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Genetic analysis of fenugreek (*Trigonella foenum-graecum*) accessions using morphometric and ISSR markers

GURINDER JIT RANDHAWA[1], MONIKA SINGH[2], K K GANGOPADHYAY[3], GUNJEET KUMAR[4] and SUNIL ARCHAK[5]

National Research Centre on DNA Fingerprinting, National Bureau of Plant Genetic Resources, Pusa, New Delhi 110 012

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ABSTRACT

In a pilot study, as a prelude for characterization of the entire *Trigonella* germplasm conserved in the National Gene Bank, National Bureau of Plant Genetic Resources, New Delhi, genetic relatedness among a selection of 49 accessions of fenugreek (*Trigonella-foenum-graecum* L.) was assessed using 19 morphometric and 186 inter-simple sequence repeat (ISSR) markers. An accession of *T. corniculata* L. was also assessed as an out-group. The accessions were collected from different eco-geographical sites located in the states of Andhra Pradesh, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Uttar Pradesh, Uttarakhand, Rajasthan, Gujarat, Manipur and Bihar; and one of the accessions was imported from Eritrea. Data for 12 qualitative and seven quantitative morphometric descriptors were recorded. Significant differences within the accessions were found for all the quantitative descriptors except primary branches and seeds/pod. Shannon-Wiener Diversity Index revealed substantial diversity for all the quantitative descriptors. The morphometric data differentiated the fenugreek accessions into two clusters (at ~65% similarity). A total of 100 ISSR primers were used for initial screening, out of which only 21 primers were found polymorphic. ISSR analysis was performed with selected 21 primers to generate 186 amplicons, of which 92.4% were polymorphic. Cluster analysis put 47 accessions in a single group at ~65% similarity. Though there was no agreement between the groupings based on morphometric and ISSR markers (Mantel statistic 0.096), specific cases of geographic groupings were supported by both the markers. Phylogenetic positioning of the accessions with no passport information was found to be possible. The ISSR markers complemented the morphometric data in understanding the genetic divergence among the fenugreek accessions.

Key words: Fenugreek, Genetic relatedness, ISSR, Morphometric descriptors, *Trigonella*

The genus *Trigonella* is one of the largest genera of the tribe Trifoliate in the family Fabaceae and sub-family Papilionaceae (Balodi and Rao 1991). Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest medicinal plants, originating in India and North Africa (Basch *et al.* 2003), and indigenous to countries on the eastern shores of Mediterranean (Polhil and Raven 1981; Dangi *et al.* 2004). In India, it is extensively grown in tropical and sub-tropical regions. The different plant parts such as tender leaves and stems are used as leafy vegetable, whereas matured ripened dry seeds are consumed as seed spices. According to Ayurvedic system, it is a herbal drug that is effective against anorexia and acts as a gastric stimulant (Rajagopalan 2001). Its seeds exert hypoglycemic effects in humans (Basch *et al.* 2003) and may also lower serum triglycerides, total cholesterol and low-density lipoprotein cholesterol (Al-Habori *et al.* 1998). The leaves of Kasoori methi (*T. corniculata* L.) are generally used as a condiment for flavouring and adding special delicious taste.

Intensive agriculture and the introduction of new varieties can result in the loss of much of the genetic diversity exhibited by the local and traditional varieties of cultivated crops (Asins and Carbonell 1989). The ever-increasing demand of herbal drugs is creating shortage of raw material and putting stress on biodiversity. In order to formulate appropriate strategies for the conservation and utilization of the cultivars and their wild relatives, it is imperative to assess the genetic composition and variability of species belonging to different phyto-geographical regions.

Multi-locus DNA markers such as random amplified polymorphic DNA (RAPD) have been extensively used for

[1] Principal Scientist (e mail: gurinder.randhawa@rediffmail.com, gjr@nbpgr.ernet.in), [2] Scientist Probationer (e mail: monika_a_singh@ rediffmail.com), 95th FOCARS, National Academy of Agricultural Research Management, Hyderabad, Andhra Pradesh 500 407; [3] Senior Scientist (e mail: gangopadhyay@nbpgr.ernet.in), Germplasm Exchange Division, National Bureau of Plant Genetic Resources, Pusa, New Delhi 110 012; [4] Senior Scientist (e mail: kumar_gunjeet@yahoo.com), Directorate of Floricultural Research, IARI, Pusa Campus, New Delhi 110 012; [5]Senior Scientist (e mail: sarchak@nbpgr.ernet.in), OIC Aris Cell, National Bureau of Plant Genetic Resources, Pusa, New Delhi 110 012

assessment of genetic diversity in Indian medicinal plants. ISSR markers have been more informative than RAPDs for the genetic diversity studies in medicinal species such as fenugreek (Dangi *et al*. 2004)*, Gaultheria fragrantissima* Wall. (Apte *et al.* 2006)*, Abrus precatorious* (Randhawa *et al.* 2007), and *Tribulus terrestris* (Sarwat *et al.* 2008).The National Gene Bank located at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, has a collection of 571 fenugreek germplasm accessions. The NBPGR aims to characterize all the accessions using morphometric as well as molecular markers. However, before undertaking the large-scale application of molecular markers in genetic characterization studies, it is crucial to select the molecular markers in conjunction with morphometric analysis. This approach is of coupling molecular markers with morphometric markers is expected to offer greater precision to the genetic analyses of the target population. As a pilot study, a random sample of 50 fenugreek accessions was selected to carry out genetic analysis employing both molecular (ISSR) as well as morphometric markers. Here, we report an assessment of the utility of combined marker approach in characterizing fenugreek germplasm.

