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Assessment of genetic divergence in fenugreek (*Trigonella foenum-graecum* L.) based on biological characters

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Abstract

Genetic divergence analysis following Mahalanobis D^2 statistics disclosed considerable genetic diversity among sixty genotypes of fenugreek (*Trigonella foenum-graecum* L.) for all the twelve quantitative characters. Sixty genotypes were grouped into eight distinct clusters depending upon the similarities of their D^2 values following Tocher's method. The clustering pattern of studied genotypes usually did not follow the geographical distribution. Appreciable diversity within and between eight clusters were observed. Among eight clusters, cluster V consisted of 16 genotypes, followed by cluster III (12), cluster I (9) and VII (7), clusters VI (6), cluster IV (5), cluster II (4) and cluster VIII was monogenotypic. Intra-cluster distance was highest in cluster IV followed by cluster VII. Inter cluster distance was observed maximum in cluster IV and VIII followed by cluster II and VIII and minimum distance was observed in cluster I and III. On the basis of cluster mean performance, cluster VIII and V can be considered better for selecting superior genotypes for most of the characters. The study also revealed that for acquiring heterotic response and better segregants, inter-mating between genotypes of diverse clusters may be undertaken in breeding programmes for improvement of yield and quality traits.

Keywords: D^2 statistic, genetic divergence, fenugreek, *Trigonella foenum-graecum*

Introduction

Trigonella foenum-graecum L., commonly known as fenugreek and locally as "Methi". As Per Balodi and Rao (1991) [3] the genus *Trigonella* is one of the largest genera of the tribe *Trifoliata* in the family Fabaceae and sub-family Papilionaceae. Among *Trigonella* species, *Trigonella foenum-graecum* (commonly known as fenugreek) is an annual species, with autogamous flowers occasionally visited by insects. It is indigenous to countries on the Eastern shores of the Mediterranean, but widely cultivated in India, Egypt, Ethiopia, Morocco and occasionally in England (Polhil and Raven, 1981; Davoud *et al.*, 2010) [13, 5]. The principal use of fenugreek is that it is being used as a rotation crop, it improves both the soil structure and fertility and it also fetches high revenue for farmers and producers. Fenugreek seeds supplies dietary proteins for vegetarians that lack animal and fish protein in their diet. Furthermore, fenugreek has several medicinal benefits (Sharma, 1990; Srinivasan, 2014) [15, 16].

One important factor is that very little information is available about its genetic diversity which restricts its large-scale production and development of better varieties. Therefore, to maximize the utilization of the available genetic wealth, disclosing the information on the extent and nature of genetic diversity of the population that would help in formulating efficient scheme of selection based on multiples of traits is of utmost importance. To overcome such a technical gap, this piece of research work was undertaken at CCS HAU, Hisar to address the major objectives subsequently given.

Methods and Materials

The experimental material for present study comprised of sixty diverse genotypes from various origin and these genotypes were evaluated in randomised blocked design. The experiment was laid out at Vegetable Research Farm of CCS HAU, Hisar (29°15'N, 75°69'E) in *rabi* 2015-16 and 2016-17. Each genotype was raised in a double row plot of 3.00 m length X 0.50 m width. All recommended agronomical practices and plant protection measures were followed timely for successful cultivation of the crop. Randomly ten competitive plants were tagged to record the observation for twelve quantitative characters namely field emergence index, days to 50% flowering, plant height (cm), pods per plant, number of branches per plant, pod length (cm), number of seeds per pod, seed yield (q/ha), test weight, seed germination(%), seed vigour index-I and seed vigour index-II.

Seed yield was recorded on the plot basis and further calculated in q/ha. One thousand seeds

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were counted in each replication of every genotype and weighed for calculating test weight (g). Rolled towel method (BP) was used for seed germination test. Four hundred seeds in four replications of each genotype were taken to record the seed germination. First count of normal seedling was taken on 5th day and final count on 14th day.

Seedling vigour indices: seedling vigour indices were calculated by following Baki and Anderson (1973) [1] method: method:

1. Vigour index-I = Standard germination (%) X Average seedling length (cm)
2. Vigour index-II = Standard germination (%) X Average seedling dry weight (g)

The genetic divergence in the germplasm was assessed by adopting Mahalanobis D² statistics (Mahalanobis, 1936) [10]. The genotypes were clustered on the basis of minimum generalized distance using Tocher's method as described by Rao (1952) [14]. The average intra and inter cluster distances were calculated by the formula given by Singh and Chaudhary (1979) [17].

