ORIGINAL ARTICLE

Improvement in the nutraceutical properties of fenugreek (*Trigonella foenum-graecum* L.)

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Abstract

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Fenugreek (*Trigonella foenum-graecum* L.) leaves and seeds have been used extensively for medicinal purposes. Fenugreek seed is known to exhibit anti-diabetic and anti-nociceptive properties and effects such as hypocholesterolaemic, anti-cancer and thyroxine-induced hyperglycaemia. Our research objectives have been to identify the chemical constituent(s) responsible for the health effects in human and to develop a strategy for improving these constituents in fenugreek plants. We have observed considerable variability among fenugreek genotypes. They differ in morphology, growth habit, biomass and seed production capability. Chemical constituents of the seed, e.g. saponins, fibre, protein, amino acids and fatty acid contents also differ markedly. This variability is most often overlooked or underestimated in clinical trials. Our research suggests that the genetic variability and the genotype by environmental interaction will play a significant role when the crop is used by the nutraceutical industry in Canada where high quality seed production

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is at present difficult. Our multi-disciplinary approach aims at understanding the processes involved in the genetic improvement of fenugreek and use the new knowledge to improve the crop. We have developed a fenugreek cultivar "Tristar" for western Canada that can produce very high quality forage and will now concentrate on producing cultivars having improved nutraceutical value. Our research results indicate that the variability for important traits in fenugreek have a genetic base, making selection for improved levels of these traits possible.

Key words : Fenugreek, cultivar, Tristar, nutraceutical, chemicals, *Trigonella foenum-graecum* L.

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the legume family. This crop is native to an area extending from Iran to northern India, but is now widely cultivated in China, north and east Africa, Ukraine and Greece (Petropoulos, 2002). In parts of Asia, the young plants are used as potherbs and the seeds as a spice or as herbal medicine (Lust, 1986 and Petropoulos, 2002). The species name "*foenum-graecum*" means "Greek hay" indicating its use as a forage crop in the past (Petropoulos, 2002). According to Lust (1986) fenugreek is one of the oldest known medicinal plants in the recorded history.

Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses (Basch *et al*., 2003). Fenugreek is reported to have anti-diabetic, anti-fertility, anti-cancer, anti-microbial, anti-parasitic and hypo-cholesterolaemic, effects (Al-Habori and Raman, 2002). In India, fenugreek is used as a lactation stimulant (Tiran, 2003). Fenugreek seed in powder or germinated form exhibits anti-diabetic properties (Broca *et al*., 2004; Devi *et al*., 2003; Hannan *et al*., 2003; Tahiliani and Kar, 2003a; Thakaran, 2003 and Vats *et al*., 203), hypocholesterolaemic effect (Suboh *et al*., 2004; Thompson Coon and Ernst, 2003 and Venkatesan, 2003), anti-cancer effect (Devasena and Menon, 2003), effect on thyroxine-induced hyperglycaemia (Tahiliani and Kar, 2003b) and protective effect on ethanol toxicity (Thirunavukkarasu *et al*., 2003). Hooda and Jood (2003), on the basis of their studies on the physiological, rheological and organoleptic characteristics of wheat-fenugreek supplemented blends have found encouraging results with respect to the increase in protein and fat contents of their supplemented blends. Bust enhancing property of fenugreek is also known but Fugh-Berman (2003) correctly points out the lack of clinical evidence to support this claim.

Interest in cultivating this crop with potential nutraceutical value in temperate climates, such as that in western Canada, has increased because the crop is adapted to dryland growing conditions (Moyer *et al*., 2003). This interest in fenugreek is further enhanced by the release of the first forage cultivar "Tristar" developed at the Lethbridge Research Centre (LRC) in close collaboration with researchers from Alberta Agriculture, Food and Rural Development.

Over the past ten years, LRC researchers have shown that fenugreek can be a very useful legume crop for incorporation into short-term rotations (Moyer *et al*., 2003). Tristar fenugreek can produce high yield (Mir *et al*., 1993) and high quality forage, can be grown for hay or silage (Mir *et al*., 1998), contains animal growth promoting substances such as diosgenin not present in other forage legumes, but does not cause bloat (Mir *et al*., 1997) like many forage legumes.

