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Abiotic stress tolerance of native *Rhizobium meliloti* strain and its effect on fenugreek (*Trigonella foenum-graecum* L.) growth under saline condition

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Abstract

The present investigation was carried out for screening abiotic stress tolerance potential of the native *Rhizobium melilotii* strain isolated from root nodules of fenugreek (*Trigonella foenum-graecum* L.) plant grown in Rajasthan, India. For assay of sodium chloride salt tolerance by the rhizobium a yeast extract mannitol broth was adjusted for different concentrations of salts by adding sodium chloride to get 300, 200, 100, 50, 20, 10 and 0.0 millimolar (mM) in growth medium. After 7days of incubation absorbance at 540 nm and bacterial population was recorded. For tolerance of pH levels the yeast extract broth media having different pH (4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) was used for enumeration of bacterial growth under laboratory conditions. To determine the impact of salt conc. and native rhizobium strain inoculation on fenugreek plants, a pot experiment was designed using pre-sterilized soil which was alternatively watered with sodium chloride solution of different conc. (300, 200, 100, 50, 20, 10 and 0.0 mM) and normal underground irrigation water. The rhizobial population growth data revealed that the *R. melilotii* preferred lower salt conc. and grow better than at the higher salt conc. of sodium chloride. The data revealed that the native fenugreek *R. melilotii* strain can grow in a yeast extract mannitol broth having pH rage 5-8. The highest seed yield plant^{-1} (2.55 g) was observed with control followed by application of 10 mM (2.43 g) sodium chloride salt solution in pots. The seed yield of fenugreek plants increased from 0.54 g plant^{-1} at 300 mM to 1.36 g plant^{-1} at 100 mM salt conc. and below 50 mM there was no significant difference in the yield of fenugreek.

Key words : Abiotic stress tolerance, fenugreek, *Rhizobium meliloti, Trigonella foenum-graecum.*

Introduction

Soil salinity is a major problem affecting plant growth and crop production. Alleviation of the adverse effects of salinity stress is desirable for development of salinity tolerant crops. Salinity causes osmotic stress, ionic stress and oxidative stress in plants and disturbs cellular homeostasis. Plants' ability to tolerate osmotic stress depends on multiple biochemical pathways that facilitate retention of water and maintains ion homeostasis. Soil salinity is defined as high concentration of solute salts including Na^+, Ca^{++}, and Mg^{++} in soils, causing more than 4 dS m^{-1} for soil electric conductivity, which is comparable to 0.2 MPa of osmotic potential produced by 40 mM sodium chloride (NaCl) in the solution (Rengasamy, 2002). This definition of soil salinity derives from the ECe that significantly reduces the yield of most crops (Rengasamy, 2002; Munns and Tester, 2008). Salinity is considered as one of the major abiotic stresses for crop production worldwide. At present, more than 6% of the world's total land area is adversely affected by salinity. This includes approximately, 20% of cultivated land and nearly half of all irrigated land. In case of India, the area of saline cultivated land is increasing due to canal irrigation and exploitation of ground water resources in arid and semi-arid regions. The problem is that, due to constantly deteriorated quality of irrigation water in agricultural practices and other causes such as industry pollution, salinity has become a more serious issue, posing a great threat to agricultural sustainability. Facing the challenge of sustainable crop production affected by salinity, development of salt-tolerant varieties is considered as one of the most effective ways for effective utilization of salted soil. However, the progress in developing salt-tolerant crops is significantly hampered by the physiologic and genetic complexity of this trait. Hence, a thorough understanding of salt-tolerant mechanisms in different kinds of crops is prerequisite for alleviating salt injury to crop growth and development by improving cultural practices and developing salt-tolerant varieties. Endophytes are micro-organisms including bacteria and fungi that survive within healthy plant tissues and promote plant growth under stress. The source of endophytic

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colonization ranges from transmission via seeds and vegetative planting material to entrance from the surrounding environment such as the rhizosphere and phyllosphere. High similarity between microbes found internally in root tissue with those found in the rhizosphere has been indicated that the rhizosphere may be one of the main sources of endophytic colonization (Lata *et al.,* 2018). Ali *et al.* (2018) showed that inoculation with *Sinorhizobium meliloti* and *Pseudomonas fluorescens* significantly improved the morphophysiological and phytochemical traits of fenugreek (*Trigonella foenum-graecum* L.) under different water availability levels, especially under the conditions of soil water restriction. With this background knowledge, present investigation was carried out to find the sodium chloride salt and pH tolerance potential in the native *R. melilotii* strain isolated from fenugreek plant root nodules.

