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RESEARCH ARTICLE

Assessment of Diversity in Kasuri Methi (*Trigonella corniculata L.*) Using Morphological and Molecular Markers with Physiological Character Analysis

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ABSTRACT

Kasuri methi (*Trigonella corniculata* L.) is an annual herb that is primarily grown in Northern India during the rabi season. The demand for Kasuri methi is increasing every year,but the production is decreasing due to the decreasing availability of water. The genetic divergence evaluation in Kasuri methi germplasm is essential to increase its adaptability in other areas and breed drought tolerant varieties. The selection of breeding parents and the conservation of germplasm depend on the availability of genetic diversity among Kasuri methi genotypes. Therefore, it was important to characterize available germplasm of Kasuri methi using morphological, physiological, and molecular parameters. Environmental factors and crop development stages impact a plant's morpho-physiological traits. Compared to morphological and physiological examination, molecular indicators take far less time and are not affected by the environment or the stages of plant development The study was done with 18 genotypes for morpho-physiological and 24 genotypes were used for molecular analysis. For morphological studies, the minimal descriptors provided by NBPGR, New Delhi, were used. Relative water content (RWC), chlorophyll, carotenoid content, and membrane stability index were recorded during the flowering stage for physiological studies. The RWC and MSI showed significant variation among genotypes, while carotenoid and chlorophyll content showed non-significant variation. Based on the results of the study, it can be concluded that morphophysiological characters, along with molecular markers such as SSR markers, are an efficient method for analysing the biodiversity among Kasuri methi genotypes, and sufficient diversity exists in the genotypes. The maximum dissimilarity was observed between JKM13 and JKM6 based on the morphological characters. Based on molecular characters, the maximum dissimilarity exists between JKM6 and PS2. Both cases JKM6 stand out from the others in the dendrograms, which ranged from 0.600 to 0.967. The maximum similarity between PS6 and PS7 was found, and the minimum similarity between JKM6 and PS2. Both cases JKM6 stand out from the others in the dendrograms.

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INTRODUCTION

Kasuri methi (*Trigonella corniculata* L.), an annual herb used as a spice, belongs to the Leguminaceae family, sub-family Papilionaceae. It is a small-seeded, diploid, self-pollinated annual legume plant with chromosome number 2n=16. It is a bushy and slow-growing crop. It is a cold-weather crop that is generally grown in the Rabi season. It is an aromatic crop used as a spice and flavour enhancer. A notable non-spice application is the prospective use of fenugreek as a source of diosgenin. It is also regarded as one of recorded history's earliest known medicinal plants. The seed treats chronic cough, diarrhoea, dysentery, dropsy, liver and spleen enlargement, gout, diabetes, and arthritis. The Indian subcontinent and the Eastern Mediterranean region are two centres of origin for Trigonella spp.A variety ofvarious regional names, including Champamethi and Marwari methi in Hindi, Piring in Assami, and sickle-fruited fenugreek in English know it. India, Pakistan, Nepal, Bangladesh, and China are major producers of kasuri methi.

Kasuri methi is only cultivated in the northern regions of India. However, due to increased utilisation and guaranteed payment, there is a need to expand the area under this spice crop. It is vital to find or develop genotypes with high yields for growing in unusual locales. To understand the variability, one needs to evaluate nearby or related genotypes. Nothing can be achieved if there is inadequate variability, and the breeder will need to enrich the genotypes or germplasm or may resort to hybridization, mutation, or polyploidy breeding to increase diversity. The genetic makeup of the plant and the environment in which it is growing primarily regulate the phenotypic expression of the plant. It is necessary to use relevant criteria, such as the genotypic and phenotypic coefficient of variation, heritability, and genetic progress, to separate the observed phenotypic variability into its non-heritable and heritable components. When there is plenty of genetic variety, selection may be able to identify superior genotypes in populations. In addition to genetic variety, heritability and genetic advancement also play a significant role in any character's improvement (genetic gain). On Kasuri methi, there is a limited amount of data available. This plant was diversified from *Trigonella foenum-graecum* in the 21st century, as it stands out from all the other genotypes of Methi in the dendrogram (Shazid *et al.,* 2011).

