

## Performance of fenugreek bioinoculated with *Rhizobium meliloti* strains under semi-arid condition

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### Abstract

*Rhizobium meliloti* strains were isolated from the fields of S.D. Agricultural University (Gujarat, India) and were maintained in the Congo Red Yeast Extract Mannitol Agar medium. These strains were tested for their effectiveness for fenugreek crop grown under semi-arid condition. Among the six *Rhizobium* strains, FRS-7 strain showed best plant growth parameters like shoot length, shoot dry weight, shoot total nitrogen, root length, root dry weight, root total nitrogen, seed yield, 1000 grain weight, number of root nodules, and nodules fresh and dry weight. The performance of this strain was better as compared to 20 kgN ha<sup>-1</sup> treatment through urea and was even far better over control plot. Seed yields obtained with FRS-7 during two years were 10.14 and 9.66 q ha<sup>-1</sup>; which was about 36.8% and 45.9% high over control. This strain resulted in saving of about 20 kgN ha<sup>-1</sup> accompanied with better crop yield and soil health. Results of the present experiments can be utilized in integrated nutrient management for cultivation of fenugreek in semi-arid areas to provide sustainability to agricultural productivity in such regions.

### Key words

Bacterization, Bioinoculants, Fenugreek, *Rhizobium meliloti*

### Introduction

Fenugreek (*Trigonella foenum-graecium* L.) is a dicotyledonous plant belonging to subfamily Papilionacea, family Leguminosae (=Fabaceae). It is native of mediterranean region and is grown as an important spice and annual legume crop in most part of the world (Petropoulos, 2002). Semiarid regions witness remarkable environmental stress mainly due to erratic rainfall and low soil fertility which result in low crop productivity with high uncertainty. However, fenugreek has emerged as one of the important crops of these areas (Ali *et al.*, 2012) and has attracted attention of producers to meet the manufacturing demands for "functional food" additives and natural health products (Fitzpatrick, 2004).

*Rhizobium* belongs to a well known group of bacteria that act as primary fixer of nitrogen in symbiotic relationship with legume plants (Mishra *et al.*, 2011; Singh, 2013). Microbes used as biofertilizers are generally regarded as

plant growth promoting rhizobacteria (PGPR) that positively influence plant growth by colonizing the plant roots, increased multiplication in rhizosphere, competing with other microflora, counteracting soil borne plant pathogens, increasing the availability of nutrients (nitrogen, phosphorous), and production of plant growth regulators like IAA, gibberellins and cytokinins (Singh, 2013). Nitrogen supply plays an important role in crop production but under intensive agriculture use of chemical fertilizers alone for long period may deteriorate the soil fertility and produce quality. However, use of biofertilizers, in combination with chemical fertilizers, has been recommended for balancing soil fertility and crop productivity (Singh *et al.*, 2013).

*Rhizobium meliloti* is a type of PGPR that shows symbiotic association with root nodules of fenugreek for nitrogen fixation (Ali *et al.*, 2012). Environmental stress are considered major force governing food production in arid and semi-arid regions. Strains of root nodulating rhizobia vary widely in their tolerance to major environmental stresses,

nodulation efficiency, and ability to fix nitrogen (Elboutahiri *et al.*, 2010). Therefore in view of better quality, higher demand, more economic returns, and suitability of various strains of *R. meliloti* to fenugreek, the present study was carried out to test the performance of fenugreek bioinoculated with *Rhizobium meliloti* strains under semi-arid condition.

### Materials and Methods

**Rhizobium strains :** *Rhizobium meliloti* strains FRS-2, FRS-6, FRS-7, FRS-8, FRS-9 and FRS-12 were isolated from root nodules of *Trigonella foenum graecum* (fenugreek) grown in the fields of S.D. Agricultural University, North Gujarat (India).

