

The effect of *Rhizobium* inoculation and chemical fertilization on seed quality of fenugreek

M.E. Abdelgani^a, E.A.E. Elsheikh^{a,*}, N.O. Mukhtar^b

^aDepartment of Soil Science, Faculty of Agriculture, University of Khartoum, Shambat, Sudan

^bAgricultural Research Corporation, Wad Madani, Sudan

Received 9 January 1998; received in revised form and accepted 16 March 1998

Abstract

Proximate composition and quality of seeds of two fenugreek cultivars were examined for their response to inoculation, nitrogen fertilization and/or molybdenum application in two field experiments. Mo significantly ($p \leq 0.05$) increased 1000-seed weight and protein content of seeds. N had no significant effect on the quality of the seeds. Inoculation significantly ($p \leq 0.05$) increased fat, fibre and protein contents. The results indicate that fenugreek inoculation with compatible and effective rhizobia can improve the seed composition and quality. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Fenugreek (*Trigonella foenumgraecum* L.), like other legumes, is a good source of dietary protein for consumption by human and animals. The crop has attracted much interest as a cheap source of good protein and as a protein supplement to sorghum and corn flour. Seeds of fenugreek are used locally as a yellow dye, for cosmetics and for medicinal purposes. Fenugreek is a good soil renovator and is widely used as a green manure. The response of fenugreek to fertilization was reported to vary from no response to highly significant depending upon the fertilizer, cultivar and soil type (Saeed and Elsheikh, 1995).

Inoculation of legumes is widely practiced with the objective of increasing production of the legume in question. *Rhizobium* inoculation of fenugreek has been reported to increase seed production (Poi et al., 1991). Fenugreek was reported to fix 48% of its total N during the growing season (Desperrier et al., 1985).

Supplying adequate amounts of high quality protein to the increasing population in developing countries is not an easy endeavor. Inoculation, fertilization and breeding programmes were recently initiated in the Sudan to improve the seed quality and composition of various crops. *Rhizobium* inoculation and N fertilization

were found to improve the protein content and decrease the carbohydrate content of faba bean seeds (Babiker et al., 1995; Elsheikh and Elzidany, 1997). The objective of this study was to assess the effect of inoculation by two *Rhizobium* strains, fertilization with nitrogen and molybdenum on the composition and quality of fenugreek seeds.

2. Materials and methods

2.1. Seeds, *Rhizobium* strains and fertilizers

Seeds of the fenugreek cultivars Baladi and Rubatab were supplied by the Ministry of Agriculture, Sudan. *Rhizobium* strain ENRRI 11 was locally isolated, whereas, strain TAL 380 was supplied by NifTAL Project, Paia, HI. The strains were maintained at 4°C on yeast extract mannitol agar (YEMA) slopes. Nitrogen was applied in the form of urea at the rate of 50 kg N ha⁻¹, whereas molybdenum was applied in the form of ammonium molybdate at the rate of 1.0 kg Mo ha⁻¹.

2.2. Field experiments

The field experiments were carried out at the Demonstration Farm of the Faculty of Agriculture, Shambat, University of Khartoum (latitude 15°40' N,

* Corresponding author.

longitude 32°32' E), for two consecutive seasons during 1994/1995 and 1995/1996 in a factorial design with four replicates. The land was prepared by deep ploughing, harrowing and levelling. Then the area was ridged and divided into 3x4 m plots. Treatments used were:

1. Control (uninoculated).
2. Inoculated with *Rhizobium* sp. strain TAL 380.
3. Inoculated with *Rhizobium* sp. strain ENRRI 11.
4. Fertilized with 50 kg N ha⁻¹.

Each of these treatments was either treated or not treated with 1.0 kg Mo ha⁻¹. Plots were immediately irrigated after sowing and then, subsequently, irrigated at 7-day intervals.

2.3. 1000-seed weight

At harvest, the seeds were carefully cleaned and freed from dirt, stones, chips and other extraneous grain or dirt. From each sample, 1000 seeds were counted randomly in triplicate and their weight was recorded.

2.4. Non-soakers and hydration coefficient

For each treatment, 1000 seeds were selected at random and soaked in tap water at a ratio of 1 part to 4 parts of water for 16 h. The percentage of non-soakers in each sample was calculated after sorting and weighing of non-soaker seeds.