Results and Discussion

Genetic diversity plays a vital role in plant breeding because hybrids between lines of diverse origin generally display a greater heterosis than those between closely related strains. The average linkage technique of clustering produced a more understandable portrayal of the sixty fenugreek accessions by grouping them into eight clusters (Table 1), whereby different members within a cluster is being assumed to be more closely related in terms of the trait under consideration with each other than those members in different clusters. Among these eight clusters, cluster V consisted of 16 genotypes, followed by cluster III (12), cluster I (9) and VII (7), clusters VI (6), cluster IV (5), cluster II (4) and cluster VIII was monogenotypic. The results indicated that genetic divergence is not related to geographical diversity and may possibly be due to varietal diversity among the genotypes due to diversity of their pedigree along with natural and directional selection pressure for certain agronomic traits. Similar results were also reported by Mathur (1992) [11] and Kole & Mishra (2002) [8] in fenugreek. Genetic drift and selection forces under diverse environments could cause greater diversity than geographical distance (Bhatt 1970; Kole *et al.* 2003) [4,9].

The intra-cluster distances were smaller than inter-cluster distances, revealing considerable amount of genetic diversity among the genotypes and presence of narrow genetic variation within a cluster (Table 2). Cluster IV showed maximum intra-cluster distance followed by cluster VII indicates high degree of divergence within that cluster. Intra-cluster distance is the main criteria for the selection of genotypes using D² analysis. Inter-cluster distance varied from 4.102 to 9.285. Minimum inter-cluster D² value was observed between clusters V and VI (4.102) indicating the close relationship among the genotypes included in these clusters. Maximum intercluster value was observed between clusters IV and VIII (9.285) indicating maximum divergence between the genotypes of these clusters. The inter-cluster D² values were also higher between the cluster II and VII (8.744), cluster III and VIII (8.534) and clusters VIII and I (8.429). This result is also in conformation with that of Jain *et al.* (2006) [7] and Fikreselassie *et al.* (2012) [6]. Hence, these results revealed that inter-mating between the genotypes included in these diverse clusters may give high heterotic response and thus better segregants.

Cluster means for different characters revealed that cluster VIII and cluster V showed moderate to high values for almost all the characters (Table 3). Cluster VIII had the highest value for plant height, pods per plant, branches per plant, pods length, field emergence index, seed yield and seed vigour index-II; cluster V showed maximum value for seed germination, seed vigour index-I; cluster II had maximum value for seed vigour index-II and days to 50% flowering. Mean values were the minimum in cluster IV for seed germination and seed vigour index I; cluster III had minimum value for branches per plant in cluster III whereas seed yield mean value was studied in cluster I. The results are in agreement with Mathur (1992) [11] and Kole & Mishra (2002) [8]. An assessment of relative contribution of twelve characters towards total genetic divergence (Table 4) revealed that seed yield had contributed highest (31.81 %) by taking 563 times first ranking followed by seed vigour index-II (15.93 %) by 282 times, number of pods per plant (11.19 %) by 198 times, seed germination (9.21 %) by 163 times. In contrast, number of branches per plant contributed least (0.73 %) by taking 13 time.

Table 1: Cluster membership of 60 fenugreek genotypes on the basis of D² statistics

Clusters	No. of genotypes	Name of genotypes
I	9	NDM7, NDM 10, NDM 8, JFg 219, AM 301, HM 278, AM 293, AM 298 and NDM 13
II	4	AM 292, AM 304, GM1 and GM2
III	12	NDM 45, JFg 13, NDM 67, RM 16, RM 28, NDM 43, NDM 48, RM 13, NDM 61, NDM 69, NDM 37 and RM 18
IV	5	NDM 72, JFg 201, RM 194, RM 70 and JFg 15
V	16	NDM13, NDM 20, NDM 25, RM 190, RM 203, AM 302, NDM 74, JFg 240, JFg 211, HM 259, AM 300, HM 280, HM 281, HM 267, HM 273 and HM 277
VI	6	RM 196, HM 297, HM 258, HM 282, JFg 196 and HM 271
VII	7	RM 27, RM 191, HM 355, HM 103, HM 444, HM 57 and HM 346-1
VIII	1	HM 346

Table 2: Average intra- (diagonal) and inter- (above diagonal) cluster D² values in 60 genotypes of fenugreek

