

## Original Research Article

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## Assessment of Genetic Diversity among Fenugreek (*Trigonella foenum-graecum* L.) Genotypes using ISSR Markers

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### ABSTRACT

Fenugreek [*Trigonella foenum-graecum* L.] is an annual herb of Leguminaceae family and is largely cultivated in warm temperate and tropical regions. The presence of variability in breeding population is prior need for crop improvement. This calls for an evaluation of genotypes to study the variability. The study was conducted to reveal the genetic diversity among 48 fenugreek genotypes which were collected from different geographical regions of India using Inter Simple Sequence Repeat markers. Totally twenty ISSR primers were screened among which, ten primers gave good amplification and revealed a total of 48 polymorphic bands with an average of 4.8 bands per primer. The percentage of polymorphism ranged from 75.00 % (UBC-842) to 100 % (UBC-851). Highest Polymorphic Information Content (PIC) value was obtained in primers UBC-842 and UBC-860 among ten primers these proved best to assess the genetic diversity in fenugreek. 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 from one region were grouped together in some cases, while they were placed in different clusters in certain cases. The measures of relative genetic distances among varieties of fenugreek did not completely correlate with the geographical distances 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 for further crop improvement, as they are genetically diverse.

#### Keywords

Fenugreek,  
Diversity, ISSR,  
UPGMA, PIC.

#### Article Info

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### Introduction

The genus *Trigonella* is one of the largest genera of the tribe Trifoliatae in the family Fabaceae and sub-family Papilionaceae (Balodi *et al.*, 1991). Among *Trigonella* species, *Trigonella foenum-graecum* L. commonly known as fenugreek is a flowering annual with autogamous white flowers occasionally visited by insects. Indigenous to countries on the eastern shores of Mediterranean, fenugreek is widely cultivated in India, Egypt, Ethiopia, Morocco and occasionally in England (Polhil *et al.*, 1981). *Trigonella foenum-graecum* L. is

extensively grown in the tropical and subtropical regions of India. Different parts of the plant such as leaves and seeds are consumed in India and are also utilized for medicinal purpose. In India, during 2011-12 this crop occupied an area of 0.93 lakh hectares and produced 1.13 lakh MT seed spice with productivity of 1.2 MT per hectare (Anonymous, 2014).

However, in view of the low production potential of traditional fenugreek varieties, there is dire need of improvement in this crop

and the major objective lies in the development of new varieties with high yield and improved yield attributing traits. For any crop improvement the basic requirement is availability of germplasm with wide variability. Assessment of genetic divergence in fenugreek germplasm is important for long term crop improvement programme. Undoubtedly, the study of the genetic diversity of fenugreek genotypes is not only important for germplasm conservation but also in selecting the parents for breeding purposes. The morphology of plant is influenced by environment and developmental stages of crop, in this context the molecular markers provide a very accurate tool for evaluating genetic diversity of crop germplasm that focuses on understanding the genetic relationship among different

accessions of fenugreek. These markers are not influenced by environment as well as the plant development stages and are also very less time consuming compared to the morphological characterization.

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

### Genomic DNA isolation

Genomic DNA was isolated from young leaves of 48 fenugreek genotypes following CTAB (Cetyl trimethyl ammonium bromide) extraction method as given by Murray and Thompson (1980) and modified by Saghai-Maroo *et al.*, (1984). The quality and quantity of isolated genomic DNA was 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

Totally twenty ISSR 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 concentration 10mM + 500 µl of sterile distilled water), 2 µl of MgCl<sub>2</sub> (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°C, followed by 36 cycles of 1 min at 94°C for denaturation, annealing temperature varied according to primer, 72°C for 2 min and for final extension 72°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**

ISSR 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).