Fenugreek genotypes differ in morphology, growth habit, biomass and seed production capability. Chemical constituents of the seed such as saponins, fibre, protein, amino acids and fatty acid contents also differ markedly (Taylor *et al*., 1997 and Taylor *et al*., 2000). This variability is most often overlooked or underestimated in clinical trials. Our research suggests that the genetic variability and the genotype by environmental interaction will play a significant role when the crop is used by the nutraceutical industry in Canada, where high quality seed production is at

present difficult. Our multi-disciplinary approach aims at understanding the processes involved in the genetic improvement of fenugreek and to use this knowledge to improve the crop.

The new cultivar Tristar requires approximately 120 days to produce high quality seed. However under Prairie conditions growing seasons are short (~ 100 frost free days) and so it is necessary to develop cultivars with short maturity time and high seed yield. Attempt will be made to select for increasing the number of pods per node and the number of flowering nodes per plant to accentuate yield per hectare. Improvement in chemical constituents such as sapogenins, proteins, amino acids, fibers and galactomanans are important goals of our research, which has the potential to enhance nutraceutical qualities of this crop.

Materials and Methods

Genetic investigations include a search for genotypes in the world collections for high yielding, early maturing types and generation of mutants that would combine determinate growth habit with high seed yield. The objectives of our multi-disciplinary research have been to identify the chemical constituent(s) that are responsible for the health effects in human and to develop a strategy for improving these constituents in fenugreek plants.

Fenugreek accessions were procured from Plant Gene Resources of Canada, Saskatoon and from Indian spice markets. Fenugreek seed of four cultivars, namely, Amber, F-70, F-86, L-3314, were obtained from Lethbridge Research Center, AB, Canada for chemical property determinations.

Evaluation of new accessions

Seeds from new accessions collected from PGRC and Indian spice stores were seeded in early May in 2004 at LRC to determine seed yield potential. Eighty-three accessions were planted in an irrigated field while 65 of the accessions having adequate amount of seed were included in a dryland test. In both tests, the genotypes were planted as in a two times replicated randomized complete block design. In each case, plots consisted of a three-meter long single row plot and 120 seeds were planted using a custom built forage seeder. After one month (mid June) the plots were scored for proportion of the row with plants. The irrigated and dryland plots were hand harvested on September 20 and October 11, 2004, respectively. After drying the material for one week indoors, the seed were separated from the rest of the plant, cleaned and weighed. Since all the lines did not have same number of plants the seed yield was adjusted using the observation on proportion of surviving plants in mid June.

Mutation breeding

A mutation breeding study, using Tristar as base population, was initiated in the green house at LRC to look for mutants with desirable and beneficial phenological traits like determinate growth habit and/or high seed yield. EMS was used as the mutagenic agent at the level of 10, 20, 30, 40, 50, 100, 150, 200 and 300 mM concentrations. The seed were pre-soaked in water for 2, 4, 6, 8, 12, 16 and 24 h before applying different concentrations of EMS. Treated seed were planted individually in pots containing soil-free mix and were designated as M_1 plants. After 85 d in the greenhouse set to cycle 16 h long days (22ºC) and 8 h night (15ºC) the plants were desiccated with 0.4% Reglone solution. The plants were then allowed to dry for 10 d before separating the seed for yield determination. Seed from selected M_1 plants were again seeded in pots and allowed to produce M_2 seed.

Determination of crude proteins

Total nitrogen content of a 1 g ground seed sample was determined using Technicon Industrial nitrogen determination procedure 146/71A and the conversion factor of 6.25 was used to calculate the crude protein content (AOAC, 1995).

Determination of sapogenins

Defatted and dried seed powder was transferred into a test tube, sapogenins were extracted with 5 mL of 80% ethanol and hydrolyzed for 2 h

