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Genetic variation in fatty acid composition of fenugreek (*Trigonella foenum-graecum* L.) seed oil

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ABSTRACT

An analysis has been carried out to explore the genetic variation in seventeen selected varieties of fenugreek. Sixteen fatty acids were identified in seed oil. Linoleic acid (18:2; n-6) and α-Linolenic acid (18:3; n-3) were the major contributor found in the range of 26-43% in variety Lam Selection. The MUFA content varied from 3.50% in Azad Methi to 19.31 % in Hisar Madhvi. The ratio of n-6 to n-3 fatty acids indicated that fenugreek seed lipids are a good source of PUFA. PCA identified linolenic and oleic acid as the most important traits responsible for variation in presented material and improving quality through breeding. Estimates of genetic distance values showed wide range of variation among the fenugreek genotypes. The Euclidean based UPGMA clustering revealed three real and four singleton clusters. Genetic diversity showed no relationship to geographical origin. Dissimilarities obtained showed a wide variation in oil content and in composition.

Key words: Fatty acids, Fenugreek, Genetic diversity, Oil content, Seed lipids.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herbaceous crop belonging to the *Fabaceae* family indigenous to the eastern shores of the Mediterranean and largely cultivated in India, Saudi Arabia, Egypt, and Morocco (Helambe and Dande, 2012). There are significant numbers of reports stating fenugreek as functional, nutraceuticals and its utilization such as antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant, antidiabetic agent, gastro- and hepato-protective and lactation stimulant in women (Sharma, 1986; Suja *et al.,* 2002; Tiran, 2003; Saxena *et al.,* 2012; Rathore *et al.,* 2013). Extractable oil from fenugreek represents about 6-8% of the seed weight and contains ω-3(n-3), ω-6(n-6), and ω-9 (n-9) fatty acids along with many saponins, alkaloids,and sterols (Heller, 2001; El-Sebaiy and El-Mahdy, 1983). Thus fenugreek seeds may serve to be a beneficial health food if consumed regularly. For formulating an effective breeding programme based on developing fenugreek varieties with desirable fatty acid composition for edible purpose, information regarding heritability, genetic advance and correlation among fatty acid is a pre requisite. Development of underutilized crops is a possible solution for the growing and diversified nutritional needs of growing population.

Hence, present study has been conducted to explore the genetic variation in selected varieties as well as to introduce novelty of oils for developing new cultivars from non-established oil crops for innovative end-uses.

MATERIALS AND METHODS

Fenugreek seeds of seventeen varieties were collected from Gene Bank of ICAR-NRCSS, Tabji, Ajmer. Crop has been raised in farm of ICAR-NRCSS, Tabji, Ajmer in year 2012-13. A detailed description of the materials used in this study is shown in Table 1.

All reagents and fatty acid standards were procured from Sigma-Aldrich, USA and were of analytical HPLC grade. Oil content was extracted using Accelerated Solvent Extraction System (Dionex India Pvt. Ltd.). The Accelerated Solvent Extraction system accelerates the traditional extraction process by using solvent at elevated temperatures and pressures. Pressure is maintained in the sample cell to maintain the heated solvent in a liquid state during the extraction. After heating, the extract is rinsed from the sample cell into a collection vessel. Oil was obtained after evaporating the solvent in rotary evaporator. Thirty gram seed powder was utilized for oil extraction and n-hexane was used as solvent. Extraction was repeated three times. The oil extract was concentrated under vacuum in a rotary evaporator (JSGW, Amballa Cantt, India) at 35°C. Solvent free lipids were weighed to determine the lipid content and then transferred to brown glass vials and stored at -20°C until further analysis.

Methyl esters of fatty acids (FAME) were prepared according to AOCS Method CE 1-62(C.S.A.O., 2009). Diluted FAME were separated on a Agilent Series GC-MS (Agilent, USA; GC -7820 A, MS-5975) equipped with an

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Table 1: List of fenugreek genotypes used in this study.

Varieties	State
Hisar Mukta	Haryana
Hisar Madhvi	Haryana
AFg-4	Rajasthan
AFg-2	Rajasthan
Azad Methi	Uttar Pradesh
Lam selection	Andhra Pradesh
RMt-305	Rajasthan
RMt-303	Rajasthan
AFg-1	Rajasthan
Rajendra Kranti	Bihar
RMt-351	Rajasthan
CO-1	Tamil Nadu
Hisar Sonali	Haryana
GM-2	Gujarat
Pant Ragini	Uttrakhand
RMt-143	Rajasthan
AFg-3	Rajasthan

HP5(Universal column) (30mx0.325mmx0.25µm); Agilent J&W GC column with an auto sampler. A sample of 1 µL was used in split mode (20:1) with an auto sampler. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The column temperature was programmed from 50°C to 280°C with equilibrium time of 3 minutes, held for 30 min. Injector temperatures were set at 250°C.The fatty acids were identified by a comparison of their retention indices and their identification was confirmed by computer matching of their mass spectral fragmentation patterns of compounds in the NIST-MS library and published mass spectra with the help of ChemStation software (Agilent Technologies, USA).

