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Molecular and Biochemical Characterization of Different Accessions of Fenugreek (*Trigonella foenum-graecum* L.)

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Abstract: Estimation of genetic variability is important for improvement of any crop. We studied the extent of variability among the 10 accessions of fenugreek (*Trigonella foenum-graecum* L.) repository of National Bureau of Plant Genetic Resources at Jodhpur Station, using RAPD and ISSR genetic markers. Multilocus genotyping by 10 SPAR (RAPD and ISSR) primers revealed intraspecific polymorphism in banding patterns. The referenced tree generated after 100 resampling via bootstrap method showed that accession IC-373449, IC-396616 and IC-448830 are distantly placed. Two major clusters were formed from rest of the accessions, one having four accessions viz. IC-448828, IC-448832, IC-448833 and IC-448834 and other having three accessions viz. IC-448835, IC-448837 and IC-396625. Diosgenin and gum content were also estimated. Study can be useful for designing of intraspecific crosses between cultivars of these fenugreek collections with potential interest in seed spices breeding programme.

Key words: Diosgenin % Fenugreek Gum % Genetic Diversity % RAPD % ISSR % Spice Crop

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an important annual legume seed spice crop. It has been used as a culinary spice, a flavoring agent and as a medicinal plant for centuries. In Ayurvedic and Unani systems of medicine, fenugreek is used for treatment of epilepsy, paralysis, gout, dropsy, chronic cough and piles [1]. This crop is also used as vegetable, fodder and green manure, has the additional recognized potential of oleoresin and steroid production for oral contraceptives [2]. Fenugreek seed contains saponins (steroidal in nature and belong to secondary plant metabolite) with diosgenin as the main sapogenin [3]. Saponins have many flavoring, sweetening, antioxidant, foaming, complexing, anticarcinogenic and antimicrobial properties. Another important product of commerce, galactomannan (fenugreek gum; a major polysaccharide), is known to found in fenugreek seeds too. Fenugreek galactomannan is considered unique due to a 1:1 to 1.2:1 ratio of galactose to mannose (G:M) molecules. This high ratio of galactose substitution helps galactomannan to adsorb water allowing them to form highly viscous solution at relatively low concentration resulting in reduced glucose absorption within digestive tract. This property of fenugreek gum is under exploited in the global food industry. The annual volume of world trade is about 10,000 tonnes. This crop is being cultivated abundantly in India claiming 70-80% of world's export share. The state of Rajasthan supplies 83-90% of this and ranks first in fenugreek production in India [4]. Inspite of large potential, however, no reports on genetic diversity using molecular markers is available in *Trigonella spp.* native to this region. The present study was therefore aimed to focus on understanding the genetic relationship among different accession of fenugreek, which may further assist in developing and planning breeding strategies for crop improvement programs. The diosgenin content and fenugreek gum content in different seed accession of fenugreek were also aimed to estimate.

MATERIALS AND METHODS

Plant Material: Fenugreek (*Trigonella foenum-graecum* L.) seed of different accessions were obtained from NBPGR (National Bureau of Plant Genetic Resources), at Jodhpur station. These seed accessions have the variations on the basis of yield, color, plant height. Seed of 10 accessions were sown in green house conditions and after two weeks juvenile leaves were used for genomic DNA isolation. Seed sample (without sowing) were used for quantification of diosgenin and gum content. Detailed procedure for the same are as under:

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Table 1: RAPD and ISSR primers used in the present study

S.No.	Primer Name	Primer Sequence 5'-3' (Length)
1.	RAPD - OPA-02	TGCCGAGCTG (10mer)
2.	RAPD - OPAB-03	TGGCGCACAC (10mer)
3.	RAPD - OPN-02	ACCAGGGGCA (10mer)
4.	RAPD-OPK-01	CATTCGAGCC (10mer)
5.	RAPD-OPG-07	GAACCTGCGG (10mer)
6.	ISSR – 806 (UBC)	TATATATATATATATAG (17mer)
7.	ISSR – 831 (UBC)	ATATATATATATATATYA (18mer)
8.	ISSR – 838 (UBC)	TATATATATATATATARC (18mer)
9.	ISSR – 807 (UBC)	AGAGAGAGAGAGAGAGT (17mer)
10.	ISSR – 840 (UBC)	GAGAGAGAGAGAGAGAYT (18mer)

DNA Isolation: Frozen leaves were ground and powdered in a pre-chilled mortar using liquid nitrogen and the DNA was then extracted by the modified CTAB method [5] with 2% CTAB and 2% PVP. The extracted DNA was treated with RNase A and re-precipitated with pre-chilled absolute ethanol and subsequently dissolved in Tris–EDTA (TE) buffer. The quality of DNA was checked by mupid gel electrophoresis with 0.8% (w/v) agarose in TBE.