The non-soakers percentage was calculated as follows:

$$\text{Non-soakers \%} = \frac{\text{weight of non-soakers}}{\text{initial weight}} \times 100$$

The hydration coefficient percentage was calculated as follows:

$$\text{Hydration coefficient \%} = \frac{\text{weight of soaked seeds}}{\text{initial weight}} \times 100$$

2.5. Chemical analysis

Seeds were carefully cleaned then ground to pass through a 0.4 mm screen, for proximate analysis, on a dry weight basis. AOAC (1975) methods were followed in the determination of moisture (7.003), petroleum ether extracts (7.048), crude fibre (7.6), ash (14), and crude protein (7.2). Carbohydrate was calculated by difference.

2.6. Statistical analysis

Each sample was analyzed in triplicate and the figures were then averaged. Data were assessed by analysis of variance (ANOVA). The Duncan multiple range test was used to separate means. Significance was accepted at $p \leq 0.05$.

3. Results and discussion

3.1. 1000-seed weight

Rhizobium inoculation or nitrogen fertilization had no significant effect on 1000-seed weight of either of the two fenugreek cultivars (Table 1). Molybdenum significantly ($p \leq 0.05$) increased 1000-seed weight of both cultivars compared to the control. The 1000-seed weight was not affected by: inoculation or N fertilization in either cultivar. In contrast, Poi et al. (1991) found a

Table 1

Effect of molybdenum, nitrogen fertilization and *Rhizobium* inoculation on 1000 seed weight, non-soakers (%) and hydration coefficient of fenugreek seeds (average of two seasons)

Treatments	1000 seed weight		Non-soakers		Hydration coefficient	
	Baladi	Rubatab	Baladi	Rubatab	Baladi	Rubatab
<i>No Molybdenum</i>						
Control	12.28 (±0.03) ^a	12.00 (±0.10) ^a	5.3 (±0.09) ^a	5.0 (±0.04) ^a	214.5 (±0.60) ^a	212.8 (±0.35) ^a
50 kg N/ha	12.27 (±0.03) ^a	12.03 (±0.08) ^a	4.8 (±0.13) ^a	5.0 (±0.03) ^a	216.7 (±1.30) ^a	212.0 (±0.36) ^a
<i>Rhizobium</i> ENRRI 11	12.43 (±0.10) ^a	12.21 (±0.10) ^a	5.0 (±0.09) ^a	4.8 (±0.09) ^a	218.1 (±0.36) ^a	216.8 (±0.52) ^a
<i>Rhizobium</i> TAL 380	12.41 (±0.09) ^a	12.31 (±0.04) ^a	4.7 (±0.04) ^a	5.2 (±0.04) ^a	216.0 (±0.27) ^a	216.6 (±0.26) ^a
Mean	12.35	12.14	4.95	5.00	216.33	214.55
<i>Plus Molybdenum</i>						
Control	13.29 (±0.07) ^b	13.09 (±0.10) ^b	5.5 (±0.04) ^a	4.9 (±0.04) ^a	215.4 (±0.11) ^a	214.3 (±0.17) ^a
50 kg N/ha	13.26 (±0.04) ^b	13.18 (±0.05) ^b	5.1 (±0.03) ^a	5.0 (±0.04) ^a	216.0 (±0.36) ^a	218.6 (±0.87) ^a
<i>Rhizobium</i> ENRRI 11	13.80 (±0.11) ^c	13.87 (±0.04) ^c	5.0 (±0.04) ^a	5.0 (±0.03) ^a	219.0 (±0.36) ^a	217.2 (±0.35) ^a
<i>Rhizobium</i> TAL 380	13.72 (±0.06) ^c	13.93 (±0.03) ^c	4.8 (±0.09) ^a	5.0 (±0.05) ^a	217.3 (±0.17) ^a	217.4 (±0.27) ^a
Mean	13.52	13.52	5.10	4.98	216.93	216.88

Values are means (±SD). Means not sharing a common superscript(s) in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range test.

significant increase in inoculated fenugreek. However, N had no effect on the 1000-seed weight of lucerne (Nayel and Khidir, 1995). Inoculation has been found to increase 1000-seed weight of faba bean (Babiker et al., 1995).

3.2. Non-soakers and hydration coefficient

None of the treatments had any significant effect on the non-soakers or hydration coefficient (Table 1) of fenugreek seeds of either of the two cultivars. In contrast, Elsheikh and Elzidany (1997) found that hydration coefficient of faba bean was significantly affected by the organic, biological and chemical fertilization treatments. The factors that make legumes hard to cook cause serious problems. Generally, hydration coefficient is a very valuable quality factor for consumers and a low hydration coefficient indicates that the seeds are not capable of absorbing water very efficiently.

