



Estimates of Genetic Divergence in Fenugreek (*Trigonella foenum-graecum* L.) germplasm

Shubham Khichi ^{a*}, Rupal Dhoot ^a, Chandan Roy ^b and Namrata ^c

^a Department of Genetics and Plant Breeding, Agriculture University, Jodhpur, Rajasthan 342304, India.

^b College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University (Imphal), Umroi Road, Umiam, Meghalaya, 793103, India.

^c Dr. BRCARS, Mandor, Agriculture University, Jodhpur, Rajasthan 342304, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

The importance of fenugreek (*Trigonella foenum-graecum* L.) as a seed spice crop is significant as the seeds have medicinal, industrial and economical importance in India. The mode of pollination in fenugreek is self-pollination and germination of seed take place in hypogeal manner. The present study is essential to identifying genetically diverse genotype which will help in selecting parents in a hybridization program. In flower biology of fenugreek, the colour of flower is white and cleistogamous, inflorescence is racemose and flowers are bracteates, pedicellate complete, zygomorphic, bisexual and hypogynous. To identify genetically diverse genotype, an experiment was conducted with 38 genotypes in Randomized Block Design with three replications at instructional farm, college of agriculture, Jodhpur (Rajasthan) during Rabi 2024-25. Mahalonobis D²

*Corresponding author: E-mail: shubhamkhichi32@gmail.com;

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statistics grouped 38 genotypes into 9 clusters in which maximum intra cluster distance was recorded in cluster IX and maximum inter cluster distance recorded between cluster II and VII and the percentage contribution towards genetic divergence was found high for oil content. The data collected from field trial on fenugreek for thirteen characters were statistically analyzed with the help of WINDOSTAT Version 9.3 software. The characters such as oil content, protein content, number of pods per plant, 1000-seed weight, plant height were the major contributing characters. Hence, these five characters are important and should be considered while selecting genetically diverse parents for any breeding programmes. The result concludes that among all the genotypes UM-3, AM-293, UM-19, AM-310, UM-9, UM-20 and UM-21 were found superior in seed yield per plant and for other characters also based on mean performance which would be used as parental source for future breeding programme.

Keywords: Fenugreek; genotypes; diversity; divergence; cluster.

1. Introduction

India is the country famous for its spice's richness and in the list of such Indian spices, fenugreek is one of the seed spices, which is mainly used in making different types of pickles. Fenugreek's importance is not only as a spice, it has many medicinal properties also, that helps in lowering the blood sugar level, decreasing cholesterol level because of presence of an alkaloid called "Diosgenin" and neuroprotective because of an alkaloid called "Trigonelline" and has anti-carcinogenic properties, anti-fungal properties, the consumption of fenugreek on daily basis helps in curing gastrointestinal disorder. Because of the presence of "Saponin" in fenugreek it considered as steroidal significant crop. Fenugreek constitute both macro as well as micro nutrients like in 100 grams of fenugreek it contains 26.2 g of protein, 44.1 g carbohydrates, 7.2 g crude fiber, 5.8 g fat, 1.5 g minerals, 360 mg calcium, 167 mg sulphur, 76.1 mg sodium, 67 mg magnesium, 51 mg phosphorous, 17.2 mg iron, 1.1 mg niacin, 96 I.U vitamin A, and food energy 333 calories (Gupta et al., 1989). Compounds like "Epicatechin" which help in rejuvenating skin cell and preventing premature aging by free radicals, which make skin sharper and healthier, so because of this anti-aging active compound in fenugreek, it has significant cosmetologically importance also. Oil derived from fenugreek seeds used to promote hair health and growth, researchers suggest it can strengthen hair from roots, improve scalp health because the oil contain compound like "Lecithin" which moisturizes and conditions the scalp. Fenugreek consist a polysaccharide called "Galactomannan" which make fenugreek industrially important plant, because this polysaccharide acts as a stabilizer, emulsifier, thickener, and gelling agent. Hence, it is widely used in food industries (Narolia et al., 2017).