MATERIALS AND METHODS

The arbitrarily chosen selection of 50 fenugreek germplasm contained 26 accessions with complete passport data, 17 accessions with incomplete or no passport data, one accession with only collector's identification number (Table 1), one exotic accession (EC 520255) imported from Eritrea in 2003, four released varieties (AM 10, AM 35, RMt 1 and Pusa Early Bunching) and one accession (IC 143900) belonging to *T. corniculata* L. Germplasm was collected from the states of Andhra Pradesh (AP), Himachal Pradesh (HP), Jammu and Kashmir (J and K), Madhya Pradesh (MP), Uttar Pradesh (UP), Uttarakhand (UK), Rajasthan (Raj), Gujarat (Guj), Manipur (Man) and Bihar.

The fenugreek accessions were raised during October to April in 2008-09 at NBPGR, Experimental Farm, Indian Agricultural Research Institute (IARI) Campus, New Delhi (28°38'18"N, 77°9'30"E) in randomized block design (RBD) with three replications in a plot size of 3.0 × 0.6 m accommodating two 3.0 m long rows spaced 30 cm apart with plant-to-plant distance of 10 cm. A basal dose of 30 kg nitrogen, 25 kg phosphorus and 15 kg potash were applied during land preparation. Weeding was done to obtain early growth and irrigations were given at 7–10 days interval. Recommended package of agronomic practices were followed as and when required. Harvesting was done when the pods matured and turned yellow. Data were recorded on 12 qualitative and 7 quantitative descriptors (Table 2) as per NBPGR descriptors list (Srivastava *et al.* 2001) on five random plants in each plot. The leaf colour, leaf margin, leaf margin colour, leaf size, leaf tip, petiole colour and petiole pubescence were recorded at marketable leaf harvest stage; stem colour at flowering stage, stem tenderness and stem pubescence at pod-filling stage; stem shape, plant height and number of primary branches at near maturity stage; and inflorescence colour at opening of flower. Number of pods/plant, number of seeds/pod and pod length were recorded at pod-maturity stage. Number of days to 50% flowering was recorded as number of days from date of sowing to date when at least 50% plants showed first flower open. Thousand seed weight was measured as weight of 1 000 matured and dry seeds after harvest.

For ISSR analysis, total genomic DNA was extracted from the fresh leaf tissue of 2-3 weeks old seedlings of *Trigonella* accessions using DNeasy Plant Mini Kit according to the protocols supplied. The DNA samples were quantified by measuring UV absorption at 260 nm using a UV spectrophotometer DU 640. The DNA samples were diluted to a final concentration of 5 ng/ul.

A total of 100 ISSR primers (University of British Columbia, Vancouver, Canada) were used for initial screening to test for robust amplification. Among these, 21 primers were selected for further analysis. PCR amplification was performed on a PTC-200 thermal cycler in a 20 µl of reaction mixture containing 25 ng template DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.75 µM primer and 1.2 units Taq DNA polymerase. The PCR conditions included an initial denaturation at 94°C for 5 min., followed by 45 cycles of 94°C for 30 sec, 50°C for 45 sec, 72°C for 2 min; and a final extension of 10 min at 72°C. Reactions were replicated at least thrice. The amplified products were resolved in 1.2% agarose gel in 1× TAE buffer (40 mM Tris-acetate, 1 mM EDTA *p*H 8.0), stained with ethidium bromide (10 µg/ml) and visualized under UV light using Gel Documentation Unit. Amplicons were sized in comparison to 1 kb DNA ladder.

Statistical analysis was performed on quantitative descriptors and the variability parameters, viz. mean, minimum, maximum, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) were calculated using statistical package SPAR 2.0 (Ahuja *et al.* 2008). The quantitative data were converted into qualitative data by making equal class intervals and the frequencies were computed. The frequency distribution for quantitative and qualitative descriptors was computed and the phenotypic frequencies were analyzed by the Shannon-Wiener Diversity Index (H´) in order to estimate the diversity of each descriptor (Shannon and Weaver 1949). The index was calculated as follows:

$$H' = \sum_{i=1}^{n} p_i \log_e p_i$$

where p_i is the proportion of the accessions in the i^{th} class of an n-class descriptors.