with 2 mL of 1M sulfuric acid in 70% propanol. Water (3 mL) and 50 µg of 6-methyldiosgenin internal standard were added, extracted with methyl *tert*-butyl ether (3 x 1 mL) solvent, evaporated at 30ºC in a Meyer N-EVAP apparatus (Organomation Associates, Berlin, MA), weight of the residue recorded and the residue dissolved in 1 mL of toluene. A portion (2 µL) was analyzed in a GC (Hewlett-Packard 6890) equipped with an HP-5 column (30 m x 0.32 mm i.d.), an HP 6890 series autoinjector, a flame ionization detector (FID, 300ºC), and an electronic gas control. The split/splitless injection port, operated at 250ºC, was equipped with a silanized glass liner (HP part 5181-3316). The sample was injected directly (30 s) at an initial oven temperature of 200ºC and then ramped to 290ºC at a rate of 1ºC min^{-1}. The carrier gas was helium with 2 mL min^{-1} constant flow under electronic pressure control. Retention times and peak area counts were obtained with HP GC ChemStation software (ver. A.05.04). Confirmation of peak identity was obtained by mass spectrometry, using HP 5989A GC-MS as reported previously by Taylor *et al*. (2000). Sapogenin content was expressed as % (w/w) and its composition was expressed as % relative GC peak area.

Results and Discussion

Seed yield

Seed yield of the accessions were variable in irrigated and dryland locations at Lethbridge (Table 1a and b) in 2004. Seed sizes and the color were also variable (Figure 1). Two of the accessions (L3172 and 3177) had determinate growth habit. Accession 3172 was 30 cm in height, matured very early and produced about 1000 kg ha^{-1} seed yield whereas accession 3177 grew only 15 cm in height, matured very early and produced 650 kg ha^{-1} seed yield. Although these determinate lines are not as high yielding as some other lines, they can be used as parents for transferring determinate nature to new cultivars.

Top and bottom five seed yielding accessions in the two growing conditions were different (Table 1a). Only one low yielding accession (L3068) was common among five top and five bottom seed producers. All the top five and four

Table 1a. Top and bottom 5 seed yielding fenugreek lines grown under irrigated and dryland conditions at Lethbridge, Alberta in 2004.

Irrigated		Dryland	
Accessions	**Seed yield (kg ha^{-1})**	**Accessions**	**Seed yield (kg ha^{-1})**
PI 211636	2881	**PI 143504**	4119
L3312	2621	**L3308**	3952
L3671	2452	**F18**	3937
L3720	2391	**PI 138687**	3320
X92-23-3	2374	**F86**	3207
L3678	261	**L3708**	1032
L3673	253	**L3704**	884
L3674	137	**L3702**	823
L3068[1]	127	**L3710**	491
L3675	19	**L3068**	377
Mean	1254	Mean	1990
SE[2]	64	SE	104

[1] **L3068 is the only common line in this summary table.**
[2] **SE = Standard Error**

Table 1b. Five fenugreek lines that were top seed yielding under dryland and low yielding on irrigation or vice versa.

Accessions	Irrigated	Dryland
	Seed yield (kg ha^{-1})	Seed yield (kg ha^{-1})
L3718	737	2194
L3713	937	2564
L3693	1054	2216
L3699	1536	1407
L3720	2391	1731

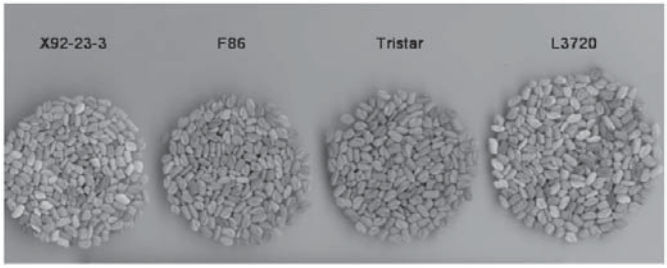

Figure 1. Seed size and color variation among four fenugreek accessions.

Figure 2. Double pods in fenugreek mutant plant.

bottom seed producing lines were different in the two conditions. Fenugreek lines grown under dryland condition produced higher seed yield than under irrigation. This can be attributed to dryland adaptation of the crop. The correlation coefficient calculated between seed yield in dryland and irrigated condition was positive and significant ($r = 0.54$; $p < 0.05$) indicating significant genotypic effect for seed yield. However, there were lines that did well under one condition but not in the other (Table 1b). The above observations indicate presence of genotype x environment interaction.

Table 2a. Observed range of different morphometric parameters of the M_1 mutant plants generated in green house at LRC, Lethbridge, AB, Canada in 2004.