Fenugreek botanically called as *Trigonella foenum-graecum* L. ($2n = 2x = 16$) and commonly known as "methi" in India, belongs to family Leguminosae and the crop is being consumed as a leafy vegetable and as a whole seed. The crop has two centers of origin, the Indian sub-continent and the eastern Mediterranean region (Vavilov, 1926). The mode of pollination in fenugreek is self-pollination and germination of seed take place in hypogean manner. In flower biology of fenugreek, the colour of flower is white and cleistogamous, inflorescence is racemose and flowers are bracteates, pedicellate complete, zygomorphic, bisexual and hypogynous. Time of anthesis ranges between 9 AM to 6 PM in which peakness achieved around 11:30 AM. Dehiscence of anthers between 10:30 AM to 5:30 PM with peak between 11:30 AM to 12:30 PM. Stigma become receptive twelve hours before flower opening and remain receptive for about 10 hours after opening of flowers. In 2023-24, fenugreek covered 1.58 lac ha area in India and 0.77 lac ha area in Rajasthan with overall all production of 2.49 lac tons in India, in which Rajasthan is the second largest producer having production of 1.02 lac tons after Madhya Pradesh (Spices Board India, 2024). Climatic conditions favor growth of fenugreek is having temperature range between of 20°C to 25°C for healthier germination and soil condition for fenugreek cultivation is, soil should be well drained, sandy loam rich in organic matter and having slightly acidic pH of around 6.0 to 7.0. However, the dominance of high yielding varieties has led to reduction in genetic diversity which ultimately makes the crop susceptible to different biotic and abiotic stresses (Singh Verma et al., 2024). For overcoming this harm germplasm evaluation is crucial to maintain the genetic diversity and for that identifying promising genotypes for breeding is crucial (Berhe et al., 2025). Several

researchers studied divergence in fenugreek genotypes in which Yadav et al. (2024) conducted an experiment to study genetic diversity with 27 fenugreek genotypes in which D² analysis grouped genotypes into 7 clusters. Therefore, in order to examine the growth characteristics and seed yield parameters of fenugreek in the context of Jodhpur, Rajasthan the present study is essential to identifying genetically diverse genotype which will help in selecting parents in a hybridization program.

2. Materials and Methods

The experiment was carried out during Rabi-2024-25 at Instructional farm, College of Agriculture, Jodhpur (Rajasthan). The experimental material consist of 38 fenugreek genotypes (Table 1) which was laid out in randomized block design with three replication planted at spacing 30 x 10 cm². "All the recommended package of practices was followed to raise a healthy crop and necessary prophylactic measures were adopted against pests and diseases. The characters under study are days to 50% flowering, days to maturity, plant height(cm), number of primary branches per plant, number of pods per plant, pod length(cm), number of seeds per pod, 1000-seed weight(g), biological yield per plant(g), harvest index(%), seed yield per plant(g), protein content(%) and oil

content(%) in which the data was recorded on five randomly selected plants from each genotype in each replication for all the characters except days to 50 percent flowering and days to maturity, where data were recorded on plot basis. protein is estimated using calculation nitrogen through Kjeldahl unit method" (J. Kjeldahl, 1883).

$$N\% = \frac{14 \times \text{normality of acid} \times \text{titration value burette}}{\text{weight of sample (g)} \times 1000} \times 100$$

$$\text{Crude protein}\% = N\% \times 6.38$$

Oil content through Soxhlet apparatus (F. Soxhlet, 1879) in which formula used for calculating oil content (Singh & Chaudhary, 1985).

$$\text{Weight of oil} = \text{Weight of flask containing oil} - \text{weight of empty flask}$$

$$\text{Percentage oil} = \frac{\text{Weight of oil}}{\text{weight of sample (g)}} \times 100$$

The data collected from field trial on fenugreek for thirteen characters were statistically analyzed with the help of WINDOSTAT Version 9.3 software (Panse & Sukhatme, 1967). For analyzing genetic divergence in fenugreek was estimated by Mahalanobis's D² statistic technique as suggested by Rao (1952).