For cluster analysis, the data on seven quantitative descriptors were subjected to linear transformation by

Table 1 Morphometric data of *Trigonella* accessions

Accession number	Source*	Plant height (cm)	Primary branches	Pods/plant	Seeds/pod	Pod length (cm)	Days to 50% flowering	1 000-seed weight (g)
IC 001978-9	AP	94.4	4.2	45.7	13	9.5	73	6.9
IC 016837	Man	71.6	5.6	54	14.3	9.5	77	5.9
IC 266838	UK	75.7	5	40.6	12.9	10.4	86	7.95
IC 331755	Guj	98.2	4.6	52.6	13	10	64	10.95
IC 332188	MP	61.3	3.6	35.4	13.6	10.2	76	13.95
IC 332236	MP	74.4	3.9	47.8	14.9	10.8	87	15.9
IC 397328	MP	78.1	3.8	29.9	12.9	10.5	82	8.05
IC 397961	HP	68.4	4.1	30.5	16.7	10.3	81	9.95
IC 398004	HP	65.4	3.4	27.4	14.5	9.5	79	7.05
IC 398093	HP	78.9	3.5	43	11.6	9.6	66	12.05
IC 398123	HP	72.6	4.3	38.5	16	10.5	86	10.05
IC 398173	MP	63	3.5	27.3	13.1	10.3	78	9.95
IC 398738	Bihar	88.4	4.1	42.9	13.5	9.9	65	11
IC 398823	Bihar	104.4	5	56.2	14.7	10.6	67	14
IC 411629	HP	85.1	5.6	58.5	13.6	9.7	81	8
IC 411675	HP	62.2	5.6	40.6	13.6	9.9	87	6.95
IC 411691	HP	74.8	4.2	41.2	15.3	9.5	83	12.05
IC 411695	HP	108.8	6.4	74.5	14.6	10.4	83	7
IC 411797	J and K	95.8	4	42.4	14.1	10.4	64	10.05
IC 421923	HP	93.7	4	53.7	15.2	10.4	64	15.05
IC 433586	UP	106.3	4.3	50.6	15.5	9.9	67	5.95
IC 433589	UP	91	4.5	51.3	14.7	9.7	80	12.95
IC 441817	UP	85.6	4.7	56.6	14.2	10.5	66	10.95
IC 467950	HP	72.5	4.3	37.5	14.3	11	88	7.05
IC 538804	HP	91.5	4.7	36.6	14.8	10.6	64	10.1
IC 538817	HP	82.4	5.2	41.8	15.3	10	67	8.95
IC 143816	NA	96.3	4.6	44.9	13.3	9.2	67	8.95
IC 143821	NA	72.9	3.5	30.5	12.8	8.9	88	9.15
IC 143845	NA	65.7	3.2	35.1	13	9.7	80	12.9
IC 143850	NA	84.9	5.1	60.8	13.6	9.5	75	8.15
IC 143889	NA	96.5	4.2	47.8	15	8.9	64	10.1
IC 143890	NA	76	3.7	27.4	16	10	82	7
IC 144245	NA	72.4	5.1	35.6	14.6	9.7	87	8
IC 144260	NA	65.1	3.4	26.1	14.6	10.2	83	8
IC 144275	NA	79.1	4.4	59.5	14.1	9.9	68	6.95
IC 144276	NA	76	4	34.1	14.4	10.2	84	13.9
IC 144290	NA	90	4.9	55.1	12.5	9.9	77	8.95
IC 144291	NA	101.2	4.8	60.8	15	11.4	72	6.95
IC 144331	NA	72.6	5.2	47.4	12.1	10.3	82	8.9
IC 149350	NA	92.3	4.8	38.4	14.6	8.4	66	9.05
IC 311282	NA	66.2	3.8	25.9	12.4	8.9	81	9.05
IC 377911	NA	92.4	5.4	43	14.4	9.9	65	6.05
IC 467951	NA	88	5.2	50.2	11.7	10.2	83	6.95
EC 520255**	Eritrea	82.2	3.9	33	15.1	10.9	80	11.15
KSS 1	NA	96.2	4.2	39.3	14.6	10.4	65	11.05
AM 10	Raj	106.1	3.7	43	15.8	10	67	6.05
AM 35	Raj	91	5.8	49.8	13.7	11.8	66	12.35
PEB	Delhi	75.9	4.7	43.2	15.1	10.4	86	7.95
RMT 1	Raj	86.8	4.1	34.5	12.9	9.2	70	11
IC 143900***	NA	76.8	5.6	485.3	5	1.7	92	0.95

* Germplasm collected from AP, Andhra Pradesh; HP, Himachal Pradesh; J and K, Jammu and Kashmir; MP, Madhya Pradesh; UP, Uttar Pradesh; UK, Uttarakhand; Raj, Rajasthan; Man, Manipur; NA, Passport Data not available

Accession imported from Eritrea in 2003; * Single accession of *T. corniculata* L.

computing the mean and standard deviation of the descriptor states of each descriptor and the values were expressed as a matrix of the deviations from the mean in standard deviation units (Bookstein 1991). A pair-wise distance matrix was generated using the average taxonomic distance coefficient as described elsewhere (Archak *et al.* 2003). The similarity matrix for morphometric markers was generated by calculating $1 - d_{ij}$.