**Isolation of *Rhizobium* and preparation of cultures :** Fresh and plump root nodules collected from fenugreek plants were surface-sterilized with 75% ethanol and 0.1% mercuric chloride and washed thoroughly with distilled water. *Rhizobium* strain was obtained by streaking the crushed root nodules on CRYEMA medium (mannitol, 10.0 g; yeast extract, 1.0 g;  $K_2HPO_4$ , 0.5 g;  $MgSO_4 \cdot 7H_2O$ , 0.2 g; NaCl, 0.1 g; agar-agar, 15g; distilled water, 1000 ml; pH 7.0) and maintained on CRYEMA slants at 29.4°C (Aneja, 2007). After 2 days of incubation, *Rhizobium* colonies were obtained. These strains were multiplied in 100 ml of the autoclaved CRYEM broth medium autoclaved in 500 ml flasks at 121°C for 30 min) on a rotary shaker at 28 °C for 7 days (Kundu and Gaur, 1980). Purity of the cultures was controlled regularly by repeated streaking. The viable cells in batches of cultures ranged from  $0.6 \times 10^9$ - $1.0 \times 10^9$  cells ml<sup>-1</sup>. These cultures were then used for preparation of bio-inoculant with autoclaved powdered lignite as carrier (Jahuri, 1988).

**Field trials and study of plant growth parameters :** North Gujarat belongs to semi-arid region and receives an average annual rainfall of 550 mm. The experimental plots consisted of sandy loam soil with 83.9% sand, 5.55% silt and 9.83% clay. Field experiments were conducted during two Rabi seasons at the agronomy instructional farm, S.D. Agricultural University (Gujarat). Soil of all the plots, irrespective of treatments, was supplied with 20 kgN ha<sup>-1</sup> (urea) and 40 kgP<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (through single super phosphate) at the time of sowing. Seeds in T1, T2, T3, T4, T5 and T6 were treated respectively with *Rhizobium meliloti* strains FRS-2, FRS-6, FRS-7, FRS-8, FRS-9 and FRS-12. However, seeds of control plots (T8) were not treated with bio-inoculant and soil of T7 were treated with 20kgN ha<sup>-1</sup> (through urea) at 30 DAS. Seeds of fenugreek (*Trigonella foenum graecum*) cv. GM-2 (seed rate 20Kg ha<sup>-1</sup>) were treated with prepared *Rhizobium meliloti* bio-inoculants (@25 g kg<sup>-1</sup> of seeds), dried in shade and sown in respective plots as per treatment (Table 1). The

experiment was conducted in randomised block design with three replications (Panse and Sukhatme, 1957).

Plant height, root length, shoot and root dry weight, total nitrogen in shoots and roots at 4 weeks, number of root nodules, their fresh weight and dry weight at flowering, seed yield and 1000 grain weight of fenugreek crops from various treatment plots and IAA production by *Rhizobium meliloti* isolates were observed. Dry weight of shoots, roots and root nodules were calculated after keeping the samples in a digital oven (NOVA Instruments Private Limited, Ahmedabad, India) at 70°C till a constant weight was achieved. The amount of total nitrogen present in shoots and roots were estimated using micro Kjeldahl method (Humphries, 1956). Statistical analysis was carried out by the method of analysis of variance (Fisher, 1958) and critical difference was calculated at 5% level of significance using M-STATC software.

**IAA production :** Overnight grown bacterial cultures in Luria-Bertani broth at 30°C in dark were removed by centrifugation at 8,000Xg for 10 min. 1 ml of this supernatant was vigorously mixed in 2 ml of Salkowski's reagent (4.5 g of FeCl<sub>3</sub> per liter in 10.8 M H<sub>2</sub>SO<sub>4</sub>) and incubated at room temperature in the dark for 30 min and absorbance was measured at 535 nm (Benizri *et al.*, 1998). Indole acetic acid concentration was determined by comparison with standard curve and the values were expressed in terms of ppm.

### Results and Discussion

Root nodule associated bacteria, isolated from the nodules of fenugreek plants, varied widely for plant growth characters like, plant height, root length, shoot and root dry weight, shoot and root total nitrogen content, and shoot/root mass ratio. Treatment with strain FRS-7 recorded highest plant height (44.53, 46 cm), shoot dry weight (1456.67, 1453.67 mg), and total nitrogen (49.87, 49.77 mg plant<sup>-1</sup>). This strain also resulted in highest root length (26.27, 26.93 cm), root dry weight (681.33, 685.67 mg), and root total nitrogen (16.5, 15.63 mg plant<sup>-1</sup>) for both the years under study. The shoot/root mass ratio obtained in treatments with all the *Rhizobium* strains was higher as compared to control plots, however, it was observed to be highest for the strain FRS-8 (Table 1). Seed treatment of fenugreek seeds with strain FRS-7 also resulted in increase of shoot dry weight, shoot total nitrogen, root dry weight and root total nitrogen by about 83%, 64%, 80%, 83% in comparison to application of 20kgN ha<sup>-1</sup> through urea at 30 DAS. Seed yield, 1000 grain weight number, fresh weight and dry weight of root nodules were higher when fenugreek seeds were treated with the *Rhizobium* strain FRS-7 as compared to control plots, as well as treatment 20 kgN ha<sup>-1</sup> (Table 2).