Table 1. List of fenugreek genotypes

Genotype	Name of Genotypes	Source	Genotype	Name of Genotypes	Source
G1	UM-3	SKNAU, Jobner	G20	UM-23	SKNAU, Jobner
G2	UM-4	SKNAU, Jobner	G21	UM-24	SKNAU, Jobner
G3	UM-5	SKNAU, Jobner	G22	UM-25	SKNAU, Jobner
G4	UM-6	SKNAU, Jobner	G23	UM-26	SKNAU, Jobner
G5	UM-7	SKNAU, Jobner	G24	UM-27	SKNAU, Jobner
G6	UM-8	SKNAU, Jobner	G25	UM-28	SKNAU, Jobner
G7	UM-9	SKNAU, Jobner	G26	UM-29	SKNAU, Jobner
G8	UM-10	SKNAU, Jobner	G27	UM-30	SKNAU, Jobner
G9	UM-11	SKNAU, Jobner	G28	AM-71	NRCSS, Ajmer
G10	UM-12	SKNAU, Jobner	G29	AM-108	NRCSS, Ajmer
G11	UM-13	SKNAU, Jobner	G30	AM-280	NRCSS, Ajmer
G12	UM-14	SKNAU, Jobner	G31	AM-281	NRCSS, Ajmer
G13	UM-15	SKNAU, Jobner	G32	AM-282	NRCSS, Ajmer
G14	UM-17	SKNAU, Jobner	G33	AM-284	NRCSS, Ajmer
G15	UM-18	SKNAU, Jobner	G34	AM-293	NRCSS, Ajmer
G16	UM-19	SKNAU, Jobner	G35	AM-310	NRCSS, Ajmer
G17	UM-20	SKNAU, Jobner	G36	RMT-143	SKNAU, Jobner
G18	UM-21	SKNAU, Jobner	G37	RMT-305	SKNAU, Jobner
G19	UM-22	SKNAU, Jobner	G38	AFG-3	NRCSS, Ajmer

3. Results and Discussion

“Mahalanobis D^2 is a useful method for assessing genetic diversity among many genotypes and on the basis of tocher method, 38 genotypes were classified into 9 clusters based

on D^2 values using Tocher's techniques” (Rao, 1952), with genotypes belonging to the same cluster having lower D^2 values on average than genotypes belonging to different clusters. The distribution of genotype into various clusters has been presented in Table 2.

Table 2. Clustering pattern among 38 genotypes of fenugreek

Cluster	Number of genotypes	Name of genotype
I	12	UM-21, AM-310, UM-18, UM-20, UM-22, UM-26, UM-23, AM-71, AM-282, AM-281, UM-6, UM-29.
II	9	UM-9, UM-24, UM-5, UM-4, UM-10, UM-13, UM-7, UM-15, UM-17
III	1	UM-8
IV	6	UM-27, UM-28, UM-25, AM-108, AM-293, RMt-143
V	1	AM-280
VI	1	UM-19
VII	2	UM-30, AM-284
VIII	1	UM-14
IX	5	UM-11, UM-12, RMt-305, AFg-3, UM-3