Due to inter-correlation of different fatty acids present in a particular genotype, it is difficult to evaluate the potential of individual genotype for breeding purposes by comparing individual fatty acid values. Hence, several ratios *viz.,* elongation ratio (ER), desaturation ratio (DR), oleic desaturation ratio(ODR) and linoleic desaturation ratio (LDR) were used additionally. The ER estimates the relative weight of the elongation pathway from oleic acid (C18:1) to eicosenoic (C20:1) and erucic acid (C22:1), while the DR estimates the relative weight of the desaturation pathway from oleic acid to linoleic (C18:2) and linolenic acid (C18:3) within the overall fatty acid biosynthetic system. The ER and DR calculation was done as per the formula suggested by Velasco *et. al.,* (1998) while ODR (efficiency of the desaturation from oleic to linoleic acid) and LDR (from linoleic to linolenic acid) are calculated as per Pleines and Friedt (1988).

ER =

(%C20: 1 + % C22:1) / (%C20: 1 + % C22:1 + % C18:1 + % C18:2 + % C18:3)

DR=

(%C18: 2 + % C18:3) / (%C20: 1 + % C22:1 + % C18:1 + %C18:2 + %C18:3)

ODR =

(% C18: 2 + %C18: 3) / (% C18:1 + % C18:2 + % C18:3)

LDR =

(% C18: 3) / (%C18: 2 + %C18: 3)

Data were analysed in completely randomized design and phenotypic and genotypic correlation coefficient for fatty acids were calculated as suggested by Panse and Sukhatme(1978). Experiment was conducted in triplicate. Comparison of fatty acid diversity for 17 varieties was done by Euclidean distance matrix which was prepared using MVSP software version 3.1. The same software was used to perform Principal Component Analysis (PCA) with the adjusted data matrix, x, which consists of n observations (rows) on p variables (columns). Genetic distance calculations was analysed by d matrices for all pairs of genotypes were constructed from the interval and ratio lipid data using the Euclidean distance method (Jerry, 2001). Cluster analysis was performed based on the genetic distance matrices generated by the Euclidean distance method to reveal the patterns of genetic relationships among genotypes.

RESULTS AND DISCUSSION

In fenugreek total oil has been isolated from the seeds through extraction process. In fatty acid profiling sixteen fatty acids were identified in fenugreek seed oil. Analysis of variance as per completely randomized design showed the significant differences among the varieties for total oil content and all its 16 components studied. It clearly shows that variability exists among the varieties for total oil content and all 16 constituent fatty acids (Table 2).

Oil content in the analyzed fenugreek varieties ranged from 4.32 to 11.62% (Table 3). Seeds from variety RMT-351 contained the highest amounts of oil whereas in Hisar Madhvi and Lam selection (respectively 4.32 and 4.36%) lowest contents were observed (Table 3). The average value among all varieties of oil contents was 6.89%. The lipid contents in the analyzed fenugreek varieties were in agreement with the data reported by previous workers (Hemavathy and Prabhakar, 1986).

Among sixteen fatty acids identified in fenugreek seed oil (Table 3), linoleic acid (18:2 n-6) was the major contributor, amount of which ranged from minimum 33.36 % in variety AFg-1 to maximum 43.41 % in variety Lam selection. This was followed by α-Linolenic acid (18:3 n-3), palmitic (16:0), stearic acids (18:0) and oleicacid (18:1 n-9).Other fatty acids*viz.,*Myristic acid (14:0), Pentadecanoic acid (15:0), *cis*-10-Heptadecenoic acid (17:1), Heptadecanoic acid (17:0), *cis*-11,14-Eicosadienoic acid(20:2 n-6), *cis*-11-Eicosenoic acid (20:1 n-9), eicosanoic acid (20:0), heneicosanoic acid(21:0), 13-Docosenoic acid (22:1 n-9), docosanoic acid (22:0) and tetracosanoic acid (24:0) were also identified in the range of 0.10% to 2.01 %. Similar composition of fatty acids was observed in all fenugreek genotypes under study (Table 3).

Table 2: ANOVA for CRD analysis of total oil and oil components

Variables (total oil and oil components)	Common name	abbreviation	Mean sum of square	
			Treatment (16)*	Error (34)*
Total oil %	-	-	10.5570**	0.6133
Tetradecanoic acid	Myristic acid	(14:0)	0.0046**	0.0009
Pentadecanoic acid	Pentadecylic acid	(15:0)	0.0082**	0.0012
Hexadecanoic acid	Palmitic acid	(16:0)	16.1360**	1.0111
Heptadecanoic acid	Margaric acid	(17:0)	0.1087**	0.0039
cis-10-Heptadecenoic acid	10-Heptadecenoic acid	(17:1)	0.0097**	0.0022
Octadecanoic acid	Stearic acid	(18:0)	12.4008**	0.4846
cis-9-octadecenoic acid	Oleic acid	(18:1 n-9)	49.5233**	0.5240
cis-9,12-octadecadienoic acid	Linoleic acid	(18:2 n-6)	19.7873**	3.5904
cis-9,12,15-octadecatrienoic acid	α-Linolenic acid	(18:3 n-3)	35.3105**	2.4648
Eicosanoic acid	Arachidic acid	(20:0)	1.0251**	0.0100
cis-11-Eicosenoic acid	11-Eicosenoic acid	(20:1 n-9)	0.0638**	0.0045
cis-11,14-Eicosadienoic acid	11,14-Eicosadienoic acid	(20:2 n-6)	0.0011**	0.0020
Heneicosanoic acid	Heneicosanoic acid	(21:0)	0.0010**	0.0005
Docosanoic acid	Behenic acid	(22:0)	0.3317**	0.0028
cis-13-Docosenoic acid	Erucic acid	(22:1 n-9)	0.0260**	0.0010
Tetracosanoic acid	Lignoceric acid	(24:0)	0.0216**	0.0022