**Table 1:** Effect of *Rhizobium meliloti* bioinoculants on different growth parameters of fenugreek

Treatments	Shoot						Root						Mass ratio	
	Plant height (cm)		Dry weight (mg)		Total N (mg plant <sup>-1</sup> )		Length (cm)		Dry weight (mg)		Total N (mg plant <sup>-1</sup> )		(Shoot/Root)	
	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09
FRS-2 (T1)	36.97	36.70	1339.00	1344.33	45.23	46.00	21.20	20.37	662.67	666.67	16.33	16.43	2.02	2.02
FRS-6 (T2)	30.73	31.17	1050.67	1047.67	28.50	28.27	17.87	18.07	513.33	514.67	12.60	12.53	2.05	2.03
FRS-7 (T3)	44.53	46.00	1456.67	1453.67	49.87	49.77	26.27	26.93	681.33	685.67	16.50	15.63	2.14	2.12
FRS-8 (T4)	28.47	30.93	1028.67	1040.33	22.63	24.13	17.67	18.10	464.67	464.33	13.20	13.40	2.22	2.24
FRS-9 (T5)	32.53	32.63	1158.67	1161.00	33.20	34.10	19.63	19.40	563.33	559.00	12.70	12.80	2.06	2.08
FRS-12 (T6)	36.57	37.07	1228.67	1236.67	41.57	42.30	18.77	18.60	610.00	616.33	14.80	14.67	2.01	2.01
20 kg N/ha (T7)	40.53	40.57	1209.33	1213.00	32.73	32.17	21.43	21.80	551.00	555.67	13.73	13.83	2.19	2.18
Control (T8)	23.80	24.53	864.67	865.00	16.93	16.07	12.40	12.50	432.67	439.33	6.87	6.70	2.00	1.97
SE (m)	1.75	1.67	8.92	8.77	0.39	1.63	0.72	0.77	3.26	8.95	0.36	0.82	0.01	0.04
CD (5%)	5.316	5.061	27.057	26.588	1.171	4.941	2.177	2.340	9.898	27.159	1.078	2.487	0.039	0.118
CV	8.858	8.269	1.324	1.297	1.976	8.274	6.407	6.863	1.010	2.756	4.615	10.719	1.080	3.226

Values for plant height, root length, shoot and root dry weight, and total nitrogen in shoot and root represent an average of twenty random samples for each treatment and three replications

**Table 2:** Performance of fenugreek crop for seed yield, 1000 grain weight, and nodulation under different treatments

Treatments	Seed yield (q ha <sup>-1</sup> )		1000 grain weight (g)		Root nodules					
					Number		Fresh weight (mg)		Dry weight (mg)	
	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09
FRS-2	9.95	8.55	12.80	12.71	34.37	32.10	302.70	304.53	17.28	17.53
FRS-6	8.34	8.12	11.74	11.75	27.27	25.60	235.10	234.03	12.56	12.37
FRS-7	10.14	9.66	13.35	13.41	39.87	34.57	356.40	364.93	18.51	18.60
FRS-8	8.17	8.21	11.56	11.63	24.53	24.43	205.73	202.07	11.56	11.30
FRS-9	8.45	8.72	12.15	12.17	27.80	28.23	253.33	248.63	12.63	12.53
FRS-12	9.90	8.81	12.71	12.68	32.93	29.37	269.23	264.83	16.52	16.47
20 kg N/ha	10.12	8.74	13.23	13.24	36.50	21.60	272.67	258.80	14.73	14.50
Control	7.41	6.62	9.89	9.87	22.95	16.57	152.90	146.93	7.61	7.37
SE (m)	0.70	0.26	0.07	0.07	4.11	2.52	7.71	6.93	0.21	0.20
CD (5%)	2.135	0.781	0.198	0.210	12.460	7.653	23.393	21.023	0.615	0.621
CV	13.455	5.289	0.927	0.983	23.117	16.455	5.218	4.743	2.457	2.565

