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Molecular Characterization of Fenugreek (*Trigonella foenum-graecum* L.) Genotypes Using Rapd Markers

N.C. Mamatha*, S.K. Tehlan, M. Srikanth, M.K. Shivaprasad and P. Karthik Reddy

Department of Vegetable Science, CCS Haryana Agricultural University, Hisar-125 004, India
**Corresponding author*

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A B S T R A C T

The study was conducted to reveal the genetic diversity among 48 fenugreek genotypes using thirty Random Amplified Polymorphic DNA (RAPD) markers among which, nineteen primers gave good amplification and revealed a total of 119 polymorphic bands with an average of 6.26 bands per primer. The percentage of polymorphism ranged from 50.00 to 91.66 per cent with an average of 79.21 % polymorphism per primer. Primer OPP-8 revealed the highest polymorphism (91.66%), whereas the primer OPC-2 exhibited the lowest polymorphism (50.00%). The polymorphic information content (PIC) value ranged from 0.66 to 0.90. Highest PIC value was obtained in primers OPP-8 followed by OPP-9, OPH-5, OPF-13, OPJ-18, OPB-5, OPC-17, OPAB-2, OPH-4 and OPN-2 and the lowest PIC value was obtained in OPC-19 followed by OPC-11 and OPG-11.UPGMA dendrogram presented into 10 clusters at 0.75 similarity coefficient. The similarity coefficient ranging from 0.59 to 1.00. Principal component analysis confirmed the results of UPGMA. The genotypes which are falling in different groups indicate the presence of genetic diversity between the members of different clusters. Crossing can be made between the members of different clusters for further crop improvement, as they are genetically diverse.

Introduction

The genus *Trigonella* is one of the largest genera of the tribe Trifoliatae in the family Fabaceae (Balodi *et al.*, 1991). It is a flowering annual, with autogamous white flowers occasionally visited by insects and is originated in the eastern shores of Mediterranean region. Fenugreek is widely cultivated in India, Egypt, Ethiopia, Morocco and occasionally in England (Polhil *et al.*, 1981). It is widely cultivated in the tropical and subtropical regions of India. Fenugreek is rich in minerals, protein, vitamin A and C. For any crop improvement program the basic requirement is availability of germplasm with wide variability. Germplasm pool is an important source of variability for developing new cultivars having good economic traits. Hence, assessment of genetic divergence in fenugreek germplasm is important for long term crop improvement programme.

Genetic diversity can be identified using morphological, biochemical and molecular markers. But, the morphology of plant is influenced by environment and developmental stages of crop, in this context

the molecular markers act as best tool for evaluating genetic diversity of crop germplasm which enables in understanding the genetic relationship among fenugreek genotypes.

Random Amplified Polymorphic DNA (RAPD) marker is the popular and widely used dominant marker which uses ten base primer to amplify the random portion of genome (Williams *et al.,* 1990) and it does not need prior information of target genome.

Materials and Methods

Plant material

Forty eight fenugreek genotypes were collected from different parts of the country *i.e*., Andhra Pradesh, Bihar, Delhi, Gujarat, Haryana, Rajasthan and Uttar Pradesh. The seeds were sown in pro trays under polyhouse and young leaves were collected for DNA extraction. List of 48 genotypes used for molecular characterization are as follows:

1. RajendraKranti
2. RM-194
3. RM-188
4. GM-1
5. GM-2
6. JFG-235
7. JFG-266
8. HM-57
9. HM-65
10. HM-103
11. HM-114
12. HM-205
13. HM-208
14. HM-221
15. HM-239-1
16. HM-242
17. HM-246
18. HM-257
19. HM-258-1
20. HM-273
21. HM-281
22. HM-291
23. HM-307
24. HM-332
25. HM-343
26. HM-346
27. HM-348
28. HM-355
29. HM-359
30. HM-444
31. HM-502
32. HM-507
33. HM-509
34. HM-517
35. HM-519
36. HM-526
37. HM-528
38. HM-536
39. HM-548
40. HM-555
41. PEB-1
42. LFC-93
43. AFG-5
44. AFG-6
45. UM-202
46. UM-354
47. NDM-69
48. NDM-72

Genomic DNA isolation

Genomic DNA was isolated from young leaves of 48 fenugreek genotypes following CTAB (Cetyltrimethyl ammonium bromide) extraction method as given by Murray and Thompson (1980) and modified by Saghai-Maroof *et al.,* (1984).