*Values in parenthesis is degree of freedom

Amount of saturated fatty acid (SFA), Mono-unsaturated Fatty Acids (MUFA), Poly-unsaturated Fatty Acids (PUFA), Essential Fatty Acids (EFA), n-3, n-6 and n-9fatty acids present in 17 fenugreek varieties are shown in Table 4. Similarly the values of elongation ratio(ER), desaturation ratio (DR), oleic desaturation ratio(ODR), and linoleic desaturation ratio (LDR) were also presented in Table 4. Perusal of the data revealed that significant variation was found in SFA content which ranged from 8.43% in Hisar Madhvi to 25.63 % in AFg-3 varieties with an average availability of 19.456%. Among SFA, Palmitic acid contributes maximum, amount of which ranged from 5.08 % in Hisar Madhvi to 13.81 % in AFg-3followed by steric acid (0.0.75% in RMT-351 to 7.62% in Lam Selection (Table 3, 4).

The MUFA content was ranged from3.50 % in Azad Methi to 19.31 % in Hisar Madhvi variety. Among MUFA, oleic acid was predominant fatty acid and member of n-9 family, content of which was ranged from a minimum of 2.53 % (Azad Methi) to maximum of 18.33 % in Hisar Madhvi.While other n-9 member 11-Eicosenoic acid is found below 1% (Table 2 & 3). Similarly, PUFA were the dominant fatty acids in fenugreek seed oil and their contribution was ranging from 66.86% in varietyAFg-1 to 82.17% in variety RMT-143. Among PUFA, level of linoleic acid, is a member of n-6 class, was maximum (43.11%) in Lam selection, while minimum (33.36%) in variety AFg-1. Other n-6 member was 11, 14-Eicosadienoic acid, that ranging between 0.19 % (AFg-4) to 0.27 % (Rajendra Kranti).Content of ±- linolenic acid, which is importent member of n-3 class, was maximum in RMT 143 (40.55%) and minimum in Lam Selection (26.77%). Level of Essential Fatty Acids (EFA) was observed maximum in RMT-143 (82.17%) followed by Pant Ragini (79.97%) while minimum amount of EFA was observed in AFg-1 (66.85 %). All fenugreek seed oils contained large amounts of linoleic acids. Fatty acids *viz.,* eicosanoic acid (20:1) acid and 13-Docosenoic acid (20:1 n-9) showed their presence above 1 % in some varieties. These results are in agreement with Ciftci *et al.,*(2011),except the amount of α-linolenic acid, which was more in our study. Similarly Sulieman *et al.,* (2008) analyzed the composition of fatty acids in Sudan fenugreek and El-Sebaiy and El-Mahdy (1983) analyzed fatty acid composition in Egyptian fenugreek also found less content of linolenic acid as compared to present study. However, content of erucic acid (9.7%) was higher, but results of present study a lower ER value, thus a lower conversion of oleic acid (18:1) to eurcic acid (22:1) (Table-4). Al-Jasass and Al-Jasser (2012) while analyzing chemical composition and fatty acid content of some spices and herbs under Saudi Arabia conditions reported 34.85% linoleic acid and 30.8% linolenic acid in fenugreek similar to present study. Variation in amounts of the fatty acids may be the effect of genotype, growing conditions and the efficacy of the analytical system applied which may lead to incomplete separation of some fatty acids. In present study the set of varieties analyzed represented a large geographical area on India.

The ratios of n-6 to n-3 fatty acids in fenugreek seed oil was found between 1.008 (AFg-1) and 1.62 (Lam selection). Results indicated that fenugreek seed lipids are a good source of PUFA and the oil is rich in n-3 and n-6 content (Table 4). Results are also supported by high LDR value (0.500) as predicted in Table 4. The balance of n-6/n-3 fatty acids is an important determinant in decreasing the risk for coronary heart disease, both in the primary and secondary prevention of coronary heart disease. Increased dietary intake

