizosphere environment is vital to legume - Rhizobium interaction, however the magnitude of the stress effects and the rate of inhibition of the stress. Mild water stress reduces the number of nodules formed on the roots of soybean, while moderate and severe water stress reduces both the number and size of the nodules (Williams and De mallorca, 1984). Athar and Johnson (1993) reported that nodulation, growth and N2 fixation in alfalfa can be improved by inoculating plants with competitive and drought tolerant rhizobia. This could be an economically feasible way to increase the production in water limited environment.

PEG concentrations reduced the level of IAA and EPS production significantly. Among the four isolates tested, SBJ-14 produced desirable amount of IAA (5.00μg ml^{-1}) and EPS (280 - 20μg ml^{-1}) at 30% PEG. Isolates SBJ-2, SBJ-23 produced least amount of IAA and EPS, SBJ-10 did not produced IAA and EPS at 30% PEG (Table. 2).

In general the increase in PEG concentration from 0-30% proportionally decreased the nodulation, nodule ARA and nodule N content of soybean plants. Among the four drought tolerant isolates, SBJ – 14 recorded highest nodule number of 24.00 $plant^{-1}$, Nodule ARA of 192.20 C_2H_4 h^{-1} g^{-1} and nodule nitrogen content of 6.85% in soils containing of 30% PEG concentration (Table. 3).

Table.1 Screening of the strains of Bradyrhizobia for Drought tolerance

S.No.	Strains	YEM liquid medium supplemented with PEG 6000		
		0%	15%	30%
1	SBJ-1	1.26	0.99	0.48
2	SBJ-2	1.80	1.45	1.30
3	SBJ-3	1.40	1.02	0.32
4	SBJ-4	1.82	1.38	1.02
5	SBJ-5	1.68	1.02	0.38
6	SBJ-6	1.36	0.32	0.18
7	SBJ-7	1.42	1.05	0.51
8	SBJ-8	1.60	0.46	0.21
9	SBJ-9	1.63	0.32	0.18
10	SBJ-10	1.74	1.20	1.18
11	SBJ-11	1.14	0.62	0.20
12	SBJ-12	1.24	1.00	0.19
13	SBJ-13	1.77	0.52	0.10
14	SBJ-14	1.98	1.64	1.53
15	SBJ-15	1.88	0.92	0.18
16	SBJ-16	1.16	0.48	0.12
17	SBJ-17	1.25	0.62	0.10
18	SBJ-18	1.08	0.52	0.25
19	SBJ-19	1.57	0.72	0.16
20	SBJ-20	1.52	0.60	0.22
21	SBJ-21	1.48	0.25	0.10
22	SBJ-22	1.10	0.34	0.11
23	SBJ-23	1.78	1.28	1.28
24	SBJ-24	1.48	0.92	0.25
25	SBJ-25	1.47	0.72	0.41
26	SBJ-26	1.36	0.25	0.12
27	SBJ-27	1.41	0.98	0.22
28	SBJ-28	1.25	0.41	0.18
29	SBJ-29	1.89	0.62	0.25
30	SBJ-30	1.28	0.32	0.12

Table. 2 Effect of PEG concentration on the production of Indole acetic acid (IAA) and Exopolysaccharides (EPS) by Drought tolerant *Bradyrhizobia* strains

S.No.	Strains	IAA Production (µg ml^{-1})			EPS Production (µg ml^{-1})		
		PEG Concentration (%)			PEG Concentration (%)		
		0	15	30	0	15	30
1.	Reference strain	5.80	4.70	0.25	320.00	290.20	120.10
2.	SBJ-2	5.25	4.20	0.12	300.00	270.30	110.10
3.	SBJ-10	3.85	2.92	0.00	265.00	100.20	-
4.	SBJ-14	6.10	5.95	5.00	340.00	300.30	280.20
5.	SBJ-23	4.95	4.05	0.10	280.00	210.50	95.30

Table. 3 Effect of PEG concentration on nodulation, nodule ARA and nodule N content of Soybean inoculated with drought tolerant Bradyrhizobia

S. No.	Strains	Nodule number (Number of nodules $plant^{-1}$)			Nodule ARA (n moles C_2H_4 h^{-1} g^{-1} nodule)			Nodule N content (%)		
		PEG Concentration (%)			PEG Concentration (%)			PEG Concentration (%)		
		0	15	30	0	15	30	0	15	30
1.	Reference strain	26.00	21.00	16.00	200.00	189.10	174.30	6.20	5.85	4.60
2.	SBJ-2	22.00	18.20	14.10	197.00	185.00	170.00	5.75	5.00	4.10
3.	SBJ-10	20.00	14.50	12.00	176.25	170.00	160.00	3.50	3.10	2.90
4.	SBJ-14	28.00	25.90	24.00	215.50	200.00	192.20	7.44	7.10	6.85
5.	SBJ-23	21.00	15.10	13.70	187.00	179.20	168.42	4.95	4.50	4.00

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