Qualitative and quantitative estimation of DNA

The quality and quantity of isolated genomic DNA will be estimated by UV Spectrophotometer (A260/A280 absorbance) and the DNA was also tested by submerged horizontal agarose (0.8%) gel electrophoresis.

Polymerase chain reaction (PCR) amplification

Thirty RAPD primers were used for divergence studies (Table 1). PCR amplification was carried out in touch down Q-thermo cycler. The PCR reactions were carried out in 23.5 µl of reaction mixture containing 13.5 µl of sterile distilled water, 2.5 µl (10 x colorless buffer) reaction buffer, 2 µl dNTP (500 µl dNTP of concentation10mM + 500 µl of sterile

distilled water), 2 µl of $Mgcl_2$ (25Mm), 2 µl of primer (10 µl primer + 90 µl of nuclear free water), 1 µl template DNA and 0.5 µl of Taq DNA polymerase (5U/µl). These were added into PCR tube in same sequence as above. In PCR amplification program for initial denaturation 4 min at 94^0 C, followed by 36 cycles of 1 min at 94^0 C for denaturation, annealing temperature varied according to primer, 72^0 C for 2 min and for final extension 72^0 C for 8 min is followed. Amplified DNA fragments were resolved by submerged horizontal agarose gel electrophoresis in 1.5 % (w/v) agarose gel and visualized by staining with ethidium bromide. Agarose solution was prepared in 1X TBE and ethidium bromide (10mg/ml) was added in the gel at a concentration of 3 µl per 100 ml of gel and then mixed gently. It was poured in gel casting tray with appropriate comb with required well number and size. PCR products were mixed with loading dye and loaded into wells. The gel was run at the voltage of 100 watts for 1 hour and is seen under UV light for presence of bands. And photo was taken in gel documentation system.

Allele scoring

The total number of bands within each line and number of polymorphic bands were noted. Each DNA fragment amplified by a given primer was considered as a unit character and the RAPD amplification profiles were scored visually, based on presence (taken as 1) or absence (taken as 0) of bands for each genotype. Only clear and unambiguous bands were scored. The size of amplified bands was determined based on its migration relative to standard molecular weight markers.

Cluster analysis

The scored band data was subjected to statistical analysis using the computer programme NTSYS (version 2.02). The resultant similarity matrix was used to generate a tree by UPGMA. Dendrogram was constructed by using distance matrix in SAHN sub-programme of NTSYS-pc software by the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) algorithm. Principal Component Analysis (PCA) was done to construct 2 and 3 dimensional diagrams.

Results and Discussion

Primer selection and polymorphism exhibited

The analysis of the prescreened data using 48 fenugreek genotypes and thirty RAPD primers showed that nineteen primers amplified unambiguous, readable and reproducible polymorphic bands. A total of 146 amplification products were produced from the selected 19 primers out of which 119 bands were polymorphic. The number of bands varied from 3 to 12 and the size ranged from 150 to 1500 bp. The highest number of bands (12) obtained in primer OPP-8, while the lowest number of bands (3) was observed with primer OPC-19 with an average of 7.68 bands per primer. Highest number of polymorphic bands was observed in primer OPP-8 and the lowest number of polymorphic bands was observed in primer OPC-19 and OPC-2. The percentage of polymorphism ranged from 50.00 to 91.66 per cent with an average of 79.21 % polymorphism per primer. Primer OPP-8 revealed the highest polymorphism (91.66%), whereas the primer OPC-2 exhibited the lowest polymorphism (50.00%). The polymorphic information content (PIC) value ranged from 0.66 to 0.90. Highest PIC value was obtained in primers OPP-8 followed by OPP-9, OPH-5, OPF-13, OPJ-18, OPB-5, OPC-17, OPAB-2, OPH-4 and OPN-2 and the lowest PIC value was obtained in OPC-19 followed by OPC-11 and OPG-11 (Table 2).

It clearly indicated that the primers used in the present study contributed moderate to high as far as PIC value is concerned. Their relative contribution is assessed accordingly and must be utilized in the light of PIC value. So, the primers OPP-08, OPP-09, OPH-05, OPF-13, OPJ-18, OPB-05, OPC-17, OPAB-02, OPH-04 and OPN-02 proved best to assess the diversity in fenugreek. Similar results were reported by Dangi *et al*., (2004), Sundaram *et al*., (2011), Choudhary *et al.*, (2013) and Tomar *et al*., (2014). Since there was no relationship between the total number of bands generated by a primer and amount of polymorphism produced, we cannot assign the ranking to a particular primer simply based upon the total number of bands it produces. In literature there is no report indicating the existence of any such correlation.