Clusters	I	II	III	IV	V	VI	VII	VIII
I	3.904	4.483	4.261	6.191	4.580	4.878	6.361	8.429
II		3.611	4.867	5.369	4.425	4.576	5.961	8.744
III			3.506	5.104	4.566	4.589	6.196	8.534
IV				4.270	5.333	5.132	6.126	9.285
V					3.320	4.102	4.635	6.754
VI						3.371	4.866	7.662
VII							4.256	5.806
VIII								0.000

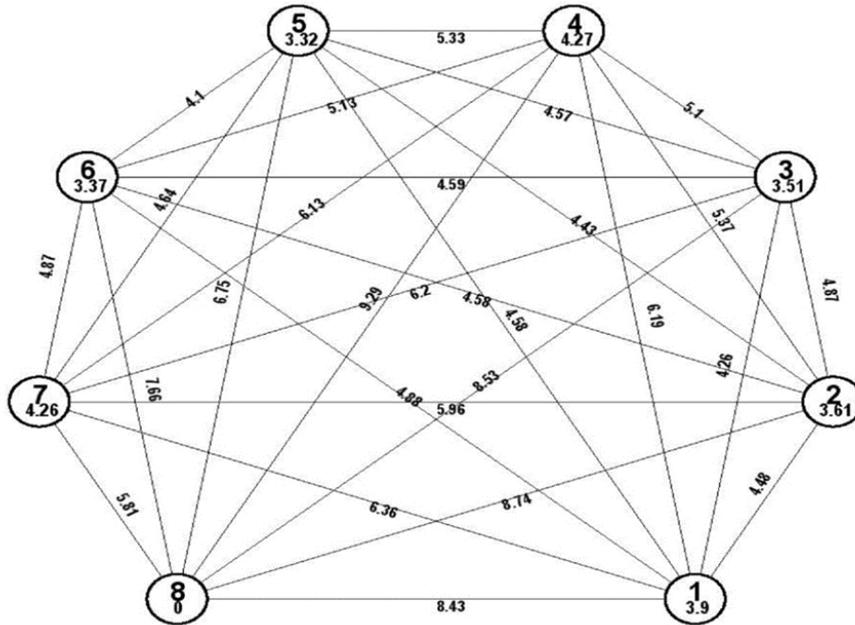


Fig 1: Average intra- (in circle) and inter- (between circle) cluster D^2 values in 60 genotypes of fenugreek

