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# PERFORMANCE OF *TRIGONELLA FOENUM-GRAECUM* L. AND ITS *RHIZOBIUM* IN THREE CULTIVATED SOILS OF AJMER

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Performance of *Trigonella foenum-graecum* L. (Fenugreek) and its *Rhizobium*, has been determined in three different cultivated soils of Ajmer, by assessing vegetative, reproductive and nodular characters. Physico-chemical characteristics of the soils revealed that soil of Ghughra was most suitable for overall growth of *Trigonella* because of it's higher macronutrient and organic matter percentage. Characterisation of rhizobial isolates from plants in three soils, indicated occurrence of more than one rhizobial substrain in each type of soil. Among the three studied isolates, the isolate $H_1$ showed better salt and pH tolerance as compared to isolates $B_1$ and $G_1$.

**Key Words** : Fenugreek, *Rhizobium*, Soil Nutrients.

In recent years, there has been a considerable increase in our knowledge on legume-*Rhizobium* symbiosis and the factors that control it. The role of soil in nitrogen fixation has been studied mostly in traditional crops, but little efforts have been made with *Trigonella foenum-graecum* L. (Fenugreek, Methi). It is an annual winter legume, grown in Northern India, while in East Rajasthan where irrigation facilities are available, it is a major winter legume crop. Apart from nitrogen fixation, it is rich in protein (18.6-40.9%) which make it a good vegetable and fodder crop. As a condiment, fenugreek seeds add nutritive value to food and are good appetizer. Therefore, an attempt has been made to evaluate the relative efficacy of the soils of the three sites, by assessing the vegetative, reproductive and nodular characters of *T. foenum-graecum* L. Amongst the three studied rhizobial isolates from plants in the three soils, the rhizobial strain having better salt and pH tolerance were also identified.

## MATERIALS AND METHODS

Seeds of *T. foenum-graecum* L. var. UM 34, were obtained from Dr. S.C. Jain, Department of Botany, University of Rajasthan, Jaipur. Soil samples were collected from the following sites of Ajmer district.

Barlea ($BAS_1$) - 8 km south of Ajmer. Gulab Bari ($GBS_2$) - a locality of eastern Ajmer, Ghughra ($GHS_3$) - 6 km North of Ajmer.

Surface sterilized seeds of *T. foenum-graecum* were sown in earthen pots containing the experimental soils in the Botanical Garden of Mahrishi Dayanand Saraswati University, Ajmer. The climatic conditions during the cultivation period (mean temperature range 17.10°C-21.72°C; humidity range 24.10-64.40) were quite favourable for the growth of *T. foenum-graecum.* Five replicated populations of twenty plants each of *Trigonella,* were raised per soil sample in earthen pots. Sowing of seeds was done in the last week of November'93 and the vegetative (plant height, dry weight, number of lateral roots and leaves) reproductive (number of flowers and pods) and nodular (number of nodules) data were recorded after 85 days of seed sowing. Estimation of total nitrogen content was carried out using micro-Kjeldahl method.

The rhizobial isolates obtained from the effective root nodules were maintained on Yeast Extract Mannitol Agar (YEMA) medium at 28±1°C and were purified (Gaur, 1975). Fifteen rhizobial isolates were subjected to nodulation test under bacteriologically controlled condition and after confirming their nodulation effectivity, they were characterised as per the procedure described by Vincent (1970) and Subba Rao (1989). Their sensitivity towards specific antibiotics was determined using Hi-media antibiotic discs and by assessing the size of bacterial inhibition zone around the disc (Bauer, 1966; PSADST, 1984). Out of the fifteen rhizobial isolates, tolerance of salt [(sodium chloride (NaCl), sodium sulphate ($Na_2SO_4$), Potassium chloride (KCl) and potassium sulphate ($K_2SO_4$)] and pH (3.00-10.00) of three rhizobial isolates ($B_1$, $G_1$ and $H_1$), one from each soil sample was determined using spectrophotometry. The physico-chemical characteristics of the three soil samples were determined as per Trivedi *et al.* (1987).