The ISSR amplification products were scored for the presence (1) and absence (0) of bands across the accessions to generate a binary matrix. Genetic similarity between pairs of accessions was estimated by the Jaccard's similarity coefficient and the accessions were clustered using the Sequential Agglomerative Hierarchical Nested (SAHN) cluster analysis as unweighed pair groups using arithmetic averages (UPGMA) procedure of NTSYS-pc version 2.11 (Rholf 2005). The polymorphism information content (PIC) which provides an estimate of the discriminating power of a locus by taking into account not only the number of alleles expressed but also their relative frequencies. Calculations were made using following formula: $PIC = 1-\Sigma f_i^2$ where f_i is the frequency of i^{th} allele (Smith *et al.* 1997). PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency). In the case of ISSR, the PIC was considered to be $1-p^2-q^2$, where p is band frequency and q is no band frequency (Ghislain *et al.* 1999).

The degree of the relationship between similarity matrices (based on ISSR and morphometric markers) was computed using the 2-way Mantel correspondence test with the product-moment correlation coefficient. Testing the significance of the correlation was performed using Mantel's t-test based on 1 000 random permutations. These computations were performed using NTSYS-pc version 2.11 software (Rohlf 2005).

RESULTS AND DISCUSSION

Fenugreek occupies a unique status as a spice, vegetable and medicinal plant. An assessment of genetic diversity within a species is a prerequisite for efficient conservation and utilization of the germplasm. Recording morphological descriptors has remained the primary means of identification, characterization and evaluation of the genotypes (Phillips and Vasil 2001). However, while handling large germplasm holdings with a continuum of variation, accuracy and consistency of interpretations based on morphological data can be enhanced by employing DNA markers (Archak *et al.* 2003). However, care should be taken as DNA marker data and morphometric data may not show a direct correlation in each case. In the present study, a sub-set of 49 accessions of fenugreek germplasm along with one accession of *T. corniculata* L. (as an out-group), being conserved at the National Gene Bank, NBPGR, New Delhi, was assessed for genetic diversity using morphometric and ISSR markers.

Morphometric divergence in fenugreek germplasm

This study revealed significant variation for all the quantitative descriptors. The variability parameters, namely, mean, minimum, maximum, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) of quantitative descriptors and Shannon-Wiener Diversity Index (H´) of qualitative descriptors are presented in Table 2. There was a wide range of variation for all the quantitative descriptors. The differences between PCV and GCV were low for all descriptors indicating that the descriptors were least influenced by environment and are controlled genetically. The PCV and GCV for descriptors, viz. pods/plant and 1 000-seed weight were in excess of 20%, signifying variation among accessions. Allelic evenness and allelic richness are the most commonly used parameters for measuring diversity. The allelic evenness was measured by Shannon-Wiener Diversity Index (H´) whereas the allelic richness was measured by counting the descriptor states for each descriptor without considering their individual frequencies. The Shannon-Wiener Diversity Index revealed presence of large diversity (= 0.40) for plant height, primary branches, days to 50% flowering, pods/plant, seeds/pod, pod length and 1 000-seed weight (Table 2).

Amongst the qualitative descriptors (Table 3) of leaf, the predominant descriptor states were dark green leaf colour (38 accessions), serrate leaf margin (34), dark green leaf margin colour (28), medium leaf size (32), pointed leaf tip (36), green (24) and dark green (24) petiole colour and petiole pubescence (42). In stem, green coloured stem with

Table 2 Variability parameters of quantitative descriptors, and corresponding H´ values

Descriptor	Mean	Minimum	Maximum	PCV (%)	GCV (%)	H´
Plant height (cm)	82.34	61.30	108.80	15.54	10.80	0.48
Primary branches	4.46	3.20	6.40	16.42	9.58	0.45
Pods/plant	52.99	25.90	485.30	124.74	106.50	0.45
Seeds/pod	13.89	5.00	16.70	12.69	7.20	0.41
Pod length (cm)	9.82	1.69	11.40	13.87	12.70	0.40
Days to 50% flowering	76.36	64.00	92.00	11.35	11.20	0.45
1 000 seed weight (g)	9.37	0.95	15.90	30.32	30.00	0.47

streaks (41), soft (22) and brittle (25) stem; presence of stem pubescence (47); and cylindrical stem shape (45) are more frequent descriptors states observed in the germplasm studied. Creamy coloured inflorescence was predominant (49). The descriptor states not found in these accessions (rare alleles) were light green leaf colour, elliptical stem shape, and white and yellowish white inflorescence colour. The Shannon-Wiener Diversity Index revealed presence of large diversity (≥ 0.40) in leaf margin colour only (Table 3). Similar findings were also reported by Datta *et al.* (2005). They reported variability in quantitative traits namely plant height, primary branches, pod length, pods/plant and seeds/pod. Gangopadhyay *et al.* (2009) reported wide variations among fenugreek accessions for number of pods/plant, 1 000-seed weight and seed yield/plant. McCormick *et al*. (2009a) reported significant variation for a range of phenotypic traits including growth habit, flowering time, seed colour, seed size, biomass and seed yield in 205 fenugreek accessions in south-eastern Australia. Significant variation was exhibited for all traits including growth habit, flowering time, seed colour, seed size, biomass and seed yield. The diversity exhibited provides a promising basis for a genetic improvement programme in Australia and in similar environments in other countries. They also concluded that traits associated with high-yielding lines with yellow or green seed can be found in different centres of origins. In a successive report, McCormick *et al.* (2009b) illustrated that combining phenotypic correlations with the qualitative assessment of data is a useful method to determine important traits associated with high seed yield in fenugreek and to identify accessions suitable for commercial production. They reported the use of quantitative and qualitative analyses to determine the main traits associated with seed yield of fenugreek and to identify accessions suitable for commercial production. Phenotypic correlation analysis and simple graphical analysis on a population of fenugreek accessions grown in 1998 and 1999 were used to determine important traits associated with yield. Traits important for yield were early vigour, growth habit, flowering date, end of flowering date, biomass at late flowering and harvest index. Yield/plant and seed weight were also important. Other traits considered important for commercial success included machine harvestability, and bacterial blight resistance.