Table.1 RAPD primers used in present investigation

S. No.	Primer code	Primer sequence	Length		S. No.	Primer code	Primer sequence	Length
1	OPB-05	TGCGCCCTTC	10		16	OPH-06	ACGCATCGCA	10
2	OPC-02	GTGAGGCGTC	10		17	OPJ-02	CCCGTTGGGA	10
3	OPC-05	GATGACCGCC	10		18	OPJ-04	CCGAACACGG	10
4	OPC-08	TGGACCGGTG	10		19	OPJ-06	TCGTTCCGCA	10
5	OPC-09	CTCACCGTCC	10		20	OPJ-10	AAGCCCGAGG	10
6	OPC-11	AAAGCTGCGG	10		21	OPJ-18	TGGTCGCAGA	10
7	OPC-17	TTCCCCCCAG	10		22	OPJ-20	AAGCGGCCTC	10
8	OPC-19	GTTGCCAGCC	10		23	OPN-02	ACCAGGGGCA	10
9	OPF-13	GGCTGCAGAA	10		24	OPP-08	ACATCGCCCA	10
10	OPF-14	TGCTGCAGGT	10		25	OPP-09	GTGGTCCGCA	10
11	OPF-18	TTCCCGGGTT	10		26	OPAB-01	CCGTCGGTAG	10
12	OPG-07	GAACCTGCGG	10		27	OPAB-02	GGAAACCCCT	10
13	OPG-11	TGCCCGTCGT	10		28	OPAB-03	TGGCGCACAC	10
14	OPH-04	GGAAGTCGCC	10		29	OPAB-04	GGCACGCGTT	10
15	OPH-05	AGTCGTCCCC	10		30	OPAB-06	GTGGCTTGGA	10

Table.2 Polymorphism exhibited by RAPD primers in fenugreek genotypes

Sr. No.	Primer code	Primer sequence	Length	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism (%)	PIC	Molecular weight range (bp)
1.	OPAB-2	GGAAACCCCT	10	9	1	8	88.88	0.86	350-1000
2.	OPB-5	TGCGCCCTTC	10	10	1	9	90.00	0.87	200-1000
3.	OPF-13	GGCTGCAGAA	10	9	3	6	66.66	0.88	150-1000
4.	OPF-14	TGCTGCAGGT	10	10	2	8	80.00	0.80	200-1200
5.	OPH-5	AGTCGTCCCC	10	10	1	9	90.00	0.89	200-1000
6.	OPH-4	GGAAGTCGCC	10	10	2	8	80.00	0.85	300-1000
7.	OPJ-6	TCGTTCCGCA	10	7	2	5	71.42	0.82	400-1000
8.	OPJ-10	AAGCCCGAGG	10	7	1	6	85.70	0.83	200- 800
9.	OPC-17	TTCCCCCCAG	10	6	1	5	83.33	0.87	500-1500
10.	OPJ-18	TGGTCGCAGA	10	11	2	9	81.81	0.88	400-1500
11.	OPJ-20	AAGCGGCCTC	10	8	1	7	87.50	0.85	350-1000
12.	OPP-8	ACATCGCCCA	10	12	1	11	91.66	0.90	300-1500
13.	OPP-9	GTGGTCCGCA	10	10	1	9	90.00	0.89	300-1500
14.	OPC-11	AAAGCTGCGG	10	4	1	3	75.00	0.67	500-1000
15.	OPC-19	GTTGCCAGCC	10	3	1	2	66.66	0.66	900-1000
16.	OPC-2	GTGAGGCGTC	10	4	2	2	50.00	0.74	400- 900
17.	OPG-11	TGCCCGTCGT	10	4	1	3	75.00	0.67	400- 700
18.	OPH-6	ACGCATCGCA	10	5	1	4	80.00	0.77	600-1200
19.	OPN-2	ACCAGGGGCA	10	7	2	5	71.42	0.85	200-1000
Total				146	27	119			
Average				7.68	1.42	6.26	79.21		

Table.3 Clustering of 48 fenugreek genotypes using RAPD at 0.75 similarity coefficient

Clusters	Number of genotypes	Genotypes
I	1	PEB-1.
II	2	UM-354, UM-202.
III	3	HM-307, HM-273, HM-507.
IV	5	HM-65, HM-208, HM-555, HM-258-1, HM-257.
V	3	HM-548, HM-517, HM114.
VI	4	HM-343, HM-348, HM-355, HM-239-1.
VII	1	RajendraKranti.
VIII	21	LFC-93, HM-528, HM-242, HM-205, NDM-69, NDM-72, HM-444, JFG-235, HM-502, HM-359, HM-526, AFG-6, JFG-266, AFG-5, HM-332, HM-103, HM-346, HM-536, HM-221, GM-1, GM-2.
IX	2	HM-291, HM-281.
X	6	RM-188, HM-509, RM-194, HM-246, HM-57, HM-519.