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Table 1: Chemical characteristics of three types of cultivated soil of Ajmer.

| Type of Soil | Conductivity (moh) | pH | Alkalinity (meq.g.) | Chlorides | Sulphates | Calcium ($mg.g.^{-1}$) | Phos-phorus | Carbon | Organic matter | Sodium (ppm.) | Potassium (ppm.) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $BAS_1$ | $2.52x\ 10^2$ | 7.97 | 0.001 | 1.40 | 7.30 | 19.20 | 13.20 | 16.20 | 29.00 | 0.00 | 48.00 |
| $GBS_2$ | $2.04\ x\ 10^2$ | 8.26 | 0.002 | 2.80 | 5.20 | 22.80 | 18.20 | 18.00 | 31.00 | 0.00 | 60.00 |
| $GHS_3$ | $3.60\ x10^2$ | 8.23 | 0.002 | 3.10 | 4.50 | 30.04 | 42.10 | 24.00 | 41.30 | 92.00 | 143.00 |

* $BAS_1$ = Soil from Barlea.
$GBS_2$ = Soil from Gulabbari.
$GHS_3$ = Soil from Ghughra.
Data represent mean value of 3 replicates per soil sample.

Table 2: Vegetative, reproductive and nodular characteristics of *Trigonella foenum-graecum* L. grown in three types of soil (after 85 days of seed sowing).

| Type of Soil | Shoot length (cm) ± S.E. | Root length (cm) ± S.E. | Number of leaves/ plant ± S.E. | Number of flowers/ plant ± S.E. | Number of pods/ plant ± S.E. | Number of lateral roots/plant ± S.E. | Number of nodules/ plant ± S.E. | Dry weight per plant (mg.) ± S.E. | Total nitrogen (%) content per plant ± S.E. |
|---|---|---|---|---|---|---|---|---|---|
| $BAS_1$ | 27.75 ±0.75 | 22.20 ±0.21 | 15.00 ±0.50 | 18.00 ±0.006 | 12.00 ±0.19 | 44.00 ±0.31 | 16.00 ±0.20 | 240.00 ±0.20 | 6.35 ±0.74 |
| $GBS_2$ | 24.43 ±0.82 | 20.77 ±0.19 | 16.00 ±0.36 | 18.00 ±0.06 | 12.00 ±0.01 | 45.00 ±0.25 | 20.00 ±0.15 | 245.00 ±0.52 | 6.60 ±0.54 |
| $GHS_3$ | 30.90 ±0.74 | 25.55 ±1.50 | 16.00 ±0.30 | 20.00 ±0.37 | 14.00 ±0.04 | 49.00 ±0.24 | 23.00 ±0.35 | 256.00 ±0.64 | 7.00 ±0.49 |

$BAS_1$ = Soil from Barlea.
$GBS_2$ = Soil from Gulabbari.
$GHS_3$ = Soil from Ghughra.
Data recorded on 5 replicated population of (20 plants each) per soil type.

## RESULTS AND DISCUSSION

Results of physico-chemical characteristics of the three soil samples have been tabulated in Table 1.

The observations on plant growth have been presented in Table 2. Relatively, better performance of *T. foenum-graecum* in soil $GHS_3$ as compared to that in soil $GBS_2$ and $BAS_1$, could be ascribed to its higher macronutrients and organic matter percentage. According to Howieson *et al.* (1992) the better performance of plants in the saline - alkaline soil could be due to a higher calcium content of the soil which neutralizes salinity and alkalinity to considerable extent, similar to the observation made in the present study where improved performance of symbionts was recorded in the saline-alkaline soil ($GHS_3$) with relatively high calcium content. The promotory effect on the nodule number, shoot dry weight and total nitrogen content of the *Trigonella* plants raised in soil $GHS_3$, having higher percentage of potassium, calcium, phosphorous and organic matter, supports the earlier findings (Sekhon *et al.*, 1984; Sarkar and Pal, 1986) who have also discussed the positive effect of marco nutrients and organic matter on vegetative, reproductive and nodular characters.

Morphological and cultural characters of all the fifteen rhizobial isolates revealed them as Gram negative, non acid fast, motile and rod shaped which showed visible growth on YEMA medium after 48 h or 72 h of inoculation (Table 3). All the studied rhizobia showed moderate growth in Hofer's alkaline medium, no growth on Glucose Peptone Agar medium and did not produce 3-ketolactose.