Table 3: Total oil content and fatty acid profiling of fenugreek varieties

Entries	Total oil (%)	Fatty acids (%)															
		14:0	15:0	16:0	17:0	17:1	18:0	18:1 n-9	18:2 n-6	18:3 n-3	20:0	20:1 n-9	20:2 n-6	21:0	22:0	22:1 n-9	24:0
Hisar Mukta	5.68	0.28	0.20	11.52	0.51	0.31	7.29	6.27	38.05	31.65	1.27	0.67	0.20	0.20	1.07	0.14	0.28
Hisar Madhvi	4.32	0.19	0.20	5.08	0.10	0.30	1.97	18.33	38.12	33.91	0.39	0.58	0.20	0.18	0.12	0.10	0.21
AFg-4	7.00	0.21	0.16	12.77	0.49	0.33	5.77	3.14	40.67	31.95	2.01	0.66	0.19	0.15	0.85	0.11	0.29
AFg-2	7.95	0.27	0.17	11.40	0.43	0.24	5.96	5.15	37.09	34.27	2.02	0.72	0.20	0.14	0.92	0.10	0.33
Azad Methi	4.45	0.26	0.18	12.06	0.49	0.26	6.78	2.53	38.61	33.65	2.07	0.59	0.20	0.17	0.78	0.12	0.36
Lam selection	4.36	0.16	0.21	12.66	0.68	0.46	7.62	4.00	43.41	26.77	1.20	0.75	0.20	0.18	0.95	0.14	0.34
RMt-305	7.31	0.23	0.25	12.59	0.62	0.40	6.55	4.00	41.25	29.54	1.56	0.73	0.20	0.20	1.12	0.24	0.38
RMt-303	8.78	0.18	0.21	11.94	0.58	0.31	6.65	8.00	35.43	32.64	1.98	0.25	0.21	0.20	0.66	0.20	0.30
AFg-1	6.80	0.16	0.20	10.53	0.43	0.26	5.25	12.45	33.36	33.30	1.63	0.24	0.20	0.19	0.89	0.21	0.32
Rajendra Kranti	7.47	0.16	0.15	11.48	0.46	0.27	6.54	7.00	37.47	32.04	1.28	0.57	0.27	0.20	1.03	0.48	0.37
RMt-351	11.62	0.20	0.16	12.24	0.47	0.29	0.75	8.00	39.68	33.65	2.01	0.65	0.24	0.19	0.90	0.19	0.27
CO-1	8.95	0.21	0.04	7.68	0.11	0.39	3.38	10.90	39.88	35.17	0.66	0.61	0.20	0.17	0.22	0.06	0.19
Hisar Sonali	6.35	0.19	0.21	9.25	0.15	0.29	3.20	5.00	40.34	38.82	0.71	0.52	0.20	0.18	0.27	0.18	0.21
GM-2	5.06	0.17	0.28	12.40	0.53	0.32	6.59	5.50	40.70	29.79	1.86	0.45	0.20	0.19	0.54	0.17	0.21
Pant Ragini	7.39	0.20	0.21	8.92	0.16	0.30	3.95	4.00	41.64	38.13	0.75	0.58	0.21	0.20	0.26	0.10	0.15
RMt-143	6.54	0.17	0.24	7.77	0.14	0.32	3.63	3.30	41.42	40.55	0.73	0.54	0.20	0.21	0.30	0.15	0.11
AFg-3	7.05	0.16	0.12	13.81	0.47	0.26	7.02	4.60	36.28	32.17	1.93	0.55	0.20	1.37	0.62	0.18	0.14
Average	6.89	0.20	0.19	10.83	0.40	0.31	5.23	6.60	39.02	33.41	1.42	0.57	0.21	0.25	0.68	0.17	0.26
CV (%)	27.24	61.56	28.92	21.42	47.66	18.36	38.88	61.56	6.58	10.27	41.31	25.49	9.32	10.31	49.17	55.19	32.46
CD	1.299	1.201	0.058	1.669	0.103	0.078	1.155	1.201	3.144	2.605	0.166	0.112	0.075	0.037	0.088	0.052	0.077
Error CV (%)	11.37	10.97	18.7	9.28	15.58	15.13	13.31	10.97	4.85	4.7	7.07	11.84	21.69	11.98	7.84	18.67	17.74

Table 4: Different group of fatty acids and utility ratios of fatty acid found in different varieties of fenugreek

