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Performance of clusterbean rhizobial isolates under drought conditions

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ABSTRACT

The legumes grown under arid and semi-arid lands require drought tolerant rhizobia to form effective symbiosis.Clusterbean growing in drought stress regions leading to poor nodulation, lowering yield and productivity of this commercially important crop. In the present study ten clusterbean root nodule isolates of Rhizobium were isolated from arid and semi-arid regions of Haryana. All ten rhizobial isolates were screened for their drought tolerance potential. Among the 10 rhizobial isolates, five isolates viz., GB-7a, GB-17b, GH-1b and GM-4a were selected as potential drought tolerant isolates. The effect of Polyethylene glycol (PEG) concentration on production of Indole-3-acetic acid was also studied. Rhizobial isolate GB-17b grew up to 40% PEG concentration and produced maximum amount of IAA under drought conditions. Thus, rhizobacterial cultures GB-17b could act as better inoculants for enhancing nodulation as well as plant growth of clusterbean under arid and semi-arid conditions.

Key words: Microorganisms, Drought, PEG, IAA, clusterbean

INTRODUCTION

Clusterbean(*Cyamopsis tetragonoloba* (L.) Taub.) is economically important and drought-tolerant *kharif* legumes growing in arid and semi-arid zone. Clusterbean is a rich source of high-protein and contain mannogalactan gum which is in huge demand in the world market for its multipurpose uses in textiles, foods and cosmetics industries (Kumara *et al.* 2015). This legume is also used as an excellent soil improvement cash crop as nitrogen fixing legume in arid and semi-arid regions (Sharma *et al.* 2015). Drought stress is one of the major limitations to clusterbean crop plant productivity. It is the most common stress affecting plant growth in arid and semi-arid regions. Thus, it is necessary to improve the level of efficiency in plant to capture and use of water and nutrients. Inoculation of plants with native drought tolerant rhizobacteria may increase drought tolerance of plants growing in arid or semi-arid areas (Marulanda *et al.,* 2007). Abiotic stress tolerance in soil rhizobacteria has been studied to provide a biological understanding of the adaptation and survival of living microorganisms in extreme environments. Shortage of water compromises plant and rhizobial growth and is a major cause of nodulation failure and low N_2 fixation. Water stress affects rhizobial morphology, survival, and growth and population structure in soil. Symbiotic N_2 fixation of legumes is also highly sensitive to soil water deficiency. A number of temperate, tropical and shrub legumes exhibit a reduction in nitrogen fixation when subjected to soil moisture deficit. This is due to the fact that water stress affects the formation and longevity of nodules, synthesis of leghaemoglobin and nodule function. In general, the wide range of moisture levels characteristic of ecosystems where legumes have been shown to fix nitrogen suggests that rhizobial strains with different sensitivity to soil moisture can be selected. Studies have shown that sensitivity to moisture stress varies for a variety of rhizobial strains. Thus, it can be assumed that rhizobial strains can be selected with moisture stress tolerance within the range of their legume host. The modification of rhizobial cells by water stress will eventually lead to a reduction in infection and nodulation of legumes. Optimization of soil moisture for growth of the host plant, which is more sensitive to moisture stress than bacteria, results in maximal development of fixed-nitrogen inputs into the soil system by the legume-*Rhizobium* symbiosis(Tate, 1995). Moreover, relatively little information is available concerning how drought affects the symbiotic relationship between nitrogen fixing soil rhizobia and the host plant (Ferguson *et al.,* 2010). The crop growing environment is the semi-arid highly variable due to erratic spacing and timing of season rainfall. The legumes grown under arid and semi-arid lands require drought tolerant rhizobia for

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effective symbiosis. The present study aimed to isolate drought tolerant rhizobia and to characterize their performance under drought conditions.

MATERIALS AND METHODS

Collection of root nodules and isolation of rhizobia The present study was conducted at the Department of Microbiology, CCS Haryana Agricultural University, Haryana, India. A total of 10 soil samples were collected from Bhiwani, Hisar and Mahendergarh districts of Haryana. The seeds of clusterbean variety HG-563 were sown in10 pots containing 2 kg of each soil sample and each pot was containing 3 or 4 clusterbean plants. All the pots supported the growth of clusterbean plants and the nodule formation was observed in all pots. After 45 days of growth, when nodule formation took place on the roots of clusterbean plants, 2 or 3 healthy pink nodules were collected from each plant and surface sterilized by using 0.1% $HgCl_2$ and ethanol as described in material and methods section. The nodules were crushed and streaked on Yeast extract Mannitol medium (YEMA) medium plates containing Congo red dye. The colonies from each nodule were purified by streaking 2-3 times on same media. In total 10 rhizobial isolates were obtained. Pure cultures were obtained with one or more further sub-culturing steps. These rhizobial isolates were further purified and maintained on YEMA slants and were stored at 4^{o}C on slants for further studies.

Authentication of rhizobia by plant infectivity test: All the 10 rhizobial isolates were authenticated by plant infection test using clusterbean seeds (HG-563) under sterilized conditions in coffee cups (Giri and Dudeja, 2013). Seeds were surface-sterilized with a 0.2% $HgCl_2$ followed by 70% ethanol and finally rinsed in five changes of sterile water. Sterilized seeds were inoculated with log phase growing rhizobial cultures (10^4-10^5 cfu/seed) and sown in sterilized coffee cups containing sand in triplicate. Seedlings were watered with sterilized tap water. Nodule formation was scored after 45 days.