Table 3 Qualitative descriptor, descriptor's states and their frequencies and corresponding H´ values

Descriptor	States (Frequency)	H
Leaf colour	Green (12), Dark green (38)	0.24
Leaf margin	Entire (16), Serrate (34)	0.28
Leaf margin colour	Light green (5), Green (27), Dark green (28)	0.40
Leaf size	Small (9), Medium (32), Large (9)	0.39
Leaf tip	Pointed (36), Rounded (14)	0.26
Petiole colour	Light green (2), Green (24), Dark green (24)	0.36
Petiole pubescence	Absent (8), Present (42)	0.19
Stem colour	Green (3), Green with streak (41), Red (6)	0.25
Stem tenderness	Soft (25), Brittle (25)	0.30
Stem pubescence	Absent (3), Present (47)	0.10
Stem shape	Triangular (5), Cylindrical (45)	0.14
Inflorescence colour	Creamy (49), Yellow (1)	0.04

Cluster analysis based on morphometric data

The single accession IC 143900 belonging to *T. corniculata* appeared as a separate cluster. The remaining 49 accessions of *T. foenum-graecum* was differentiated into two clusters I and II comprising 28 and 21 accessions, respectively (Fig 1). Three released varieties, viz. AM 10, AM 35 and RMt 1 were grouped in one cluster, while Pusa Early Bunching was in another cluster. Out of 12 accessions from Himachal Pradesh, six accessions in cluster II (three accessions out of five from Bilashpur, two accessions out of four from Kullu, and one accession from Chamba); and six accessions comprising two accessions out of five from Bilashpur, two accessions out of four from Kullu, one accession each from Hamirpur and Solan were grouped in cluster I. All the accessions from Bihar (two), Uttar Pradesh (three) are grouped in cluster I and Madhya Pradesh (four) in cluster II.

Molecular genetic divergence in fenugreek germplasm

After screening 100 ISSR primers, 21 primers (Table 4) that generated scorable, unambiguous and polymorphic bands were selected for the genetic analysis. ISSR profiling of fenugreek accessions generated 186 amplicons, out of which 172 were found to be polymorphic (92.4%). The size of amplified DNA products ranged from 250 to 2000 bp. The number of scored amplicons for each primer varied from 3 (UBC 820) to 13 (UBC 885, UBC 887 and UBC 899), with a mean number of 8.9 amplicons/primer. The primers UBC 811, UBC 815, UBC 817, UBC 820, UBC 840, UBC 842, UBC 846 (Fig 2), UBC 848, UBC 849, UBC 856, UBC 885, UBC 886 and UBC 897 produced 100% polymorphism (Table 4). Polymorphic PCR amplicons produced using primer UBC 846 have been shown in Fig 2. The pair-wise similarity values on the basis of Jaccard's similarity coefficient ranged from 0.37 to 0.93. The calculated PIC values for ISSR markers ranged from 0.2556 to 0.4988. The highest mean PIC value (0.4988) is given by primer UBC 846 (Table 4) indicating that this primer is most polymorphic.

The utility of DNA markers in molecular genetic analysis of fenugreek has also been demonstrated (Dangi *et al.* 2004, Sundaram and Purwar 2011). Sundaram and Purwar (2011) evaluated genetic diversity and species relation among two species of fenugreek, *T. foenum-graecum* with 59 accessions

and *T. corniculata* with two accessions from different locations of Uttarakhand, using 18 random primers.

Dangi *et al.* (2004) analyzed 17 accessions of *T. foenum-graecum* and nine accessions of *T. caerulea* representing various countries using ISSR and RAPD markers. Genetic diversity parameters (average number of alleles per polymorphic locus, per cent polymorphism, average heterozygosity and marker index) were calculated for ISSR, RAPD and ISSR+RAPD approaches. The UPGMA analysis showed that plants from different geographical regions were distributed in different groups in both the species. Based on genetic similarity indices, higher diversity was observed in *T. caerulea* as compared to *T. foenum-graecum*.

Cluster analysis based on ISSR data

The accessions were grouped by subjecting the similarity values to UPGMA clustering (Fig 3). IC 143900 representing *T. corniculata* was a clear out-group (at 20% similarity). ISSR data grouped 49 fenugreek accessions into two major clusters: cluster I with only two accessions, viz. IC 398173 and IC 397961 and cluster II comprising the remaining 47 accessions. Cluster II was further divided into two groups; group IIa had only 2 accessions, viz. PEB from Delhi, and IC 144245 from 'no passport data' group, whereas rest of 45 accessions was grouped into group IIb. Although accessions in the group IIb appeared to be further clustered, such apparent grouping was at or beyond the similarity level of 80%. ISSR markers were found highly polymorphic showing 92% polymorphism and displayed high level of genetic diversity (average PIC value was 0.46).