EMS conc. (mM)	Plant height (cm)	No. of nodes	No. of pods	No. of single pods	No. of double pods	Pod length (cm)	No. of seed	Seed weight/ plant (g)
10	18.8-67.6	6-21	6-25	0-14	0-12	0.3-15.9	9-336	0.44-4.2
20	30.3-81.2	8-19	4-18	0-13	0-7	1.3-19.8	68-271	0.56-4.1
30	31.3-74.4	9-24	6-21	0-17	0-7	0.5-19.5	56-306	0.28-2.8
40	32.4-72.2	11-27	5-22	0-17	0-6	0.4-21.6	59-324	0.47-2.8
50	30.2-65.8	5-24	5-27	1-15	0-7	0.2-21.1	3-176	0.03-3.4
100	33.2-72.7	12-23	6-36	1-26	0-9	0.7-24.3	5-190	0.01-2.5
150	28.7-75.1	11-29	8-67	1-29	0-19	0.6-21.6	4-289	0.05-2.4
200	25.7-73.6	7-26	4-90	0-36	0-27	0.2-18.8	5-1352	0.01-10.1
300	7.5-35.7	4-26	0-18	0-7	0-6	0.8-11.4	0-173	0-1.7

Table 2b. Observed range of different morphometric parameters of selected M_2 mutant plants generated in green house at LRC, Lethbridge, AB, Canada in 2004.

EMS conc. (mM)	Plant height (cm)	No. of nodes	No. of pods	No. of single pods	No. of double pods	Pod length (cm)	No. of seed	Seed weight/ plant (g)
10	11.6-42.4	11-44	10-38	4-23	1-13	2.6-6.9	98-388	1.2-4.6
20	6.7-45.6	13-56	2-52	4-34	2-15	1.5-8.6	89-556	1.2-6.1
30	16.7-67.3	18-51	17-52	3-32	2-14	2.3-11.2	98-467	1.2-5.2
40	6.9-45.6	9-41	5-37	1-28	0-8	3.5-13.7	124-756	1.6-8.3
50	33.9-83.4	28-77	23-82	2-44	3-23	1.7-11.5	0-654	1.4-5.8
100	11.9-45.3	9-39	7-41	3-22	0-13	3.2-9.1	0-667	1.2-5.0
150	9.1-35.1	6-29	4-27	4-21	0-5	2.5-10.8	125-636	1.5-5.3
200	17.5-48.9	15-54	10-58	3-34	2-18	3.2-12.8	122-552	1.8-5.9
300	5.6-28.9	3-31	5-48	3-23	0-14	1.9-13.2	163-759	1.1-9.8

Seed yield of EMS treated plants and other morphometric parameters measured from M_1 and M_2 plants are presented in Table 2 a and b. The M_1 and M_2 plants were variable in spite of the fact that M_2 were selected for seed yield. Lower value for range of seed number and seed weight are higher in M_2 compared to M_1. This may be due to the selection practiced for higher seed yield in M_1 generation. Stability of seed yield performance in the mutants will most probably be visible in later generations (M_4 or M_5). In M_2 frequency of double pods increased. Since double pod characteristics (Figure 2) is linked to high diosgenin content, it is expected that some of these mutants may produce more diosgenin in addition to producing high seed yield (Petropoulos, 2002). High diosgenin producing lines will probably be preferred by the nutraceutical industry. Raghuvanshi and Singh (1981) observed high heritability estimates and genetic advance for double pod trait implying that selection will be effective for improvement in this trait.

Chemical constituents

Crude protein content in seed of lines grown in Southern Alberta (Lethbridge) was significantly higher than that of the Indian line (Table 3). Protein content of the four Lethbridge grown lines was not significantly different. This was expected as these lines were selected for their ability to produce high forage yield and had very similar growth habit, morphological features and were grown under

Table 3. Composition of Fenugreek Seeds (%, w/w, dry basis)[1]

Seed components	Fenugreek Lines				
	Amber	F-70	F-86	L-3314	Indian
Crude proteins	31.6±0.8^{a}	28.7±0.3^{b}	30.1±0.5^{ab}	31.6±0.2^{a}	26.0±0.3^{c}
Soluble fiber	18.8±0.2^{b}	21.7±0.3^{a}	16.1±0.3^{c}	18.2±0.3^{b}	17.5±0.8^{bc}
Insoluble fiber	25.8±0.3^{d}	25.8±0.4^{cd}	32.3±0.5^{a}	27.4±0.6^{bc}	28.1±0.1^{b}
Sapogenins	0.4±0.0^{a}	0.3±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}	0.5±0.0^{a}
Diosgenin	47.8±1.6^{a}	41.0±5.1^{a}	43.9±2.9^{a}	44.6±2.1^{a}	43.8±3.2^{a}

[1] **Means sharing the same superscript in a row are not significantly different (p>0.05) from one another.**

Table 4. Diosgenin levels from 10 accessions of fenugreek seed grown in 1998 and 1999 at Saskatoon, SK; Lethbridge, AB and Agassiz, BC. Table adopted from Taylor *et al.* (2002).