Materials and methods

Root nodules from healthy fenugreek plants were collected from the fields of ICAR- NRCSS. These root nodules were first washed by running tap water followed by sodium hypochlorite (1 per cent) for 30 seconds and distilled water for 60 seconds and finally washed into ethyl alcohol (70 per cent) for 30 seconds. The nodules were then crushed in a drop of sterile water on a glass slide with help of pre-sterilized scalpel and forceps. This suspension was streaked on congo red yeast extract mannitol agar (CRYEMA) media (Hi-media). The *R. melilotii* culture obtained was purified through repeated sub-culture on YEMA perti-plates. The stock culture of *R. melilotii* was prepared by streaking the YEMA slant test tubes and incubating it at 28 ±2 ^{0}C in a BOD incubator for 48 hours and stored in a refrigerator (4-6^{0}C) for use in the experiment. A bacterial suspension from the stock culture of Rhizobium was inoculated into four replicates of yeast extract mannitol broth in 50 ml Erlenmeyer flasks. The broth was adjusted for different concentrations of salts by adding sodium chloride to get 300, 200, 100, 50, 20, 10 and 0.0 millimolar (mM) of sodium chloride in growth medium. The initial bacterial population was adjusted to get approximately x10^3 colony forming units (cfu) per ml and incubated in a BOD incubator cum shaker at 28 ±2 ^{0}C for 7 days. Observations were recorded for optical density by spectrophotometer at 540 nm wavelength and total viable bacterial population by serial dilution and plating technique. To assay the impact of pH on the growth of *R. melilotii*, the above process was modified using nutrient broth having different pH adjusted by 0.5 M NaOH/HCl solution and pH meter. To determine the impact of salt conc. and rhizobium inoculation on fenugreek plants, a pot experiment was designed using pre-sterilized sandy loam soil. The surface sterilized seeds of fenugreek variety Ajmer fenugreek-3 were soaked with nutrient broth of Rhizobium culture. The treated seeds were dried by using pre-sterilized talc powder and carboxy methyl cellulose mixture under aseptic conditions and sown in pots immediately. After germination the plant population was maintained to 10 per pot through thinning. These posts were alternatively watered with sodium chloride solution of different conc. (300, 200, 100, 50, 20, 10 and 0.0 millimolar) and normal underground irrigation water as per requirement. Plant growth parameters were recorded and mean of three random plants were used for analysis of observation.

Results and discussion

There was increase in absorbance of the fenugreek rhizobium growth medium broth with decreasing sodium chloride concentration from 300mM to 10 mM salt concentration of sodium chloride, which ranged from 0.10 to 0.38 at 540 nm. At higher than 100mM salt conc., the absorbance of growth medium broth was lower than mean absorbance of all salt conc. (Table 1). Similarly, when

Table 1. Effect of salt concentration on native fenugreek rhizobium strain under in-vitro condition

Salt conc. (mM of NaCl)	Absorbance (at 540 nm)	Population count (x 10^5 cfu ml^{-1})
300	0.10	0.18
200	0.12	0.72
100	0.26	2.36
50	0.25	2.08
20	0.35	3.20
10	0.38	3.12
control(0.0)	0.30	2.47
Mean	0.26	2.01
SD	0.09	1.15