**Note:** 1000 grain weight, number of root nodules/plant, nodules fresh and dry weight represents an average of twenty random samples for each treatment and replication (n=3)

The bacterium *Rhizobium meliloti* is defined by an inherent ability to stimulate the development of root nodules in symbiotic association with *Trigonella* (fenugreek) (Jordan, 1984). The amount of indole acetic acid (IAA) produced by the experimental strain (FRS-7), in the present study, was 24.25 ppm. Better performance of FRS-7 treated seeds might be due to the fact that *Rhizobium* inoculation leads to better root development, nodulation and nutrient availability resulting in vigorous plant growth and dry matter production, which in turn results in better flowering and pod formation thereby giving higher yield attributes and yield over control (Mehta and Patel, 2011). The differences

observed among treatments for performance of fenugreek might be due to differences in number of nodules formed on the plant roots and biological nitrogen fixation (El-Kramany *et al.*, 2000).

The semi arid environment is marked by frequent abiotic stresses like drought, salinity and temperature. Sometimes, a given abiotic stress has more than one effect for example, salinity on one side creates water stress and reduces the photosynthetic rate while on the other side it leads to both osmotic and ionic stress in plants (Munns, 2002) as well as in the symbiont. However, normally, *Rhizobium* bioinoculants

obtained from semi-arid and arid regions are well adapted to such adverse environmental conditions. Legume crop generally performs better if *Rhizobium* strains grow rapidly in the rhizosphere and effectively colonize, the root system and show better tolerance level to unfavorable conditions (Abdel-Wahab *et al.*, 2002).

*Rhizobium* belongs to a group of bacteria referred to as “plant growth promoting rhizobacteria (PGPR)” which can affect plant growth and yield by nitrogen fixation, phosphate solubilization, production of IAA, ACC deaminase, chitinase,  $\beta$ -1,3-glucanase, and siderophores these play an important role in rhizosphere and are reported to directly and indirectly facilitate proliferation of plant host (Chandra *et al.*, 2007). *Rhizobium* inoculation of fenugreek has earlier been observed to increase the biomass of the plant and seed production (Poi *et al.*, 1991). Kumar *et al.* (2011) observed efficient colonization of fenugreek roots by PGPR strains which were able to produce IAA,  $\beta$ -1,3-glucanase, and ACC deaminase, resulting in increased vigour index, nodule number and root and shoot biomass and increased grain yield by 35% and 36% over control in fenugreek.

Growth parameters like plant height, number of branches and seed yield were significantly higher under substitution of fertilizer nitrogen with biofertilizer (Dutta *et al.*, 2011). Their results indicate saving of 25% inorganic nitrogen with addition of biofertilizer in fenugreek. Sulfab *et al.* (2011) observed that inoculation of groundnut seeds with *Rhizobium* plus basal application of 20kgN ha<sup>-1</sup> increased shoot and root dry weight, nodulation, early pod formation, shoot N and P accumulation and pod yield over control. *Rhizobium* acts as a bioenhancer and increases the uptake of primary nutrients like nitrogen, phosphorus and potassium by producing IAA, and subsequently increases the plant root system (Etesami *et al.*, 2009). Extracellular polysaccharides excreted by bacteria promote binding of soil particles into small aggregates and thus improve structural integrity of soil. These bacteria improve soil aggregate stability, water infiltration, water holding capacity and thus protect it from erosion and improve the availability of large number of nutrients (Czarnes *et al.*, 2000; Singh and Dhar, 2010).

Application of bioinoculants together with organic manure and chemical fertilizer is known to improve shoot and root fresh and dry weights test weight and overall yield of crop (Singh *et al.*, 2013). Chemical fertilizers are supposed to have deleterious effect on soil fertility leading to unsustainable yields; whereas integration of chemical fertilizers with bio fertilizers may be an amenable strategy in order to maintain soil fertility and sustain crop productivity (Jeyabal *et al.*, 2000). It is therefore concluded that effective use of biofertilizer supplemented with inorganic nutrients play a vital role in increasing and sustaining agricultural

production. Rhizobial strains showing important plant growth promoting attributes proved to be efficient in enhancing various growth parameters of fenugreek and thus, these strains can be useful in formulation of new inoculants for fenugreek.

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