Entries	SFA	MUFA	PUFA	EFA	n-3	n-6	n-9	n-6/n-3	ER	DR	DR/ER	ODR	LDR
Hisar Mukta	22.617	7.391	69.901	69.901	31.652	38.249	7.080	1.208	0.010	0.908	87.059	0.917	0.454
Hisar Madhvi	8.432	19.313	72.223	72.223	33.907	38.317	19.013	1.130	0.007	0.791	105.938	0.797	0.471
AFg-4	22.712	4.239	72.809	72.809	31.952	40.857	3.910	1.279	0.010	0.949	95.183	0.959	0.440
AFg-2	21.641	6.213	71.560	71.560	34.270	37.290	5.970	1.088	0.010	0.923	87.913	0.933	0.480
Azad Methi	23.152	3.508	72.463	72.463	33.650	38.813	3.246	1.153	0.009	0.957	102.238	0.966	0.466
Lam selection	24.009	5.354	70.385	70.385	26.774	43.612	4.893	1.629	0.012	0.935	79.475	0.946	0.381
RMt-305	23.490	5.374	70.993	70.993	29.543	41.450	4.974	1.403	0.013	0.934	73.527	0.947	0.417
RMt-303	22.705	8.756	68.280	68.280	32.637	35.643	8.451	1.092	0.006	0.890	151.841	0.895	0.479
AFg-1	19.605	13.158	66.857	66.857	33.297	33.560	12.897	1.008	0.006	0.838	150.389	0.843	0.500
Rajendra Kranti	21.664	8.324	69.785	69.785	32.043	37.742	8.053	1.178	0.013	0.896	66.481	0.909	0.461
RMt-351	17.195	9.130	73.566	73.566	33.646	39.920	8.839	1.186	0.010	0.892	88.151	0.902	0.459
CO-1	12.672	11.960	75.245	75.245	35.170	40.075	11.570	1.139	0.008	0.866	112.215	0.873	0.469
Hisar Sonali	14.359	5.990	79.360	79.360	38.820	40.540	5.700	1.044	0.008	0.933	113.200	0.941	0.490
GM-2	22.765	6.435	70.698	70.698	29.793	40.905	6.119	1.373	0.008	0.920	114.442	0.928	0.423
Pant Ragini	14.802	4.980	79.978	79.978	38.131	41.847	4.680	1.097	0.008	0.945	117.534	0.952	0.478
RMt-143	13.304	4.310	82.170	82.170	40.547	41.623	3.990	1.027	0.008	0.954	119.004	0.961	0.495
AFg-3	25.630	5.591	68.654	68.654	32.174	36.480	5.332	1.134	0.010	0.928	94.068	0.937	0.470
Mean	19.456	7.649	72.643	72.643	33.412	39.231	7.336	1.186	0.009	0.909	103.450	0.918	0.461

Table 5: Correlations among total oil and major fatty acids in fenugreek varieties

	Total oil %	16:0	18:0	18:1	18:2	18:3	20:0
Total oil %	1	0.131034	-0.41456	0.032769	-0.17044	0.204771	0.24555
16:0		1	0.66497**	-0.60459*	-0.06743	-0.63046**	0.846507**
18:0			1	-0.47378	-0.12629	-0.61089**	0.460875
18:1 n-9				1	-0.46157	0.011559	-0.37413
18:2 n-6					1	-0.00148	-0.33515
18:3 n-3						1	-0.46331
20:0							1

*, ** Means significant at P0.05 and 0.01 respectively

of linoleic acid leads to oxidation of low-density lipoprotein (LDL), platelet aggregation, and interferes with the incorporation of eicosapentaenoic acid (EPA) in cell membrane phospholipids. Both omega-6 and omega-3 fatty acids influence gene expression. Dietary intake of omega-3 fatty acids may prevent the development of disease, particularly in persons with genetic variation (Artemis and Simopoulos 2008).

A lower ratio of n -6/ n-3 is desirable to reduce the risk of some chronic diseases, while the optimal ratio of n -6 to n-3 is presumed to be between 1 and 5 (Simopoulos *et al.,* 1999). This ratio was reported as 1.8 by El-Sebaiy and El-Mahdy, (1983). In the current western diet the ratio of n -6 to n -3 is in the range of 10 to 20 (Maillard, 2002). In this perspective fenugreek seed lipids could be a useful ingredient to control it in the diet. In the light of above facts fenugreek seed lipids may emerge as a potential source to control n-6: n-3fatty acids in human diet. Mustard oil also has ideal n-6:n-3 (1.2) but contained significant amount of erucic acid. The fenugreek seed lipids apart from having near to ideal n-6: n-3 with negligible erucic acid.

Values of ER and DR with average values for the different varieties are shown in Table 4. Varieties RMt-303 and AFg-1 showed minimum ER value (0.006), while maximum (0.013) value observed in RMt-305 and Rajendra Kranti. This lower ER estimates the relative weight of the elongation pathway from oleic acid to longer chain fatty acids i.e. eicosenoic and erucic acid, which are less desirable in food. Value of DR was however, maximum in Azad Methi (0.957) and minimum in Hisar Madhavi (0.791) with an average of 0.909. DR is an estimates of relative weight of the desaturation pathway from oleic acid to PUFA *i.e.* linoleic and linolenic acid which are more desirable in food for n-3 and n-6 content. The Ratio of DR to ER was maximum in RMT-303 and AFg-1 being observed 151.84 and 150.38 respectively. Higher DR, ODR and LDR are the indicator of the efficiency of desaturation systems from C18:1 to C18:2, and from C18:2 to C18:3, respectively. Variety AFg-1, Hisar Sonali and RMT-143, showed high LDR (0.500, 0.490 and 0.495) and ODR (0.843, 0.941 and 0.961) respectively. Variety RMT-143 showed maximum PUFA (82.17%) with the higher side combination of DR, ODR and LDR value 0.954, 0.961 and 0.495 respectively. Contrary to this, higher efficiency of the elongation pathway in RMT-305 led to a considerably lower side (29.54%) α-linolenic acid content. Similar pattern was observed in mustard genotypes (Velasco *et al.,* 1998).