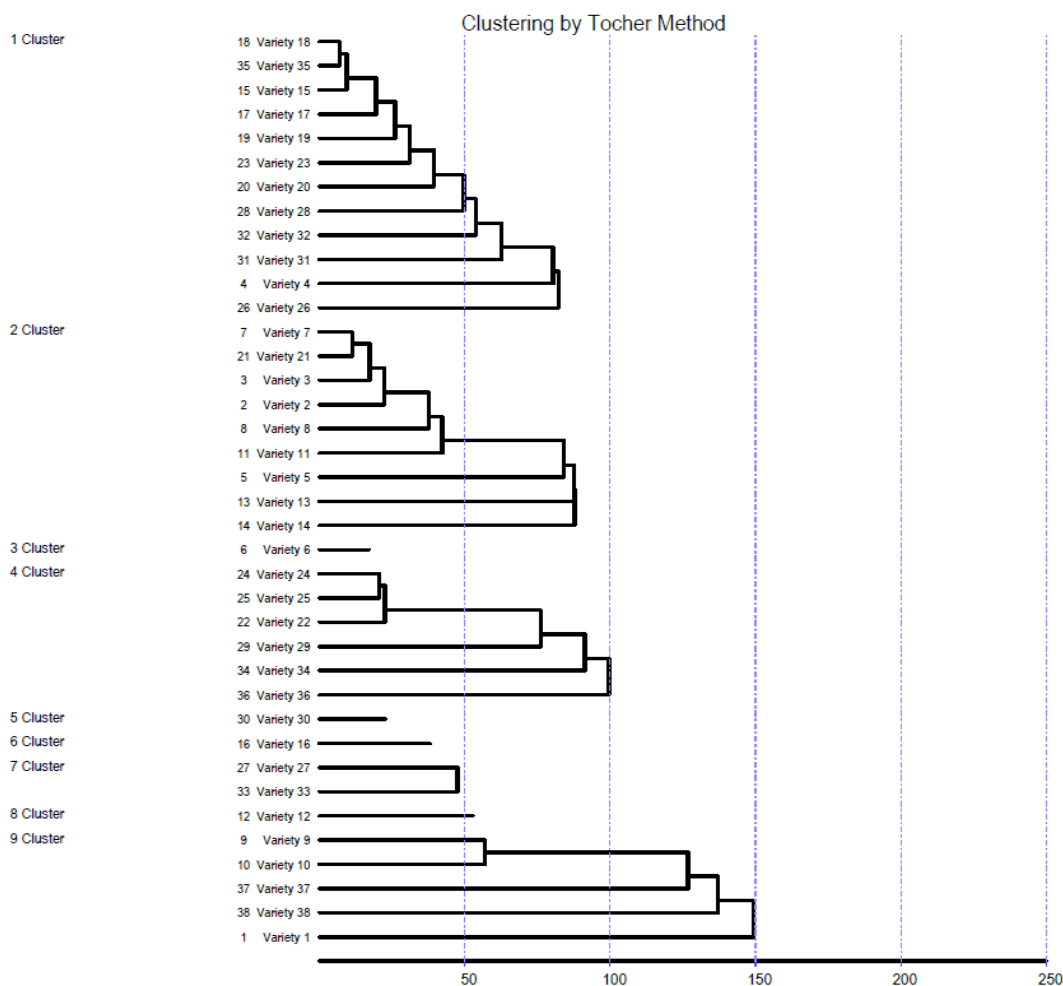


Fig. 1. Dendrogram representing clustering of 38 fenugreek genotypes into nine cluster using tocher method

Table 3. Average intra and inter cluster distances of D² values of 38 genotypes of fenugreek

Cluster	C I	C II	C III	C IV	C V	C VI	C VII	C VIII	C IX
Cluster I	61.96	438.19	190.45	170.63	111.63	196.41	927.52	198.89	315.18
Cluster II		74.35	125.21	934.19	220.09	126.64	2445.91	177.21	237.13
Cluster III			0	516.96	75.19	546.74	1705.98	82.87	94.56
Cluster IV				96.47	351.8	546.74	452.33	517.25	642.42
Cluster V					0	108.63	1403.03	149.73	189.01
Cluster VI						0	1777.71	64.3	161.54
Cluster VII							47.54	1644.88	1858.1
Cluster VIII								0	196.34
Cluster IX									173.37

All the nine cluster have certain number of genotypes in which cluster I was the largest comprising of 12 genotypes followed by cluster II with 9 genotypes, clusters IV with 6 genotypes, cluster IX with 5 genotypes, cluster VII with 2 genotypes. Cluster III, V, VI and VII were the clusters having single genotype (Table 2) that means they all are mono-genotypic cluster. The similar results were reported by Jain et al. (2006), Patahk et al. (2014) and Meena et al. (2025) as they reported maximum cluster were mono-genotypic.

The range of intra cluster distance recorded was 47.54 – 173.37 (Table 3) in which maximum intra cluster distance was observed in cluster IX (173.37) followed by cluster IV (96.47), cluster II (74.35), cluster I (61.96) and cluster VII (47.54) indicated that some genetic divergence still existed among the genotype within the clusters and selection within such cluster might be conducted based on maximum mean value character. The maximum inter cluster distance was recorded between cluster II and VII (2445.91) followed by cluster VII and IX (1858.1) cluster VI and VII (1777.71), cluster III and VII (1705.98), cluster VII and VIII (1644.88), cluster V and VII (1403.03), cluster II and IV (934.19) and cluster I and VII (927.52). The distance between two clusters is directly proportional to their genetic diversity. Highly divergent genotypes would be quite useful in a recombination breeding effort to produce highly desired recombinants. The lowest inter cluster divergence was recorded between cluster VI and VIII (64.3) followed by cluster III and V (75.19), cluster III and VIII (82.87), cluster III and IX (94.56) and cluster V and VI (108.63) demonstrating that the genotypes contained in these clusters were closely linked. Selection should be carried out in genetically varied populations to preserve a relatively broad genetic base.