Determination of drought tolerance rhizobia: The effect of drought on rhizobia-growth was studied using polyethylene glycol (PEG) 6000. One hundred microliters of YEM overnight culture was transferred to 10ml of the same YM broth supplemented with 10, 20, 30, 40 and 50% PEG, after incubation at 30^{o}C with shaking at 120 rpm for five days the bacterial growth was measured spectrophotometrically The growth was measured spectrophotometrically at OD 420 nm (Abdel-salam *et al.*, 2010).

Effect of PEG concentration on the production of IAA by drought tolerant isolates Among the 10 isolates tested, the four drought tolerant isolates (GB-7a, GB-17b, GH-1b and GM-4a) were selected for further studies. YEM broth was prepared at different level of PEG concentrations *viz*., 0 to 40% and inoculated with drought tolerant isolates. The production of Indole acetic acid (IAA) was estimated by Tien *et al.,* (1979).

RESULTS AND DISCUSSION

Isolation and authentication of rhizobia

A total of ten rhizobial isolates obtained from different nodules of clusterbean roots were characterized by using Gram staining and plant infectivity test. It was observed that all the isolates were found to be Gram negative with small rods. Moreover, the isolates obtained from same soil samples showed identical cell shape and size. For plant infectivity test, all rhizobial isolates were able to nodulate clusterbean plant under sterilized conditions. Thus, on the basis of Gram staining and plant infectivity test, all rhizobial isolates were selected for drought tolerance.

Effect of drought on rhizobial growth and production of IAA: In the present study growth of rhizobial isolates were measured after their exposure to 10 to 50% PEG 6000, for five days. Tolerance to drought stress is a very complex phenomona that involves not only the bacterial ability to tolerate the stress but also the swiftness to respond and adapt to the environmental change. In the current research, diminished growth of rhizobial isolates with rising PEG concentration was observed. As much as 60% of legume production in the developing world occurs under conditions of significant drought stress (Graham and Vance, 2003; Zhang *et al.*, 2007). The effect of drought on growth of rhizobia was studied using polyethylene glycol (PEG) 6000. So all the rhizobial isolates were tested for drought tolerance in YEM broth supplemented with 0, 10, 20, 30, 40 and 50% PEG. Most of the rhizobial isolates were able to grow up to 20% PEG, however, there was drastic decrease in their growth rate with increasing concentration of PEG. Out of 10 rhizobial isolates, only 5 and 1 isolates were able to grow at 30 and 40 % PEG, respectively and none of them was able to grow at 50% PEG concentration (Table 1). The present results are conformity with the results of Abdel-Salam *et al.* (2010). Uma *et al.* (2013) studied 30 isolates using YEM broth supplemented with PEG. All the 30 isolates grew well in YEM broth without PEG. As the concentration of PEG increased, the growth was found to decrease. The isolates SBJ-2, SBJ-10, SBJ-14 and SBJ-23 were found to grow at 30% PEG 6000.

Table 1: Growth of rhizobial isolates at different PEG concentrations

Rhizobial Isolate No.	PEG concentration (%)				
	0	10	20	30	40
GB-7b	0.738	0.251	0.116	0.068	-
GB-16c	0.645	0.268	0.093	0.002	-
GB-17b	0.751	0.370	0.238	0.047	0.025
GB-17d	0.671	0.458	0.084	0.029	-
GB-31a	0.858	0.463	0.230	0.032	-
GH-1b	0.756	0.319	0.209	0.068	-
GH-4d	0.670	0.392	0.155	0.022	-
GH-6a	0.562	0.347	0.104	0.008	-
GM-2a	0.760	0.394	0.078	-	-
GM-4a	0.632	0.258	0.067	0.004	-

The growth and persistence of *Rhizobia* and *Bradyrhizobia* in soils are negatively impacted by drought conditions (Cytryn *et al.*, 2007). A favorable rhizosphere environment is indispensible to legume-*Rhizobium* symbiosis, 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 as well as size of the nodules (Williams and De mallorca, 1984). Athar and Johnson (1996) reported that nodulation, growth and N_2 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 severe drought conditions. The effect of PEG concentrations on production of IAA also studied. It was observed that drought conditions reduced the level of IAA significantly. Among the four isolates tested, GB-17b produced desirable amount of IAA (14.3 gml^{-1}) at 30% PEG. Rhizobial isolate GH-1b produced least amount of IAA, rhizobial isolates GB-7b and GM-4a did not produced IAA at 30% PEG concentration (Table 2). Similar results were observed by Uma *et al.* (2013). In the present work, it was observed that drought conditons affect the production of IAA. The rhizobial isolates survived at higher drought conditions were able to produce maximum amount of Inodle-3-acetic acid. It was concluded from the present study that the only one *Rhizobium* isolates GB-17b survived at highest concentration of PEG i.e. 40% and having significant nodulation as comparison to control. This indicates that these rhizobial isolate had good tolerance to above tested adverse conditions.

Table 2: Effect of different PEG concentration on the production of indole acetic acid (IAA) by drought tolerant rhizobial isolates

Rhizobial Isolate No.	IAA Production (μg ml^{-1})			
	PEG concentration (%)			
	0	10	20	30
GB-7b	05.4	05.0	03.2	0.11
GB-17b	20.5	19.3	17.9	14.3
GH-1b	08.2	07.7	03.9	0.20
GM-4a	06.8	06.2	02.3	0.12

The results of present study suggest that rhizobial isolates GB-17b tolerant to almost all concentration of PEG and produced significant IAA at drought conditions and would be ideal microorganism for further study in pot culture and field experiments to exploit their PGPR potential for a good biofertilizers production.

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