In correlation studies (Table 5), a significant (P>0.01) positive correlation was found among palmitic acid with stearic acid and arachidic acid while a significant negative correlation was found with linolenic acid. A significant (P>0.05) negative correlation was also observed between stearic acid and oleic acid. A negative correlation between stearic acid and oleic acid, oleic acid and linoleic acid, linolenic acid and archidic acid were also observed. While stearic acid was observed positively correlated with archidic acid.

PCA analysis of fatty acids are presented in Table 6. The first principal component accounted for 46.71% of the total variance, the second a further 30.28%, the third 13.19%, the fourth 8.25% of the total variance. The components can be interpreted in terms of the variables, which have the highest component loadings.

Table 6: Eignvector, eignvalue, the percent of variance and cumulative variance obtained from principal component analysis

Total oil and fatty acids	PC 1	PC 2	PC 3	PC 4
Total oil	0.054	0.076	-0.371	-0.762
14:0	-0.001	0.001	-0.001	0.001
15:0	-0.003	0.001	0.005	0.007
16:0	-0.404	-0.215	-0.314	-0.234
17:0	-0.029	-0.03	-0.011	-0.013
17:1	-0.003	-0.002	0.016	-0.007
18:0	-0.307	-0.23	-0.109	0.465
18:1 n-9	0.759	-0.473	0.154	-0.069
18:2 n-6	-0.206	0.307	0.78	-0.297
18:3 n-3	0.336	0.755	-0.318	0.223
20:0	-0.068	-0.056	-0.126	-0.06
20:1 n-9	-0.011	0.006	0.029	-0.018
20:2 n-6	0	0	-0.002	-0.004
21:0	0	0	0	0
22:0	-0.041	-0.043	-0.034	-0.029
22:1 n-9	-0.003	-0.006	-0.01	-0.003
24:0	-0.006	-0.012	-0.003	-0.003
Eigenvalues	**22.635**	**14.673**	**6.393**	**4**
Percentage	**46.719**	**30.286**	**13.194**	**8.256**
Cum. Percentage	**46.719**	**77.005**	**90.2**	**98.455**

The first component had high positive loadings from oleic acid and linolenic acid and high negative loadings from palmitic acid stearic acid. This component PC I then describing the general trend of correlations resulting from the high increase rate of oleic acid, the lower increase of linolenic acid, the highest decrease rate of palmitic and stearic acid. There is a positive loading indicates, a positive correlation between the component and the variable.

The second component, accounting for 30.28% of the total variance, had high positive loadings from linolenic acid and linoleic acid, while high negative loadings from oleic and stearic acid. The component is used to describe the trends in correlations resulting from the highest rate of increase in linolenic acid and highest rates of decrease in oleic acid.

The third component, explaining 13.19 % of the total variance, had high positive loadings from linoleic acid and high negative loadings from total oil percentage followed by palmitic acid.The fourth component, explaining 8.25% of the total variance, had high positive loadings from stearic acid and high negative loadings from total oil percentage followed by linoleic acid. PCA identified linolenic and oleic acid as the most important traits responsible for variation in presented material. Variation in linolenic and oleic acid content of fenugreek oil indicates the possibility of improving quality through breeding as describe earlier in safûower oil (Fernandez-Martinez *et al*. 1993).

The data matrix of oil content and fatty acid composition formed the basis of Euclidean genetic distance calculations for all genotypes (Table7). In all varieties, the estimates of Euclidean genetic distance value ranged from 2.222 (between Pant Ragini and Hisar Sonali) to 19.376 (between Lam Selection and Hisar Madhvi). Estimates of genetic distance values showed wide range of variation among the fenugreek varieties. This information can be used to exploit genetic diversity.

The clusters from the UPGMA clustering method of the 17 fenugreek varieties are depicted in Figure 1. Mean and standard deviation of the quantitative phenotypic oil traits for each cluster are presented inTable 3. These are descriptive statistics once the clusters are given. At a cut off 6.694 the denderogram revealed seven distinct clusters (Figure 1). Three are real clusters and four are singletons. These singletons are also called clusters even though they consist of only one variety.

Cluster I consists of six varieties i.e. Hisar Mukta, Rajendra Kranti, AFg-2, AFg-3, RMt-303 and Azad Methi. This cluster contains high SFA includes myrstic acid, stearic acid, heptadecaenoic acid,arachidic acid, behenic acid and tetracosanoic acid. Cluster II consists of 4 varieties namely AFg-4, RMt-305, GM-2 and Lam selection. It is mainly

Table 7: Euclidean distance matrix among fenugreek varieties based on total oil and fatty acid composition