The diversity of morphological forms offers a greater perspective on the phenotypic features present in a given crop. However, as they are more susceptible to environmental impacts and aid in establishing trait-marker links with repeated efforts, the exact needs to be investigated and confirmed using molecular markers. Microsatellites are small tandem-repeating motifs with a variable number of repeats at a specific locus, often known as simple-sequence repeats (SSRs) (Tautz, 1989). One of the numerous advantages SSR markers offer over other molecular markers is genetic co-dominance. They are widely dispersed across the multi-allelic genome and easily and mechanically tested (Powell *et al.,* 1996). Hence, SSR primers have been used for the biodiversity analysis.

MATERIALS AND METHODS

The materials were collected from different institutions and locations of Rajasthan (Table 1). The seeds were collected from these locations and grown in the Net house of the Department of Plant Physiology, SKN College of Agriculture, in the Rabi season 2022-23.

Morphological descriptors

The morphological data were observed and recorded per the minimal descriptor provided by ICAR-National Bureau of Plant Genetic Resources, New Delhi, at different growth stages and scored accordingly.

Physiological characters

Relative water content (RWC):

Fresh leaves were selected, and their weight was measured. Leaf discs were soaked in water to reach maximum turgidity, blotted dry, and weighed again. Dry weight was obtained by oven drying, and R.W.C. was calculated using specific formulas.

% R.W.C. = [(Fresh weight - Dry weight) / (Turgid weight - Dry weight)] X 100

Chlorophyll and carotenoid content:

Total chlorophyll and carotenoid contents were calculated using Arnon's (1949) and Duxbury and Yentsch's (1956) techniques. The sample extract was homogenized 500 mg of leaves in 10 mL of 80% acetone for 10 minutes at 5000 rpm. The clear supernatant was transferred to a volumetric flask of 50 mL. Adding acetone reduced the final volume of supernatant to 50 mL. Finally,

Table 1. List of the genotypes used in the study

	Genotypes	Source
G1	JKM-2	AICRP on Seed Spices, Dept. GPB, S.K.N. COA, Jobner
G2	JKM-3	,,
G3	JKM-4	,,
G4	JKM-5	,,
G5	JKM-6	,,
G6	JKM-7	,,
G7	JKM-8	,,
G8	JKM-9	,,
G9	JKM-10	,,
G10	JKM-11	,,
G11	JKM-12	,,
G12	JKM-13	,,
G13	JKM-14	,,
G14	Nagori Panmethi	Dept. of Horticulture, SKNCOA, Jobner
G15	Pusa Kasuri	,,
G16	MR	Merta Road, Rajasthan
G17	JN	Janana, Rajasthan
G18	PHA	Phalodi, Rajasthan

the optical density of chlorophyll at 663 and 645 nm and carotenoids at 480 and 510 nm was determined using a spectrophotometer. The following formula was used to compute chlorophyll estimate (mg g-1 fresh leaf tissue) and carotenoid content (mg g-1 fresh leaf tissue):

Chlorophyll a = $[12.7(A_{663})- 2.69(A_{645})] \times V/1000xW$

Chlorophyll b = $[22.9(A_{645})- 4.68(A_{663})] \times V/1000xW$

Total chlorophyll = $[20.2(A_{645}) +8.02(A_{663})] \times V/1000xW$

Carotenoids = $[7.6 (A_{480}) + 1.49 (A_{510})] \times V/1000xW$

A= Optical density

V= Final volume of 80% acetone (in mL)

W= Weight of leaf material (in g)

Membrane Stability Index (MSI):