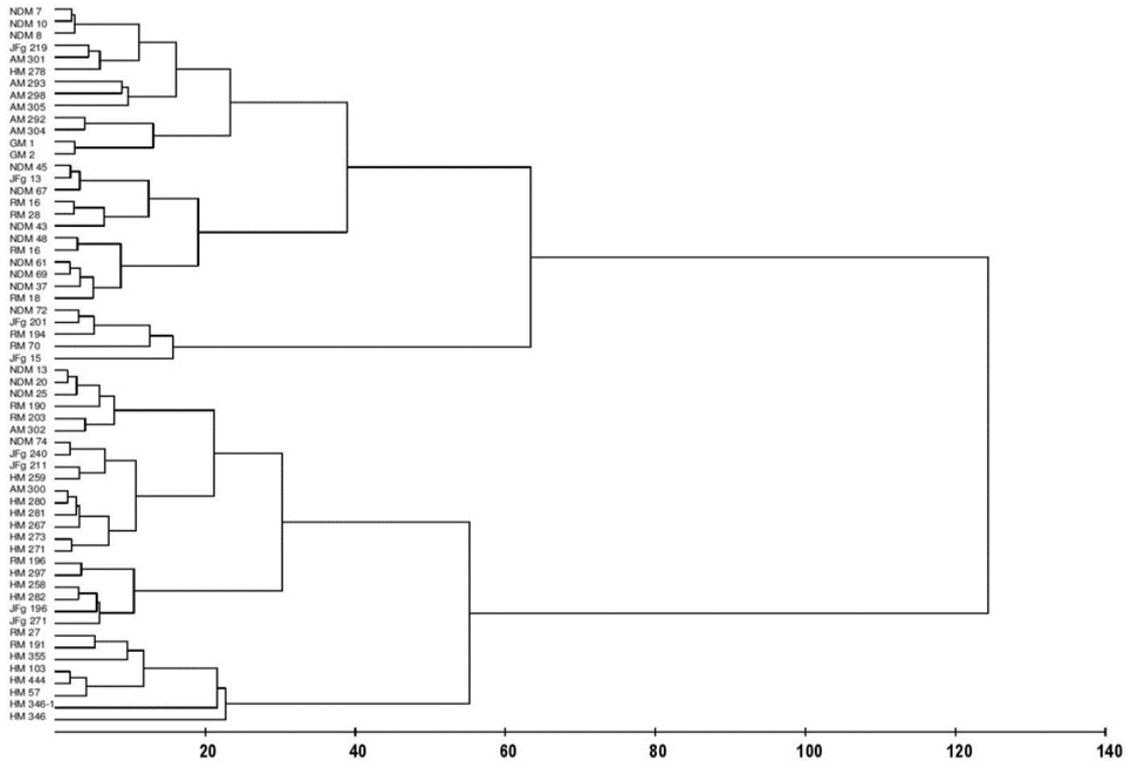


Fig 2: Clustering by Tocher Method

Table 3: Mean values of different clusters for twelve characters in fenugreek

	FEI	DF	PH	BPP	PPP	PL	SPP	TW	SG	SVI	SVII	SY
Cluster I	10.001	55.306	83.937	5.381	68.213	9.093	16.387	10.55	95.519	1357.034	56.217	14.709
Cluster II	9.651	55.999	85.655	5.382	67.413	9.002	16.201	10.59	93.973	1297.037	59.553	15.270
Cluster III	9.941	55.092	83.991	5.282	64.703	9.304	16.552	10.19	94.768	1323.266	49.970	14.475
Cluster IV	9.515	55.180	84.441	5.373	67.729	9.116	16.328	10.05	91.301	1268.831	46.057	14.965
Cluster V	9.647	54.678	86.817	5.471	70.246	9.307	16.647	10.54	94.667	1330.541	53.394	16.042
Cluster VI	9.739	54.522	87.187	5.459	66.656	9.403	16.062	11.01	93.139	1309.507	51.922	14.759
Cluster VII	9.624	54.410	87.992	5.633	76.257	9.597	16.957	10.96	92.913	1287.993	52.080	15.517
Cluster VIII	10.013	52.567	92.190	5.810	79.270	9.863	18.200	10.57	93.390	1322.223	59.540	16.143

Note:- Field Emergence Index: FEI; Days to 50% flowering: DF; Plant Height: PH; Pods per plant: P/P; Branches per plant: B/P; Pod length: PL; Seeds per pod: S/P; Test Weight: TW; Seed germination: SG; Seed Vigour Index-I: SVI; Seed Vigour Index-II: SVII

Table 4: Percentage contribution of each trait toward divergence

S. No	Characters	Times ranked 1 st	Contribution towards divergence (%)
1	Field Emergence Index	118	6.67%
2	Days to 50% Flowering	139	7.85%
3	Plant Height	41	2.32%
4	Branches per plant	13	0.73%
5	Pods per plant	198	11.19%
6	Pod Length	53	2.99%
7	Seeds per pod	60	3.39%
8	Test Weight	85	4.80%
9	Seed Germination	163	9.21%
10	Seed Vigour-I	55	3.11%
11	Seed Vigour-II	282	15.93%
12	Seed Yield	563	31.81%

Conclusion

The selection of parents for hybridization depends on their genetic diversity. Precise information in the nature and degree of genetic divergence would help the plant breeder in selecting the particular parents for hybridization. The expression of heterosis is highly affected by genetic diversity of parents. It is general belief that more diverse the parents within overall limits of fitness, the maximum are the chances of obtaining higher amount of heterosis expression in the F₁s and a broad spectrum of variability in segregating generations (Arunachalam, 1981) [2]. In general, the level of heterosis increases with the increase in parental diversity up to some extent and decreases with further increase in parental diversity owing to cross-ability barriers. Thus maximum heterosis occurs at an optimal or intermediate level of parental diversity. Further, the occurrence of heterosis cannot be predicted on the basis of genetic divergence alone (Matzinger and Werusman, 1958) [12]. Apart from the high degree of divergence, the mean performance of genotypes and the characters with maximum contribution towards divergences should also be given due consideration. The best combination of parents for improvement in various economic characters can be recommended on the basis of *per se* performance of the genotypes and intercluster divergence. Therefore, it can be concluded from the present study that hybridization among genotypes of these cluster combinations is expected to enhance variability in fenugreek for the targeted traits.

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