PCR Amplification: DNA amplification reactions were assembled in 25 µl volumes containing 75 ng of template DNA, 1× PCR buffer (100 mM Tris (pH 9.0), 500 mM KCl, 1% Triton-X-100 with 15 mM $MgCl_2$), 0.2 mM each of dATP, dCTP, dGTP and dTTP (Bangalore Genei, India), 1.5 mM $MgCl_2$ and 1.5U *Taq* DNA polymerase (Bangalore Genei, India). The primers were added to a final concentration of 0.2 µM. The samples were amplified in a thermal cycler (Eppendorf 5331) through initial denaturation for 4 min at 94°C; 40 cycles of denaturation for 30 seconds at 94°C; annealing for 1 min at 35°C; elongation for 2 min at 72°C followed by final extension for 10 min at 72°C. The amplification products for all samples were resolved on 1.4% agarose gel. From the preliminary screening, 5 RAPD primers and 5 ISSR that could amplify visible bands were used for this study (Table 1).

Molecular Data Analysis: Each amplification product was considered a DNA marker and was scored across all samples. Bands were recorded as present (1) or absent (0). Faint bands with low intensity were not considered for final scoring. The data were scored cumulatively for all the primers and were combined together for the final Neighbor-Joining (NJ) analysis. A pair-wise matrix of distances between genotypes was determined cumulatively using Jaccard coefficient [6] by the Free Tree program [7]. This distance matrix was used to compute a single NJ-tree after allowing a 100 replicate bootstrap test using the same program. The tree was viewed, annotated and printed using Tree View (ver. 1.6.5).

Estimation of Diosgenin Content: For estimation of diosgenin content, the sample seed were ground in pestle and mortar to form a fine powder and these samples were defatted using n-hexane. Saponin was extracted from these defatted samples by methanol. Diosgenin was estimated from this crude saponin. Entire procedure has been described by Chapagain and Wiesman [8].

Extraction of Fenugreek Gum: Hundred mg of fine powder were placed in test tube and fenugreek gum was extracted in water at a ratio of 1:30 w/v, on an agitator for 4 hours at 60°C. The slurry that formed was centrifuged at 8000 rpm for 15min (Sigma 3K15 Refrigerated Centrifuge), to yield heavy and light components. The light phase was precipitated by mixing with equal amount of anhydrous ethanol, the precipitated gum produced thereby then recovered by further centrifugation. The recovered crude gum was washed twice by anhydrous ethanol. The recovered clean gum solids were then dried at room temperature.

RESULTS AND DISCUSSION

Fenugreek (*Trigonella foenum-graecum*) is receiving global attention due to its unique medicinal properties of significance to human health. Gene banks possess scanty germplasm and very little background information regarding its genetic variability that has hampered its improvement. Estimation of genetic diversity is very important for any crop improvement program. In the present study, an attempt has been made to examine the level of genetic variation within *T. foenum-graecum* accessions obtained from germplasm collection at NBPGR Jodhpur Station. Using 10 primers (5 RAPD and 5 ISSR), 36 bands were produced with an average of 3.6 bands per primer. Primer OPA-02 and UBC-840 amplified minimum and maximum number of bands where were 2, 6 bands respectively. A total of 248 scorable bands were generated from these RAPD and ISSR primers. Multilocus genotyping by 10 SPAR primers detected intraspecific variations in banding patterns. The referenced tree generated after 100 resampling via bootstrap method shows accession IC-373449, IC-396616 and IC-448830 are distantly placed from rest of