The MSI was calculated using a method developed by Permchand *et al.,* (1990). 100 mg leaf discs were washed and rinsed to remove surface electrolytes. These discs were then put in a test tube with 10 mL of double-distilled water and treated to a 40 °C water bath for 30 minutes before being measured for electrical conductivity (C_1). The same leaf sample was then water bathed in a 100 °C water bath for 10 minutes, and the electrical conductivity (C_2) was measured. The MSI was then computed using this formula:

$MSI = [1- (C_1/C_2)] \times 100$

Molecular Characterization:

Collection of Plant Material:

The seeds of 18 genotypes were collected and grown under pot culture conditions. Ten plants were raised in each pot, but after thinning, 5 plants were maintained in each pot. The young and tender leaves are collected and kept in liquid nitrogen before storing at -80 °C, which is used for further DNA extraction.

DNA Isolation, Purification, and Quantification:

CTAB buffer was preheated at 60°C for 30 minutes, then PVP was added (at 10% of the CTAB buffer volume), with β- marcapthanol (at 1/1000 volume of the CTAB buffer). Each sample's young leaves (1-2 g) were homogenized to a fine powder with a mortar and pestle with liquid nitrogen. The homogenized leaf was transferred to a 30 mL centrifuge tube containing 10 mL CTAB buffer, vortexedmLmL, and incubated for one hour in a water bath at 60°C with periodic mixing. 10 mL of chloroform: isoamyl alcohol (24:1) was added, gently mixed, and centrifuged at 13,000 rpm for 15

minutes at 25 °C. After transferring the aqueous phase to a new centrifuge tube, 0.6 volume of cold isopropanol was added and kept at -20 °C overnight. The DNA was pelleted by centrifugation at 13,000 rpm for 15 minutes at 25 °C, washed with 70% ethanol, air dried, and suspended in 500 µL of TE (10 mM:1 mM).

The DNA was incubated at 37 °C for one hour with RNase at a concentration of 6 µg/100 µl of DNA solution. A combination of phenol, chloroform, and isoamyl alcohol 25:24:1 was added to the DNA solution, gently mixed, and centrifuged at 13,000 rpm for ten minutes at 25 °C. The DNA was precipitated by adding 1/10 volume of 3M sodium acetate (pH 5.6) and 2 volumes (v/v) of cooled absolute alcohol, which was kept at -20 °C overnight, followed by centrifuging at 13000 rpm for 15 minutes. Then washed twice with 70% ethanol, air dried, and dissolved in 500 µL of 1X TE (10:1) buffer at room temperature and kept at 4 °c.

The quality and quantity were checked with 0.8% agarose gel electrophoresis. Concentration was determined using a Nanodrop spectrophotometer. The DNA sample was diluted with 10x TE Buffer to 5 ng/µL and stored at 4 °c.

SSR primers screening and amplification:

Fourteen primers were screened with different concentrations of PCR mixture and temperatures to obtain the optimal result. The annealing temperatures ranged from 57 °C to 59 °C, depending upon the primer; the PCR mixture with optimum result is given in Table 2. After the amplification, the PCR product was loaded in a 3% agarose gel with 2 µL loading dye that contained bromophenol blue and xylene cyanol.4 µl mix ladder DNA marker (50 bp StepUp ™ladder) was loaded in the final gel lane to measure recognized bands. Then, the patient was placed in a 0.5X TBE buffer for 3.30 hours at 120 V to complete electrophoresis. The gel was imaged with a gel documentation system (Syngene, UK).

Data analysis:

The morphological data were scored as per the minimal descriptor NBPGR released, including vegetative, floral and yield attributes. The software XLSTAT was used to calculate the dissimilarity between genotypes with Euclidean distance.