	Saskatoon		Lethbridge		Agassiz	
Accession	1998 [a]	1999 [a]	1998 [a]	1999 [a]	1998 [a]	1999 [a]
19062	0.284	0.727	0.420	0.392	0.398	0.923
19063	0.411	0.347	0.642	0.246	0.347	0.458
19064	0.431	0.291	0.400	0.361	0.206	0.333
19065	0.519	0.402	0.478	0.355	0.528	0.391
19066	0.448	0.243	0.473	0.209	0.286	0.373
19067	0.685	0.663	0.453	0.800	0.354	0.906
19068	0.592	0.498	0.522	0.366	0.328	0.464
19069	0.542	0.352	0.539	0.248	0.383	0.302
19070	0.801	0.506	0.467	0.369	0.633	0.543
19071	0.730	0.555	0.535	0.341	0.747	0.613
Mean %	0.544	0.458	0.493	0.369	0.421	0.531

[a] **Average values (N=3) determined by single analysis of three sub-samples of defatted and dried seed material.**

uniform growing conditions. Seed used in this study had a high protein content as compared to 24% observed in seed from Egypt (Gerhartz, 1987), and 26% in seed from India (Sharma, 1986), but they were slightly lower in proteins than the 34% reported by Brummer *et al*. (2003). Differential crude protein content in seed in this experiment cannot be attributed directly to the genotypic potential of the lines. Higher levels of protein in seed grown in southern Alberta may have been due to better nutrient status of the Lethbridge soil compared to the soil where the seed was grown for the spice market in India.

Sapogenin content of Amber (0.4) and the Indian seed (0.5) were significantly higher ($p< 0.05$) than the other three lines tested (Table 3). The lines F-70, F-86 and L-3314 had about 0.3% sapogenin. Diosgenin was found to be the predominant steroidal sapogenins in all lines but were not significantly ($p>0.05$) different from each other in the five lines tested. Diosgenin levels observed in these samples were within the range of values observed earlier by Taylor *et al*. (2002) in a more detailed study. In the Taylor *et al*. (2002) study, the same genotypes were grown in three locations in western Canada in two consecutive years (Table

4). In 1998, Saskatoon and Lethbridge sites produced higher mean value for diosgenin than 1999. Whereas 1999 harvested seed contained higher mean diosgenin than 1998 in Agassiz site indicating genotype x environment interaction for this trait. It is interesting to note that even within a location some genotypes did not follow the general yearly trend. For example, accessions 19062 and 19067 did better in 1999 than 1998 in Saskatoon and Lethbridge, respectively. Reverse trend was noticed for the accessions 19065 and 19070 in Agassiz.

Conclusion

From our field and green house trials we can conclude that there is variability among fenugreek accessions for the traits studied. The accessions differ in their ability to produce good quality seed within 90 to 100 frost-free days. The present set of experiments identified genotypes with determinate growth habit and ability to produce high seed yield under western Canada growing conditions. Genotypes with determinate growth habit by themselves do not seem to be suitable for commercial seed production. These genotypes, however, may be valuable as parent material for improvement of high yielding cultivars in this crop. It is also encouraging to note that mutants are showing signs of high proportion of double or twin pods indicative of high diosgenin content in the seed. If these mutants are stable, we will be able to improve nutraceutical property of fenugreek.

The other important conclusion is that for most traits studied there was evidence of genotype x environment interaction. This is important for a crop that has potential to be used as a nutraceutical. Such interaction indicates that seed produced in all environments will not be of similar quality and all genotypes will not produce high quality seed every year. To maintain high quality in nutraceutical product we need to produce stable cultivars showing least amount of genotype x environment interaction and continue monitoring quality every year.

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