Out of 18 accessions without passport data (geographical location), seven were found to be within the single sub-group (75% similarity) based on ISSR data. Four more such accessions (IC 143821, IC 144275, IC 143889, and IC 144331) were placed together in a sub-group based on ISSR data that is pronouncedly away from other fenugreek

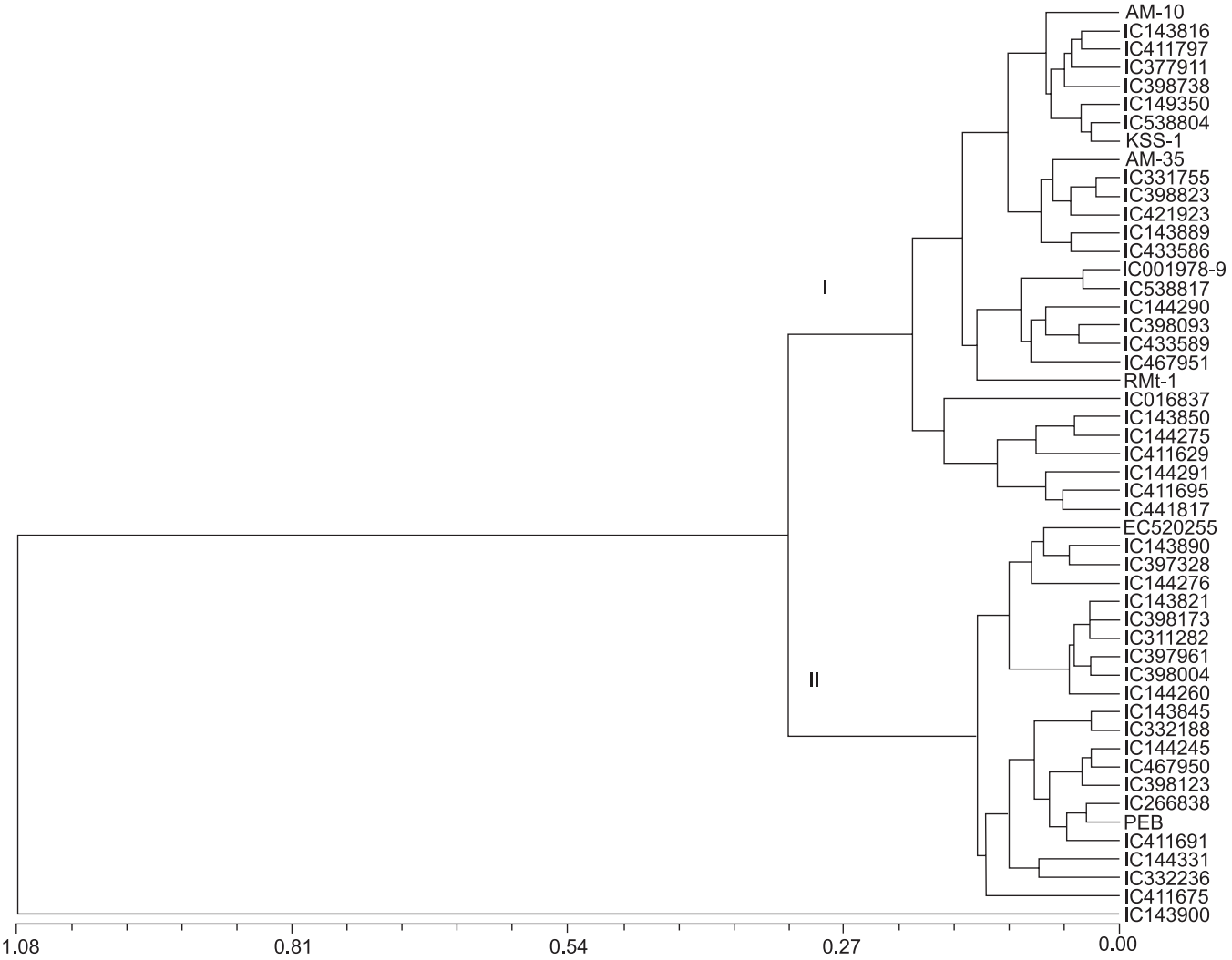

Fig 1 Dendrogram of 50 *Trigonella* accessions based on morphometric markers

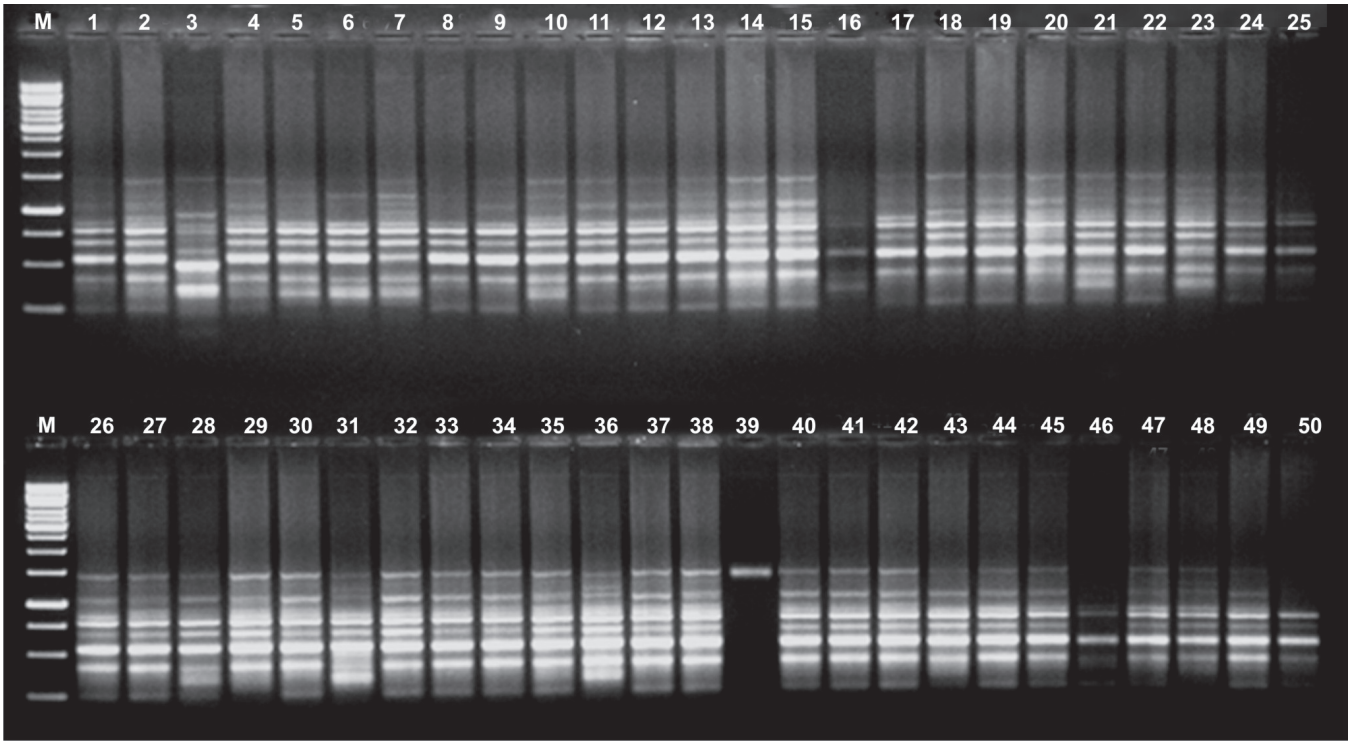

Fig 2 PCR amplification of 50 *Trigonella* accessions using ISSR primer UBC 846
M: Gene RulerTM 1 kb DNA ladder; 1-50: *Trigonella* accessions IC 001978-9, IC 016837, IC 266838, IC 331755, IC 332188, IC 332236, IC 397328, IC 397961, IC 398004, IC 398093, IC 398123, IC 398173, IC 398738, IC 398823, IC 411629, IC 411675, IC 411691, IC 411695, IC 411797, IC 421923, IC 433586, IC 433589, IC 441817, IC 467950, IC 538804, IC 538817, IC 143816, IC 143821, IC 143845, IC 143850, IC 143889, IC 143890, IC 144245, IC 144260, IC 144275, IC 144276, IC 144290, IC 144291, IC 144331, IC 149350, IC 311282, IC 377911, IC 467951, EC 520255, KSS 1, AM 10, AM 35, PEB, RMT 1, IC 143900

Table 4 Polymorphism in *Trigonella* using ISSR markers