3.3. Moisture content

The average moisture content of fenugreek seeds was found to be 6.5% in the control treatments (Table 2). The moisture content of fenugreek seeds was reported to vary from 4.3% (Nour and Magboul, 1986) to 10.65% (Abdel-Nabey and Damir, 1990). No treatment was found to affect the moisture content of fenugreek seeds significantly. Varietal differences also were not observed. Generally, the moisture content is affected by the relative humidity of the surrounding atmosphere at the time of harvest and during storage.

3.4. Protein content

Fenugreek seeds were reported to be rich in protein with a well balanced amino acid pattern. The protein

content ranges from 25.4 to 27.3% (Rao and Sharma, 1987; Abdel-Nabey and Damir, 1990). In this investigation, high levels of protein content were observed in the control treatments (Table 2). Inoculation with each of the two strains significantly ($p \leq 0.05$) increased crude protein content of fenugreek seeds over the control. This could probably be attributed to the increase in N_2 -fixing efficiency of inoculated plants where more nitrogen was fixed and translocated to the seeds. Moreover, inoculation enhanced the symbiotic properties of fenugreek plants and better growth and production were obtained by inoculation (Saeed and Elsheikh, 1995). Inoculation has been reported to increase seed protein content of soybean (Mukhtar and Abu Naib, 1987; Regitano et al., 1995), and faba bean (Babiker et al., 1995; Elsheikh and Elzidany, 1997).

Nitrogen fertilizer at the rate of 50 kg N ha⁻¹ slightly increased the protein content of fenugreek seeds but its effect was not significant. This is probably because the fertilizer was applied at sowing and its effect was observed only at early stages of growth and was not extended to the stage of pod filling. Similar results were obtained with soybean (Gaydou and Arrivets, 1983) where nitrogen fertilizer did not affect protein content significantly. However, 50 kg N ha⁻¹ were found to increase protein content of faba bean (Babiker et al., 1995).

3.5. Ash content

The ash content of fenugreek seeds was not significantly affected by any of the fertilization or inoculation treatments (Table 2). The average ash content of fenugreek seeds varied in the range 3.5 to 4.0% for all treatments. The ash content of fenugreek seeds was reported to vary from 3.4% (Abdel-Nabey and Damir,

Table 2

Effect of molybdenum, nitrogen fertilization and *Rhizobium* inoculation on moisture (%), protein (%) and ash (%) of fenugreek seeds (average of two seasons)

Treatments	Moisture (%)		Protein (%)		Ash (%)	
	Baladi	Rubatab	Baladi	Rubatab	Baladi	Rubatab
<i>No Molybdenum</i>						
Control	6.5 (±0.02) ^a	6.5 (±0.02) ^a	32.8 (±0.02) ^a	32.6 (±0.05) ^a	3.7 (±0.02) ^a	4.0 (±0.06) ^a
50 kg N/ha	6.4 (±0.03) ^a	6.6 (±0.04) ^a	32.9 (±0.04) ^a	33.2 (±0.04) ^a	3.6 (±0.04) ^a	3.8 (±0.01) ^a
<i>Rhizobium</i> ENRR1 11	6.9 (±0.07) ^a	6.4 (±0.03) ^a	34.2 (±0.04) ^b	34.4 (±0.01) ^b	3.9 (±0.03) ^a	3.7 (±0.03) ^a
<i>Rhizobium</i> TAL 380	6.8 (±0.04) ^a	6.2 (±0.04) ^a	34.2 (±0.02) ^b	34.3 (±0.04) ^b	4.0 (±0.05) ^a	3.8 (±0.05) ^a
Mean	6.65	6.53	33.5	33.6	3.80	3.83
<i>Plus Molybdenum</i>						
Control	6.5 (±0.02) ^a	6.4 (±0.02) ^a	34.6 (±0.06) ^b	34.6 (±0.04) ^b	3.9 (±0.06) ^a	3.7 (±0.02) ^a
50 kg N/ha	6.3 (±0.03) ^a	6.4 (±0.00) ^a	34.5 (±0.04) ^b	34.5 (±0.01) ^b	3.7 (±0.02) ^a	3.8 (±0.04) ^a
<i>Rhizobium</i> ENRR1 11	6.8 (±0.04) ^a	6.2 (±0.06) ^a	35.6 (±0.03) ^c	35.7 (±0.01) ^c	4.0 (±0.04) ^a	3.6 (±0.02) ^a
<i>Rhizobium</i> TAL 380	6.6 (±0.02) ^a	6.5 (±0.02) ^a	35.6 (±0.02) ^c	35.8 (±0.02) ^c	3.9 (±0.02) ^a	3.9 (±0.01) ^a
Mean	6.55	6.38	35.8	35.2	3.88	3.75

Values are means (±SD). Means not sharing a common superscript(s) in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range test.