these broth cultures were enumerated for Rhizobial population through dilution plate technique, the bacterial population was significantly low at higher salt conc. (300 & 200 mM) and at lower conc. (100-10 mM) the population increased due to better growth condition in the broth (Table 1).The rhizobial population growth data revealed that the native *Rhizobium melliloti* preferred lower salt conc. and grow better than at the higher salt conc. of sodium chloride. The effect of growth medium pH on the fenugreek rhizobium strain is presented in table 2. The lowest bacterial population (0.05 x 10^5 cfu ml^{-1}) was recorded at pH 4.0 followed by pH 9.0 (0.91x10^5 cfu ml^{-1}). It seems that at 4.0 and 9.0 pH, there were no or little growth of fenugreek rhizobium in the broth culture. The maximum population (2.20 x 10^5 cfu ml^{-1}) of the rhizobium strain was at 6.0 pH followed by 7.0 pH. The data revealed that the native fenugreek rhizobium melilotii strain can grow in a yeast extract mannitol broth having pH rage 5-8.

Table 2. Effect of different pH levels on native fenugreek rhizobium strain under in-vitro condition

pH	Absorbance (at 540 nm)	Population count (x 10^5 cfu ml^{-1})
4.0	0.35	0.05
5.0	0.48	0.54
6.0	0.72	2.20
7.0	0.63	2.04
8.0	0.51	1.68
9.0	0.20	0.91
Mean	0.48	1.23
SD	0.18	0.86

The pot studies on effect rhizobium strain on fenugreek growth and yield, indicated a decrease in shoot dry weight and number of pods per plant with higher salt conc. in irrigation water. The number of seeds per pod was lowest (9) at 300 mM and the same was highest (18) at 20 mM sodium chloride salt solution. Similarly, the highest seed yield per plant (2.55 g) was observed with control followed by application of 10 mM (2.43 g) sodium chloride salt solution in fenugreek pots (Table 3). The seed yield of fenugreek plants increased from 0.54 g $plant^{-1}$ at 300 mM to 1.36 g $plant^{-1}$ at 100 mM salt conc. and below 50 mM there was no significant difference in the yield of fenugreek. It is known that salt stress significantly reduces nitrogen fixation and nodulation in legumes. Hashem *et al.* (1998) have proposed that salt stress may decrease the efficiency of the Rhizobium-legume symbiosis by reducing plant growth and photosynthesis, and hence nitrogen demand, by decreasing survival and proliferation of rhizobia in the soil and rhizosphere, or by inhibiting very early symbiotic events, such as chemotaxis and root hair colonization, thus directly interfering with root nodule function. To date, some rhizobial isolates have been shown to grow under high salt conditions (4-5%) (Kucuk *et al.,* 2006). The results of present investigation indicated that *R. melilotii* was able to grow upto 100 mM NaCl containing medium but were unable to grow on higher concentrations (300-200mM), thus showing that the isolate was sensitive to the higher salt level. pH is another important parameter for the growth of the microorganisms. Slight variations in pH of medium might have enormous effects on the growth of organism. Singh *et al.* (2008) reported that *Rhizobium*

Table 3. Effect of salt concentration on fenugreek- rhizobium association and plant growth under in-vitro condition

Salt conc. (mM of NaCl)	shoot Dry weight $plant^{-1}$ (g)	no. of pods $plant^{-1}$	no. of Seeds pod^{-1}	seed yield $plant^{-1}$ (g)
300	1.20	4	9	0.54
200	2.50	5	12	0.90
100	3.08	7	13	1.36
50	5.51	9	16	2.16
20	6.65	9	18	2.04
10	6.22	10	17	2.43
control (0.0)	5.80	8	17	2.55
Mean	4.42	7.43	14.57	1.71
SD	2.12	2.23	3.30	0.78

grows the best at neutral pH *i.e.* 7 and no growth was observed in medium with pH 4.0 and 9.0. These findings are in conformity with previous studies of abiotic stress tolerance by rhizobium strains from different legumes (Kucuk *et al.*, 2006; Singh *et al.*, 2008).

It can be concluded that present investigations ascertained the abiotic stress tolerance potential of native *Rhizobium melilotii* strain from fenugreek root nodules. Further, more elaborate investigations are needed for field application of abiotic stress tolerance potential of the rhizobium strain to host crop.

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