Fig.1 Dendrogram generated using RAPD markers in 48 fenugreek genotypes

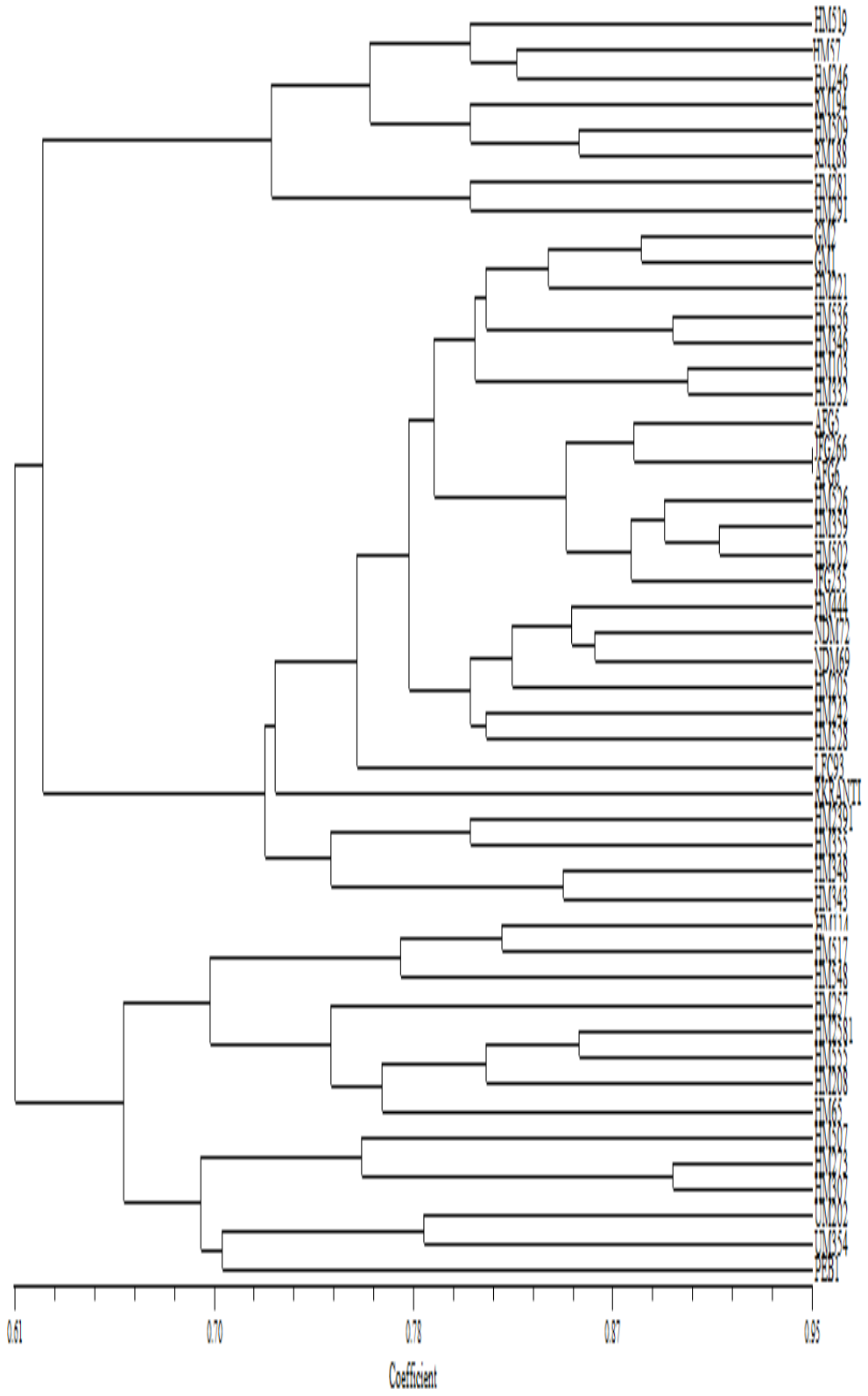


Fig.2 Two dimensional PCA scaling of 48 fenugreek genotypes using RAPD

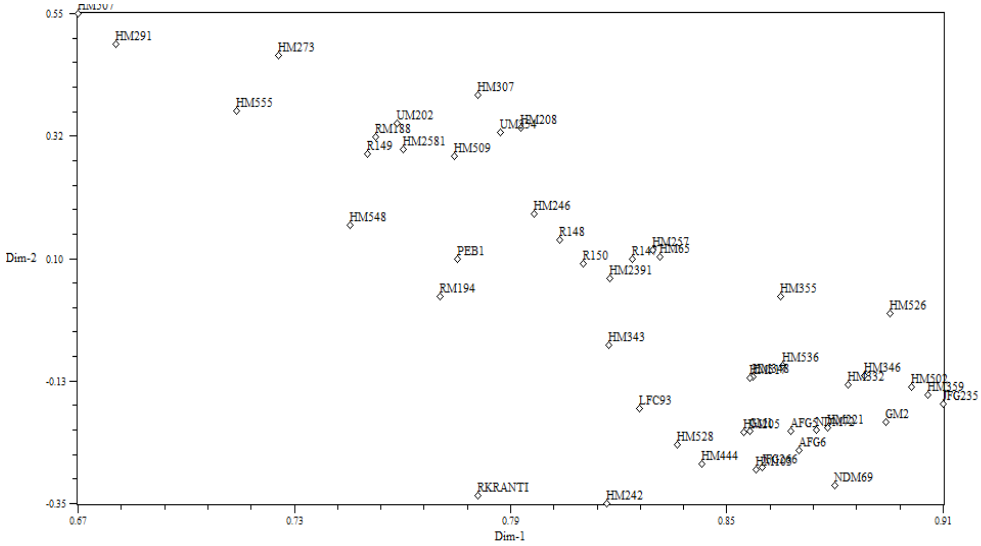
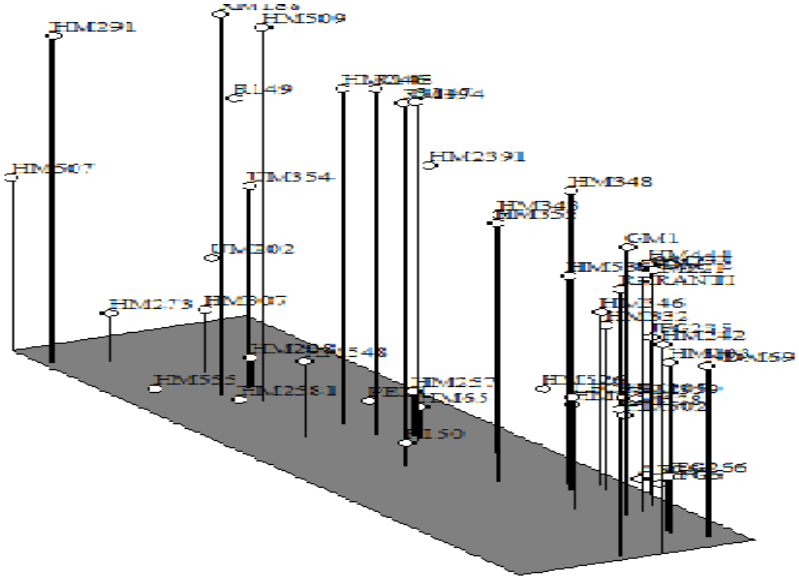


Fig.3 Three dimensional PCA scaling of 48 fenugreek genotypes using RAPD



Cluster analysis

Molecular data obtained using 19 RAPD primers was used to construct dendrogram which, divided the fenugreek genotypes into 10 clusters at 0.75 similarity co-efficient (Table 3). The similarity co-efficient ranged from 0.61 to 0.95 (Fig. 1). The two dimensional as well as three dimensional diagrams also formed using same results as of dendrogram and are presented in figures 2 and 3 respectively. The genotypes from one region were grouped together in some cases while they were placed in different clusters in certain cases. The clusters containing genotypes from same region maybe due to their pedigree relationship or they may be selected from same breeding population.

The measures of relative genetic distances among varieties of fenugreek did not completely correlate with the geographical

distances of places of their development. And the genotypes which are falling in different groups indicate the presence of genetic diversity between the members of different clusters. Crossing can be made between the members of different clusters as they are genetically diverse. Similar results were observed by Sundaram *et al*., (2011), Choudhary *et al.,* (2013) and Tomar *et al*., (2014).

It is concluded that RAPD markers proved best for assessing genetic diversity in fenugreek germplasm. This shows the clear picture of existence of diversity in genotypes at genetic level and the genotypes used in the present study can be utilized for further crop improvement programme.

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