### **Results and Discussion**

#### **Primer selection and polymorphism exhibited**

Among the twenty ISSR primers ten primers amplified unambiguous, readable and reproducible polymorphic bands. A total of 59 amplification products were produced from the selected 10 primers out of which 48 bands were polymorphic. The number of bands varied from 4 to 9 and the size ranged from 200 to 1500 bp. The highest number of bands (9) obtained in primer UBC-860, while the lowest number of bands (4) was observed with primer UBC-851 with an average of 5.9 bands per primer. Highest number of polymorphic bands (7) was observed in primer UBC-860 and the lowest number of polymorphic bands (4) was observed in primer UBC-807, UBC-834, UBC-840, UBC-851, UBC-845 and UBC-850. The percentage of polymorphism ranged from 75.00 to 100 % with an average of 83.15 % polymorphism per primer. Primer UBC-851 revealed the highest polymorphism (100 %), whereas the primer UBC-842 exhibited the lowest polymorphism (75.00 %). The polymorphic information content (PIC) value ranged from 0.56 to 0.86. Highest PIC value was obtained in primers UBC-842 and UBC-860 and the lowest PIC value was obtained with the primer UBC-851. The details are presented in table 2. It clearly indicated that the primers used in the present study contributed moderate to high as far as PIC is concerned. Their relative contribution is assessed accordingly and must be utilized in the light of PIC value. So, the primers UBC-842 and UBC-860 among ten primers proved best to

assess the diversity in fenugreek. Similar results were reported by Harish *et al.*, (2011), Nidhal *et al.*, (2009) 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** ISSR primers used in present investigation

S. No.	Primer code	Primer sequence	Length
1	UBC-807	AGAGAGAGAGAGAGAGT	17
2	UBC-834	AGAGAGAGAGAGAGAGYT	18
3	UBC-835	AGAGAGAGAGAGAGAGYC	18
4	UBC-838	TATATATATATATATARC	18
5	UBC-840	GAGAGAGAGAGAGAGAYT	18
6	UBC-841	GAGAGAGAGAGAGAGAYC	18
7	UBC-842	GAGAGAGAGAGAGAGAYG	18
8	UBC-843	CTCTCTCTCTCTCTRA	18
9	UBC-844	CTCTCTCTCTCTCTRC	18
10	UBC-845	CTCTCTCTCTCTCTRG	18
11	UBC-850	GTGTGTGTGTGTGTGYC	18
12	UBC-851	GTGTGTGTGTGTGTGYG	18
13	UBC-852	TCTCTCTCTCTCTCRA	18
14	UBC-856	ACACACACACACACACYA	18
15	UBC-857	ACACACACACACACACYG	18
16	UBC-858	TGTGTGTGTGTGTGTGRT	18
17	UBC-860	TGTGTGTGTGTGTGTGRA	18
18	UBC-882	VBVATATATATATATAT	17
19	UBC-894	TGGTAGCTCTTGATCANNNNN	21
20	UBC-896	AGGTCGCGGCCGCNNNNNNATG	22

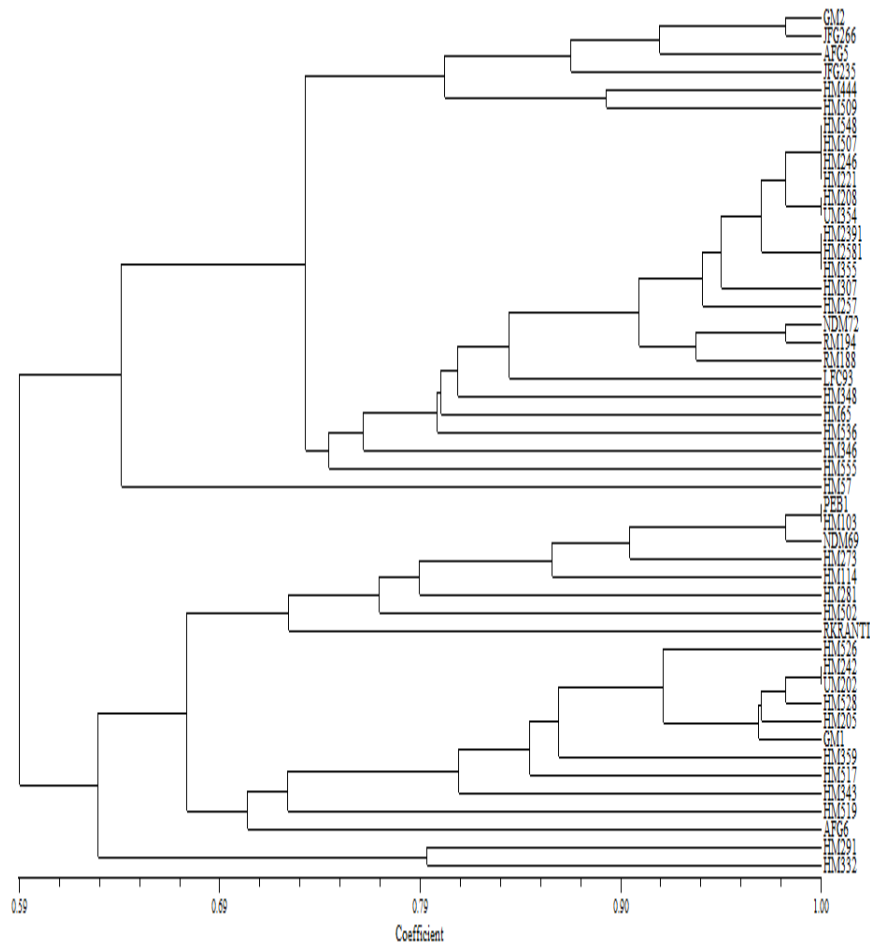
**Table.2** Polymorphism exhibited by ISSR primers in fenugreek genotypes