Primer	Total no. of bands	No. of polymorphic bands	Amplicon size range (in bp)	PIC
UBC 811	11	11	250-1 400	0.4974
UBC 815	6	6	350-700	0.4549
UBC 817	4	4	450-850	0.4928
UBC 820	3	3	750-1 000	0.3768
UBC 840	11	11	250-900	0.3857
UBC 841	8	7	250-950	0.4653
UBC 842	5	5	250-900	0.4928
UBC 846	8	8	300-1 400	0.4988
UBC 847	7	6	400-1 050	0.4935
UBC 848	11	11	350-1 200	0.4972
UBC 849	10	10	400-1 050	0.3790
UBC 850	7	5	250-700	0.4813
UBC 851	9	8	250-1 200	0.4968
UBC 856	7	7	400-1 020	0.4968
UBC 861	10	7	400-1 400	0.4959
UBC 885	13	13	250-1 400	0.4977
UBC 886	12	12	300-1 450	0.4968
UBC 887	13	10	350-1 500	0.4468
UBC 888	6	5	300-850	0.4800
UBC 897	12	12	300-1 000	0.2556
UBC 899	13	11	280-2 000	0.4923

genotypes. The accession from Eritrea was not observed to be distinct by either of the marker sets.

Moreover, the molecular data also revealed few exceptions to the relationship between genetic similarity and geographical distance, as there was no clear clustering pattern of geographically closer accessions. Both the accessions collected from the districts of Bihar and all the three collections from Rajasthan fell in different clusters based on ISSR data in spite of being geographically very close to each other.