The mean values of different characters in the nine clusters revealed that the different clusters were superior in respect of different characters (Table 4). The data indicated that the cluster mean 50 percent flowering was highest in cluster VIII (59.33) and the lowest in cluster V (47.67). Days to maturity were exhibited highest means in cluster VIII (131.33) and lowest in cluster III (123.00). Cluster V exhibited highest plant height (89.50) while lowest was in cluster III (66.80). Numbers of primary branches per plant exhibited highest and lowest means in clusters IX (7.45) and cluster VIII (5.27) respectively. In number of pods per plant Cluster IX recorded the highest mean value (59.64) and lowest was recorded in cluster V (32.60). Pod length was highest in cluster V (8.65) and lowest was recorded in cluster VII (7.20). The number of seeds per pod was highest in cluster VI (20.59) and lowest in cluster V (14.21). Cluster V (15.85) recorded highest mean value for 1000-seed weight while in cluster VIII (10.88) it was the lowest value. Mean value for biological yield per plant was highest in cluster VI (39.47) while lowest in cluster III (22.47). Cluster I (27.75) recorded the highest mean for harvest index and the lowest was recorded in cluster V (19.35). The highest protein content was recorded in cluster VIII (33.27) and the lowest was recorded in cluster IX (25.97). Cluster VII exhibited the highest oil content (6.15) and the lowest was recorded in cluster II (1.78). For seed yield per plant highest mean was recorded for cluster VI (10.33) and the lowest value was recorded for cluster V (5.33). The maximum genetic divergence was contributed by oil content (78.09%) followed by protein content (10.1%), number of pods per plant (3.7%), 1000-seed weight (2.99%), plant height (1.42%) indicated that attributes could be offered sufficient scope for selecting desired genotypes. Similar finding was earlier reported by Jain et al. (2006), Patahk et al. (2014), Panwar et al. (2018), Meena et al. (2021) and

Table 4. Cluster means of different characters in fenugreek genotypes

Characters	Days to 50 percent flowering	Days to maturity	Plant height (cm)	Number of primary branches per plant	Number of pods per plant	Pod length (cm)	Number of seeds per pod	1000-seed weight (g)	Biological yield per plant (g)	Harvest index (%)	Protein content (%)	Oil content (%)	Seed yield per plant (g)
Cluster I	47.94	124.58	80.89	5.43	44.89	8.53	15.64	14.10	27.55	27.75#	29.38	3.53	7.55
Cluster II	49.00	123.59	71.05	5.42	38.58	8.41	15.47	13.53	24.61	26.87	30.67	1.78•	6.64
Cluster III	51.00	123.00•	66.80•	5.53	44.67	7.73	16.19	12.87	22.47•	26.86	27.62	2.45	6.03
Cluster IV	48.67	123.39	82.90	6.00	49.44	8.11	14.71	15.06	29.54	23.96	28.14	4.41	7.00
Cluster V	47.67•	127.33	89.50#	6.40	32.60•	8.65#	14.21•	15.85#	27.53	19.35•	26.83	2.82	5.33•
Cluster VI	50.33	125.00	85.50	5.73	50.93	8.48	20.59#	13.07	39.47#	26.16	31.15	2.56	10.33#
Cluster VII	49.50	125.50	72.05	5.83	52.73	8.09	15.11	15.08	23.50	23.65	27.84	6.15#	5.52
Cluster VIII	59.33#	131.33#	73.20	5.27•	46.40	7.20•	18.48	10.88•	24.40	25.54	33.27#	2.68	6.17
Cluster IX	53.60	127.00	66.95	7.45#	59.64#	8.64	17.29	12.93	32.52	25.18	25.97•	2.45	8.25

•, # represents minimum and maximum values, respectively

Maurya et al. (2021). The characters such as oil content, protein content, number of pods per plant, 1000-seed weight, plant height were the major contributing characters. Hence, these five characters are important and should be considered while selecting genetically diverse parents for any breeding programmes. Out of 38 genotypes UM-3, AM-293, UM-19, AM-310, UM-9, UM-20 and UM-21 were found superior in seed yield per plant and for other characters also based on mean performance. Hence, these genotypes would be used as parental source for future breeding programme.

4. Conclusion

The highest intra-cluster distance was found in cluster IX, while the greatest inter-cluster divergence occurred between clusters II and VII, suggesting that favorable recombinants can be achieved through mating between the genotypes. Among the 38 genotypes, UM-3, AM-293, UM-19, AM-310, UM-9, UM-20, and UM-21 were identified as superior regarding seed yield per plant and other characteristics based on their average performance. Therefore, these genotypes will serve as parental sources for upcoming breeding programs

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Details of the AI usage are given below:

1. ChatGPT based on GPT -5.1 have been used during the writing or editing of manuscripts.
2. The AI tool was only used for minimal paraphrasing and not for any other purposes.

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Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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