Sr. No.	Primer code	Primer sequence 5'<----->3'	Length	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism (%)	PIC	Molecular weight range (bp)
1.	UBC-807	AGAGAGAGAGAGAGAGT	17	5	1	4	80.00	0.78	500-1000
2.	UBC-834	AGAGAGAGAGAGAGAGYT	18	5	1	4	80.00	0.79	300-950
3.	UBC-840	GAGAGAGAGAGAGAGAYT	18	5	1	4	80.00	0.77	300-1000
4.	UBC-841	GAGAGAGAGAGAGAGAY C	18	6	1	5	83.33	0.74	200-1000
5.	UBC-851	GTGTGTGTGTGTGTGTYG	18	4	0	4	100.00	0.56	700-1200
6.	UBC-842	GAGAGAGAGAGAGAGAY G	18	8	2	6	75.00	0.86	200-1000
7.	UBC-843	CTCTCTCTCTCTCTRA	18	7	1	6	85.71	0.84	600-1000
8.	UBC-845	CTCTCTCTCTCTCTRG	18	5	1	4	80.00	0.74	400-1000
9.	UBC-850	GTGTGTGTGTGTGTGTYC	18	5	1	4	80.00	0.78	450-1000
10.	UBC-860	TGTGTGTGTGTGTGTGRA	18	9	2	7	87.50	0.86	300-1500
Total				59	11	48			
Average				5.9	1.1	4.8	83.15		

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

Clusters	No. of genotypes	Genotypes
I	2	HM-332, HM291.
II	1	AFG-6.
III	1	PEB-1.
IV	9	HM-343, HM-517, HM-359, UM-354, HM-205, HM-528, UM-202, HM-242, HM-526.
V	1	R. Kranti.
VI	7	HM-502, HM-281, HM-114, HM-273, NDM-69, NDM-72, HM-519.
VII	1	HM-57.
VIII	1	HM-555.
IX	18	HM-346, HM-65, HM-348, LFC-93, RM-188, RM-194, HM-103, HM-257, HM-307, HM-355, HM-258-1, HM-239-1, GM-1, HM-208, HM-221, HM-246, HM-507, HM-548.
X	6	HM-509, HM-444, JFG-235, AFG-5, JFG-266, GM-2.

**Fig.1** Dendrogram generated using ISSR markers in 48 fenugreek genotypes





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. These results were in agreement with the findings of Harish *et al.*, (2011), Nidhal *et al.*, (2009) and Tomar *et al.*, (2014).

In conclusion present study gave the clear picture of existence of diversity in genotypes at genetic level as DNA markers are not influenced by environmental effects or plant growth stages whereas morphological markers does. ISSR markers proved best for assessing genetic diversity in fenugreek germplasm. This shows that the genotypes which were used in the present study can be utilized for further crop improvement programme.

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