Distance matrix

	Hisar Mukta	Hisar Madhvi	AFg-4	AFg-2	Azad Methi	Lam selection	RMt -305	AFg-1 -303	Rajendra	RMt Kranti	CO-1 -351	Hisar	GM-2	Pant Sonali	RMt	AFg Ragini	-143	-3
Hisar Mukta	0																	
Hisar Madhvi	14.968	0																
AFg-4	4.793	18.032	0															
AFg-2	4.006	15.71	4.771	0														
Azad Methi	4.782	18.034	4.133	4.659	0													
Lam selection	7.807	19.376	6.799	10.553	8.954	0												
RMT-305	5.065	18.016	2.869	6.51	6.288	4.642	0											
RMT-303	4.68	14.408	7.511	4.072	7.808	11.667	8.067	0										
AFg-1	8.348	10.325	12.149	8.59	11.708	15.222	12.552	5.709	0									
Rajendra Kranti	2.225	14.352	5.325	3.118	5.972	9.195	5.708	2.881	7.179	0								
RMt-351	9.424	14.787	8.622	7.411	10.912	13.345	9.417	7.892	10.325	7.775	0							
CO-1	8.9	9.497	10.442	7.993	11.244	13.99	10.983	8.114	8.147	7.671	6.933	0						
Hisar Sonali	9.04	15.162	8.507	6.803	7.644	13.851	10.649	9.918	11.922	8.759	9.005	7.594	0					
GM-2	3.664	16.318	3.873	6.398	5.66	4.593	2.921	7.502	11.094	5.014	9.979	10.407	10.322	0				
Pant Ragini	9.053	16.242	7.761	6.829	7.528	13.033	9.786	10.249	13.012	8.827	9.011	7.989	2.222	9.934	0			
RMt-143	11.343	17.204	10.318	9.034	9.256	15.491	12.481	12.413	14.593	11.222	11.356	9.749	3.078	12.451	2.924	0		
AFg-3	3.753	17.518	4.92	3.737	4.821	9.492	6.027	4.39	9.256	3.711	9.386	10.864	9.923	5.675	9.985	12.175	0	
	Hisar Mukta	Hisar Madhvi	AFg-4	AFg-2	Azad Methi	Lam selection	RMt -305	AFg-1 -303	Rajendra	RMt Kranti	CO-1 -351	Hisar	GM-2	Pant Sonali	RMt	AFg Ragini	-143	-3

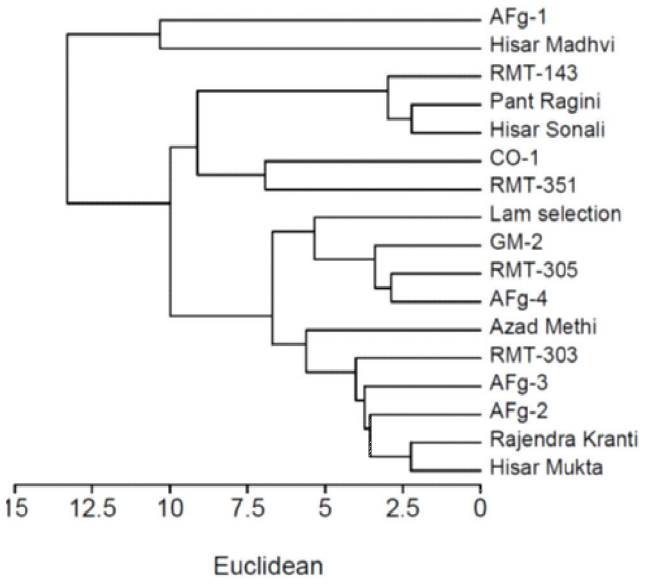

Fig 1: Dendrogram produced by UPGMA clustering based on 17 fenugreek varieties based on fatty acid composition

characterized with low LDR value, comparatively high palmitic acid and low oleic acid. Cluster III and IV consists of only one genotype namely RMt-351 and CO-1 having the cluster distance of 6.933. Cluster V consists of three entries namely Hisar Sonali, Pant Ragini and RMt-143. This group is characterized with high PUFA, EFA, high α-linolenic acid, ODR and LDR value. Cluster VI and VII also consists only one variety namely Hisar Madhavi and AFg-1 with a genetic distance of 10.325. Both the varieties in cluster VI and VII contain low oil as well as lower SFA.

Varieties in the same cluster may represent members of one heterotic group. Maximum variability for selection in segregating population may be achieved by utilizing genotypes from different clusters as parents of crosses. Genetic diversity showed no relationship to geographical origin, as in the case of morphological diversity; accessions from unknown origin were also randomly distributed over the whole dendrogram.

Dissimilarities obtained in this study show a wide variation in oil composition and total oil content in fenugreek. It is probable that greater diversity would be seen if a wider collection from more distant geographical regions was included. However, the knowledge of genetic relationships generated from this investigation will be of value in the exploitation of available germplasm.