Correlation between clustering based on morphometric and molecular markers

Both the dendrograms (Fig 1, 3) clearly differentiated the out-group IC 143900 (*T. corniculata*) from *T. foenum-graecum* accessions that helped in confident groupings of the fenugreek genotypes. The genetic similarity/distance matrices were compared using 2-way Mantel test method. The matrix correlation (r) expressed as normalized Mantel statistic (r) was 0.096. This meant no strong correlation in the way pair-wise genetic relatedness is deciphered by ISSR and morphometric markers.

In conclusion, morphometric as well as ISSR markers were found informative to estimate the genetic relatedness in

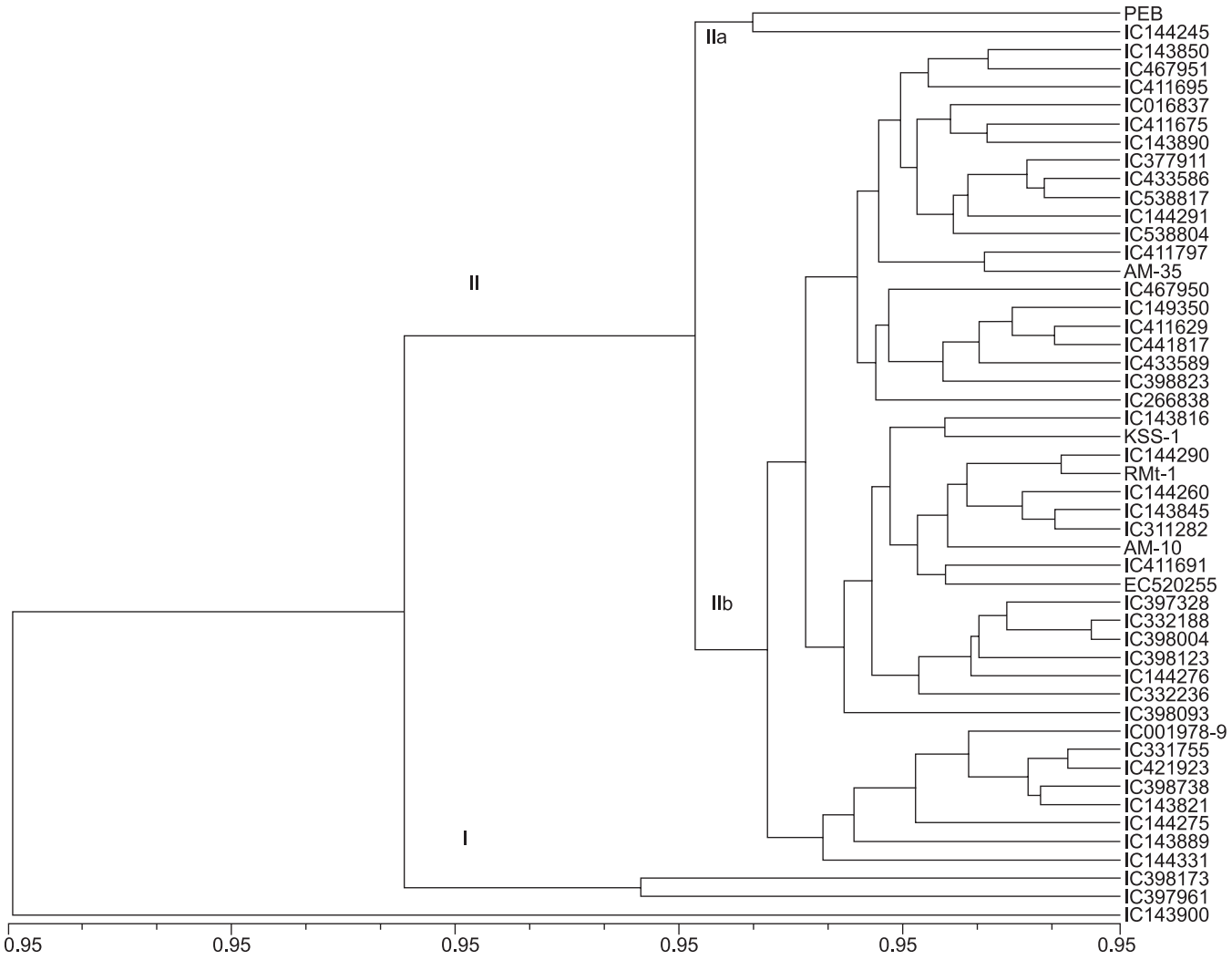

Fig 3 Dendrogram of 50 *Trigonella* accessions based on ISSR data

a sub-set of Indian fenugreek germplasm. The study revealed significant genetic diversity in fenugreek germplasm being conserved in the National Gene Bank at the NBPGR. The results also revealed that *T. foenum graecum* germplam within India constitute a broad genetic base. From the clustering pattern and genetic relationship obtained using morphometric and ISSR markers, breeders can identify the diverse genotype from different cluster and employ them in their future breeding programme. Furthermore, these data may be useful for developing strategies for the conservation of this important spice crop in India.

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