1990) to 3.8% (Nour and Magboul, 1986). The ash content of foodstuffs represents the inorganic residue remaining after the organic matter has been burnt. The ash obtained is not necessarily of exactly the same composition as the mineral matter present in the original food as there may be losses due to volatilization or as a result of some interaction between constituents.

3.6. Fat content

Inoculation with each of the two *Rhizobium* strains significantly ($p \leq 0.05$) increased fat content of fenugreek seeds of both cultivars over the control (Table 3). Differences between the two *Rhizobium* strains were not significant. None of the fertilization treatments had a significant effect on fat content. However, N and Mo each slightly increased the fat content of uninoculated fenugreek plants of the two cultivars compared to the control. The effect of inoculation with each of the two *Rhizobium* strains exceeded that of N, Mo or N+Mo application. The fat content of fenugreek seeds remained within the low fat level, even with inoculation treatments which increased the level of fat significantly. Fenugreek seeds are known to have a low crude oil content (El-Shimi et al., 1984; Hemavathy and Prabhakar, 1989). Fats are important dietary constituents, not only because of their high energy value, but also because of the essential fatty acids and vitamins which are associated with the fat of natural foods.

3.7. Crude fibre

Inoculation with each of the two *Rhizobium* strains significantly ($p \leq 0.05$) increased the fibre content of fenugreek seeds of both cultivars over the control (Table 3). Nitrogen and Mo also increased the fibre

content over control, but insignificantly. Varietal differences in fibre content was observed as it was higher in Rubatab than in Baladi cultivar. No significant difference was observed between the effects of the two *Rhizobium* strains. Molybdenum slightly enhanced the effect of inoculation on crude fibre content. According to Nour and Magboul (1986) The fibre content of fenugreek seeds was 6.7% which is comparable to the values of the control treatments of this experiment. The fibre content is an important constituent of human food and animal feed and it is needed in a reasonable proportion as it gives the bulk to the diet and helps in movement of food through the digestive tract.

3.8. Carbohydrate content

The effect of N and Mo on carbohydrate content of uninoculated fenugreek seeds was not significant (Table 3). Inoculation with either of the two inoculum strains significantly ($p \leq 0.05$) decreased the carbohydrate content (Table 3). This result is in line with that of fat, fibre and protein contents where the increase in these constituents due to inoculation was counteracted by the decrease in carbohydrate content.

4. Conclusions

Fenugreek has a potential to fix a substantial amount of atmospheric nitrogen. Inoculation of fenugreek with suitable strains of *Rhizobium* is expected to improve the quantity and quality of the produced seeds and, consequently, the nutritional and economic status of the population will be improved. Inoculation programmes should be adopted in areas with a low soil nitrogen content and deficient in native rhizobia.

Table 3

Effect of molybdenum, nitrogen fertilization and *Rhizobium* inoculation on fat content (%) crude fibre (%) and carbohydrates content (%) of fenugreek seeds (average of two seasons)

Treatments	Fat (%)		Crude fibre (%)		Carbohydrates (%)	
	Baladi	Rubatab	Baladi	Rubatab	Baladi	Rubatab
<i>No Molybdenum</i>						
Control	6.2 (± 0.04) ^a	6.3 (± 0.03) ^a	6.6 (± 0.03) ^a	7.0 (± 0.03) ^a	44.8 (± 0.02) ^a	43.6 (± 0.03) ^a
50 kg N/ha	6.8 (± 0.04) ^a	6.6 (± 0.02) ^a	6.5 (± 0.03) ^a	7.5 (± 0.02) ^{ab}	43.8 (± 0.04) ^a	42.3 (± 0.02) ^a
<i>Rhizobium</i> ENRR1 11	7.4 (± 0.06) ^b	7.4 (± 0.05) ^b	7.9 (± 0.07) ^b	8.5 (± 0.03) ^c	39.7 (± 0.02) ^a	39.6 (± 0.04) ^a
<i>Rhizobium</i> TAL 380	7.5 (± 0.02) ^b	7.5 (± 0.02) ^b	7.9 (± 0.03) ^b	8.5 (± 0.02) ^c	39.6 (± 0.04) ^a	39.7 (± 0.05) ^a
Mean	6.98	6.95	7.23	7.88	42.0	41.3
<i>Plus Molybdenum</i>						
Control	6.6 (± 0.02) ^a	6.7 (± 0.05) ^a	6.7 (± 0.02) ^a	7.4 (± 0.04) ^a	41.7 (± 0.03) ^a	41.2 (± 0.02) ^a
50 kg N/ha	6.8 (± 0.00) ^a	6.7 (± 0.03) ^a	6.9 (± 0.04) ^a	7.8 (± 0.05) ^b	41.8 (± 0.05) ^a	40.8 (± 0.05) ^a
<i>Rhizobium</i> ENRR1 11	7.5 (± 0.03) ^b	7.5 (± 0.02) ^b	7.9 (± 0.04) ^b	8.6 (± 0.05) ^c	38.2 (± 0.06) ^a	38.4 (± 0.01) ^a
<i>Rhizobium</i> TAL 380	7.5 (± 0.03) ^b	7.4 (± 0.00) ^b	7.9 (± 0.04) ^b	8.6 (± 0.03) ^c	38.5 (± 0.06) ^a	37.8 (± 0.03) ^a
Mean	7.10	7.08	7.35	8.10	40.1	39.6