Present study revealed that fenugreek oil contains a good amount of EFA (LA and ALA) in all fenugreek genotypes. The n-6: n-3 fatty acids in fenugreek seed oil is very near to ideal ratio for human consumption. Variety RMt-143 showed maximum PUFA (82.17%) with the higher side combination of DR, ODR and LDR value 0.954, 0.961 and 0.495 respectively. According to cluster analysis, genotypes Hisar Sonali, Pant Ragini and RMt-143 are characterized with high PUFA, EFA, DR, ODR and LDR value. PCA identified linolenic and oleic acid as the most important traits responsible for variation and can be used as target trait for improving quality through breeding. Genetic diversity showed no relationship to geographical origin while dissimilarities obtained in this study show a wide variation in oil composition and total oil content in fenugreek. However, the knowledge of genetic relationships generated from this investigation will be of value in the exploitation of available germplasm for crop improvement programme. Results of analysis suggested that a significant genotypic variation in fatty acids composition of fenugreek genotypes may be utilize to introduce novelty of oils for developing new cultivars from non-established oil crops like fenugreek for innovative end-uses either sole use or blended with other vegetable oil.

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REFERENCES

Al-Jasass, F.M. and Al-Jasser, M.S. (2012). Chemical composition and fatty acid content of some spices and herbs under Saudi Arabia conditions. The ScientificWorld Journal Article ID 859892, 1-5 pages doi:10.1100/2012/859892

Artemis, P.and Simopoulos, M.D. (2008). The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease.*Asia Pac. J. Clin. Nutr*.**17** (S1):131-134

Ciftci, O.N., Przybylski, R., Rudzinska, M.and Acharya, S. (2011). Characterization of fenugreek (*Trigonella foenum-graecum*) Seed Lipids.*J. Am. Oil. Chem. Soc*.**88:**1603–1610. DOI 10.1007/s11746-011-1823-y

C.S.A.O.(2009). Official and recommended practices of the American Oil Chemist's Society, 6th edn. American Oil Chemist's Society, Champaign

El-Sebaiy, A.and El-Mahdy, A.R. (1983). Lipid changes during germination of fenugreek seeds (*Trigonella foenum-graecum*). *Food. Chem*.**10:**309–319.

Fernandez-Martinez, J., Rio, M.D.and Haro, A.D. (1993). Survey of safflower (*Carthamus tinctorius* L.) germplasm for variants in fatty acid composition and other seed characters. *Euphytica* **69:**115–122.

Helambe, S.S.and Dande, R.P. (2012). Fenugreek. An overview, *Inter. J. of Curr. Pharmaceutical Review and Res.* **2:** 76-82.

Heller, L. (2001). Fenugreek. A noteworthy hypoglycemic, Pacific College of Oriental Medicine. From www.ormed.edu/newsletters/fenugreek.html.

Hemavathy, J., Prabhakar, J.V. (1986). Lipid composition of fenugreek (*Trigonella foenum-graecum* L.) seeds. *Food Chem* **31:**1–7

Jerry, H.L. (2001). Number Cruncher Statistical Systems (NCSS). Statistical Software. Kaysville, Utah.

Maillard, V., Bougnoux, P., Ferrari, P., Jourdan, M.L., Pinault, M., Lavillonniere, F., Body, G., Le- Floch, O.and Chajes, V. (2002). n-3 and n-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in tours France. *Int. J. Cancer* **98:**78–83.

Panse, V.C. and Sukhatme, P.V. (1978). Statistical Methods for Agricultural Workers.III Rev. Ed. ICAR, New Delhi.

Pleines, F.L.S.and Friedt, W.(1988). Breeding for improved C18 fatty acid composition in rapeseed (*Brassica* napus L.). *Fat Sci. Technol*. **90:** 167–171.

Rathore, S.S., Saxena, S.N.and Singh, B. (2013).Potential health benefits of major seed spices.*International J. Seed Spices*, **3:** 1-12.

Saxena, R., Saxena, S.N., Barnwal, P., Rathore, S.S., Sharma, Y.K. and Soni, A. (2012). Estimation of antioxidant activity, phenolic and flavonoid content of cryo and conventionally ground seeds of coriander (*Coriandrum sativum* L.) and fenugreek (*Trigonella foenum-graecum* L*.). International J. of Seed Spices***2:** 89-92.

Sharma, R.D. (1986). An evaluation of hypocholesterolemic factor of fenugreek seeds (*Trigonella foenum-graecum*) in rats. *Nutr. Rep. Int.***33:**669–677.

Simopoulos, A.P., Leaf, A. and Salem, N. Jr. (1999). Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann. Nutr. Metab*. **43:**127–130.

Suja, P.R., Anuradha, C.V.and Viswanathan, P. (2002). Gastroprotective effect of fenugreek seeds (*Trigonella foenum-graecum*) on experimental gastric ulcer in rats. *J. Ethnopharmacol* **81:**393–397.

Sulieman, A.M.E., Ali, A.O.and Hemavathy, J. (2008) Lipid content and fatty acid composition of fenugreek (*Trigonella foenum-graecum* L.) seeds grown in Sudan. Int.*J..Food Sci. Tech.***43**:380–382.

Tiran, D. (2003). The use of fenugreek for breast feeding woman. *Complement Ther. Nurs. Midwifery* **9:**155–156.

Velasco, L. and Goffman, D. (2000). Tochopherol, plastochromanol and fatty acid patterns in genus *Linum*. *Plant Syst. Evol.* **221:** 77-88.