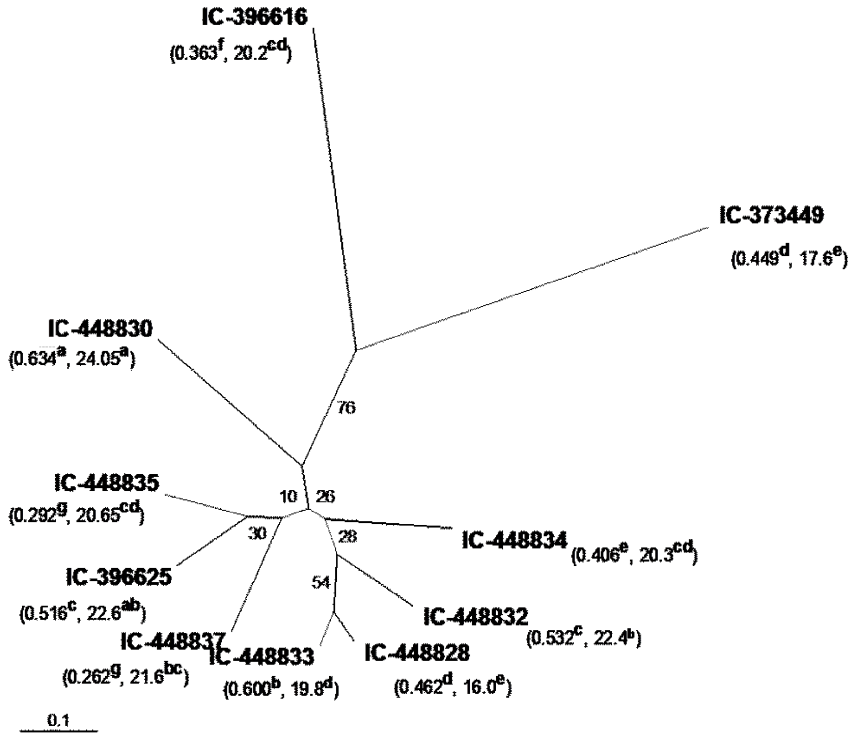

Fig. 1: Cluster analysis molecular profiling data of the 10 accessions after 100 replicate bootstrap analyses. The NJ tree was generated for cumulative band data (RAPD and ISSR). The branch lengths are based on the distance values computed using NJ method and Jaccard coefficient in the program Free Tree. The numbers at the nodes in each tree are the bootstrap percent values. First value in parenthesis is of Diosgenin content and next is fenugreek gum for ready reference. Superscript letter shows their Duncan's group

the accessions studied. Two major clusters were formed from rest of the accessions. One having four accessions viz. IC-448828, IC-448832, IC-448833 and IC-448834 and other having three accessions viz. IC-448835, IC-448837 and IC-396625 (Fig. 1). The cluster analysis revealed considerable variation present among different accession of this plant and there is scope for crop improvement by hybridization and marker assisted selection. The present study demonstrates the potential of RAPD and ISSR techniques to study the level and partitioning of genetic variations within the species. The two marker systems, RAPD and ISSR have been used as effective tools to evaluate genetic diversity and to assess the phylogenetic relationships in *Allium sacculiferum* [9], *Asimina triloba* [10, 11], *Brassica napi* [12] and *Hordeum vulgare* [13]. Genetic diversity analysis using RAPD and ISSR markers in legumes like *Cajanus* [14, 15], *Cicer* [16, 17] and *Prosopis cineraria* [18] have also been carried out. These studies have given important clues understanding species relationship, which may further assist developing and planning breeding strategies.

Natural diosgenin is mainly procured from the tubers of certain wild species of Mexican yam (*Dioscorea species)*. However, there are some alternative plants sources for production of diosgenin such as *Balanities aegyptiaca* and *Trigonella fonum-graceum.* The seeds of fenugreek are good source of diosgenin, because of its shorter growing cycle, lower production cost, consistent yield and quality. For improvement of crop with higher yield of diosgenin in filial generations, estimation of parental diosgenin content among different genotypes is prerequisite. Our analysis revealed that there is a large variation in resultant diosgenin percent among the 10 accession that were analyzed in this study (Fig. 2). The highest level of diosgenin was obtained in the IC-448830 (0.634); while the lowest for IC-448837 (0.262).

Different modifications were tried for extraction of maximum fenugreek gum. Heating treatment at 60°C for 4-h gave higher yield of fenugreek gum. Precipitation with equal volume of ethanol is found suitable for maximum recovery of gum. The highest percentage of fenugreek gum is obtained in accessions no. IC-448830 and the lowest for IC-448828.

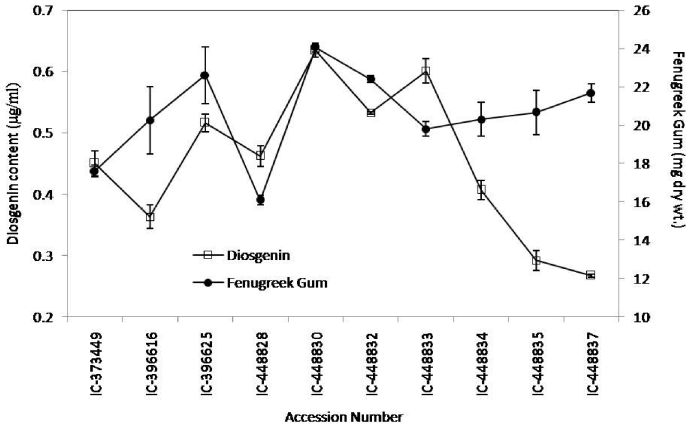

Fig. 2: Diosgenin and gum content in different accession of fenugreek

Eventually, the knowledge of their genetic relationships with biochemical characterization might contribute for the designing of intraspecific crosses between cultivars of this fenugreek collection with potential interest in seed spices breeding programme. Results showed that there is enough scope for fenugreek crop improvement using these 10 accessions.

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