Values are means (\pm SD). Means not sharing a common superscript(s) in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range test.

References

- Abdel-Nabey, A. A. and Damir, A. A. (1990) Changes in some nutrients of fenugreek (*Trigonella foenumgraecum*) seeds during water boiling. *Plant Foods for Human Nutrition* **40**, 267–275.
- AOAC (1975) *Official Methods of Analysis*, 12th ed. Association of Official Analytical Chemists, Washington, DC.
- Babiker, E. E., Elsheikh, E. A. E., Osman, A. J. and El Tinay, A. H. (1995) Effect of nitrogen fixation, nitrogen fertilization and viral infection on yield, tannin and protein content and *in vitro* protein digestibility of faba bean. *Plant Foods for Human Nutrition* **47**, 257–263.
- Desperrier, N., Baccou, J. C. and Sauvaire, Y. (1985) Nitrogen fixation and nitrate assimilation in field grown fenugreek (*Trigonella foenumgraecum*). *Plant and Soil* **92**, 189–199.
- El-Shimi, N. M., Damir, A. A. and Ragab, M. (1984) Changes in some nutrients of fenugreek seeds during germination. *Food Chemistry* **14**, 11–19.
- Elsheikh, E. A. E. and Elzidany, A. A. (1997) Effect of *Rhizobium*, organic and chemical fertilizers on proximate composition, *in vitro* protein digestibility (IVPD), tannin and sulphur content of faba beans. *Food Chemistry* **59**, 41–45.
- Gaydou, E. M. and Arrivets, J. (1983) Effects of phosphorus, potassium, dolomite and nitrogen fertilization on the quality of soybean: yield, proteins and lipids. *Journal of Agricultural and Food Chemistry* **31**, 765–769.
- Hemavathy, J. and Prabhaker, I. V. (1989) Lipid composition of fenugreek (*Trigonella foenumgraecum*) seeds. *Food Chemistry* **31**, 1–7.
- Mukhtar, N. O. and Abu Naib, S. A. (1987) Inoculation of irrigated soybean in the Sudan Gezira. *Journal of Agricultural Science (Cambridge)* **108**, 183–187.
- Nayel, B. A. and Khidir, M. O. (1995) Effect of seed rate and fertilizer on fodder and seed yield of lucerne (*Medicago sativa* L.). *University of Khartoum Journal of Agricultural Sciences* **3**, 24–44.
- Nour, A. A. and Magboul, B. I. (1986) Chemical and amino acid composition of fenugreek seeds grown in Sudan. *Food Chemistry* **22**, 1–5.
- Poi, S. C., Basu, T. K., Behari, K. and Srivastav, A. (1991) Symbiotic effectiveness of different strains of *Rhizobium meliloti* in selecting inoculants for improvement of productivity of *Trigonella foenumgraecum*. *Environment and Ecology* **9**, 286–287.
- Rao, U. P. and Sharma, R. D. (1987) An evaluation of protein quality of fenugreek seeds (*Trigonella foenumgraecum*) and their supplementary effects. *Food Chemistry* **24**, 1–9.
- Regitano, M. A., Carpi, S. M., Camara, G. M., Bagio, C. E. and Marcos, E. A. (1995). Effects of nitrogen sources on soybean (*Glycine max* (L) Merrill) oil characteristics and seed storeability. *Tropical Science*, **35**, 135–140.
- Saeed, H. A. and Elsheikh, E. A. E. (1995) Response of fenugreek (*Trigonella foenumgraecum*) to inoculation as influenced by salinity, soil texture, chicken manure and nitrogen. *University of Khartoum Journal of Agricultural Sciences* **3**, 77–90.