All the fifteen isolates showed resistance towards ampicillin (10 mcg), penicillin (10 units) and neomycin (30 mcg). However, unlike other isolates, the rhizobial isolates $B_3$ and $B_4$ exhibited resistance towards tetracy-

Table 3: Morphological and biochemical characters and antibiotic sensitivity of the rhizobial isolates obtained from *T. foenum-graecum l.* raised in three types of soil.

| Types of soil and their rhizobial isolates | | Morphological characters | Biochemical characters | | | | | | | Antibiotic sensitivity DZ of inhibition around disc (cm.) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Growth pattern | Production of hydrogen sulphide | Production of indole | Reduction of nitrate | Production of ammonia | Citrate utilization | Gelatin liquifaction | Starch hydrolysis | Streptomycin (10 mcg)* | Tetracycline (30 mcg) | Neomycin (30 mcg) |
| $BAS_1$ | $B_1$ | +++ | - | + | + | - | + | + | + | 2.00 | 2.20 | 0.80 |
| | $B_2$ | ++ | + | + | + | - | + | + | + | 1.00 | 2.60 | 0.60 |
| | $B_3$ | ++ | - | + | + | + | + | + | + | 1.40 | 1.20 | 0.70 |
| | $B_4$ | ++ | + | + | + | + | + | - | + | 0.80 | 1.00 | 0.60 |
| | $B_5$ | +++ | - | + | + | + | + | - | + | 2.00 | 2.10 | 0.70 |
| $GBS_2$ | $G_1$ | +++ | - | + | + | + | + | - | + | 1.40 | 1.60 | 0.70 |
| | $G_2$ | +++ | - | + | + | + | + | + | + | 1.60 | 1.60 | 0.60 |
| | $G_3$ | ++ | + | + | - | - | + | + | + | 1.40 | 1.60 | 0.60 |
| | $G_4$ | +++ | - | + | + | + | + | + | + | 1.40 | 1.60 | 0.70 |
| | $G_5$ | +++ | - | + | + | - | + | + | + | 1.60 | 2.20 | 0.60 |
| $GHS_3$ | $H_1$ | ++ | - | + | + | + | + | + | + | 2.00 | 2.60 | 0.90 |
| | $H_2$ | ++ | - | + | + | + | - | - | - | 1.00 | 1.80 | 0.90 |
| | $H_3$ | +++ | - | + | + | + | - | + | - | 1.40 | 2.00 | 0.80 |
| | $H_4$ | +++ | - | - | + | - | + | + | + | 1.60 | 1.80 | 1.00 |
| | $H_5$ | +++ | + | + | - | - | + | + | + | 1.40 | 1.60 | 0.80 |

++ = Visible growth after 48 h of inoculation
+++ = Visible growth after 72 h of inoculation
\+ = positive test
\- = negative test

* According to zone size interpretative chart. [Bauer *et al.* (1960), PSADST (1984].
DZ > 11 mm = Streptomycin resistant
DZ > 14 mm = Tetracycline resistant
DZ > 12 mm = Neomycin resistant.
DZ = Diameter of inhibition zone.
mcg = microgram.

cline (30 mcg) and rhizobial isolates $B_2$, $B_4$ and $H_2$ were resistant towards streptomycin (10 mcg) (Table 3). Among 15 rhizobial isolates, only five rhizobial isolates ($B_2$, $B_4$, $G_3$, $H_2$ & $H_5$) did show variations in their physiological characters including antibiotic sensitivity. Such variations were recorded within rhizobial isolates of each type of soil, thereby indicating the possibility of cohabitation of two or three rhizobial substrains in each type of soil.

Data on comparative effect of pH on the growth of three rhizobial isolates clearly indicated no growth at pH - 3.00, whereas maximum growth was recorded at pH-7.00, beyond which a decline in growth was observed and the three isolates were able to survive pH-10.00. The three rhizobial isolates exhibited differential growth pattern under the influence of a range of concentrations of four salts. Amongst the three isolates, isolate $H_1$ could tolerate a wider range of pH and various salt concentrations as compared to isolates $B_1$ and $G_1$, thus could be used for further studies to improve cultivation of *T. foenum-graecum* in saline-alkaline soils of semi-arid region around Ajmer.

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