The clear amplified bands were scored as 1 or 0 for presence or absence, and a binary data matrix was

Table 2. Scoring of Qualitative characters in different Kasuri methi genotypes

	Leaf colour	Leaf margin	Leaf margin colour	Leaf Size	Leaf tip	Petiole colour	Petiole Pubescence	Stem colour	Stem herbility
JKM2	2	2	2	3	2	1	1	1	1
JKM3	2	2	2	3	2	2	1	1	1
JKM4	2	2	2	3	2	2	1	1	1
JKM5	2	2	2	3	2	1	1	1	1
JKM6	1	2	2	3	2	1	1	1	1
JKM7	2	2	2	3	2	1	1	1	1
JKM8	2	2	2	3	2	2	1	1	1
JKM9	2	2	2	3	2	2	1	1	1
JKM10	2	2	2	3	2	2	1	1	1
JKM11	2	2	2	3	2	2	1	1	1
JKM12	2	2	2	3	2	2	1	1	1
JKM13	2	2	2	3	2	2	1	1	1
JKM14	2	2	2	3	2	2	1	1	1
MR	2	2	2	3	2	2	1	1	1
JN	2	2	2	3	2	2	1	1	1
PHA	1	2	2	3	2	1	1	1	1
NP	2	2	2	3	2	2	1	1	1
PK	2	2	2	3	2	2	1		

Table 3. Scoring of Qualitative characters in different Kasuri methi genotypes

	Stem Pubescence	Stem Shape	Inflorescence Colour	Biotic stress susceptibility	Leaf Size
JKM2	1	99	3	5	3
JKM3	1	99	3	3	3
JKM4	1	99	3	3	3
JKM5	1	99	3	7	3
JKM6	1	99	3	3	3
JKM7	1	99	3	3	3
JKM8	1	99	3	5	3
JKM9	1	99	3	3	3
JKM10	1	99	3	7	3
JKM11	1	1	3	3	3
JKM12	1	99	3	3	3
JKM13	1	99	3	7	3
JKM14	1	1	3	3	3
MR	1	99	3	3	3
JN	1	99	3	5	3
PHA	1	1	3	3	3
NP	1	1	3	3	3
PK	1	1	3	3	3

created for molecular analysis. Jaccard's similarity coefficient was established from the data matrix (Jaccard, 1908). The data analysis and dendrogram production were done using the software NTsys v2.10e. For Polymorphic information content & Heterozygosity, Gene-Calc (Bińkowski and Mike, 2018) was used.

The physiological data were statistically analyzed on a Microsoft Excel sheet using a Completely Randomized Design (CRD) with three replications..

Result and Discussion:

Morphological characterization:

The morphological characterization was done according to the descriptors provided by ICAR- NBPGR, New Delhi.

The descriptors were recorded and scored to evaluate the Euclidean distance using the unweighted pair-group average agglomeration method. In the proximity matrix of dissimilarity (Table 4), the maximum distance was found between JKM13 and JKM6 genotypes, whereas the minimum distance was between Pusa Kasuri and JKM14. The average distance was found to be 4.16.

The dendrogram was divided into four clusters, in which cluster C1 included G1, G4, and G6. The cluster C_2 included G2, G3, G7, G8, G10, G11, G13, G14, G15, G16, G17. The cluster C_3 included G5 and G18. The cluster C_4 included G9 and G12. For the cluster C1 -G1, C2- G15, C3- G5 and C4-G9was considered central objects. The maximum distance of all genotypes from the central object of C_1 was 5.03, C_2 was 4.90, C_3 was 6.22, and C_4 was 5.93. The minimum distance between all genotypes and central objects of C_1 was 2.92, C_2 was 2.10, C_3 was 3.84, and C_4 was 2.75.

The dissimilarity matrix provided us with the diversity data between genotypes. The greater the dissimilarity between genotypes, the more diverse the genotypes were, with the least similar characters, such as G12 and G5. The least matrix distance shows more similarity, such as G15 and G13. However, morphological diversity analysis can have some errors as the environmental condition plays a significant role in character development.

Physiological character analysis:

The physiological characters were analysed and presented in Table 5. The RWC (Relative water content) and MSI (membrane stability index) have shown significant variation among genotypes, whereas

Table 4. Proximity matrix of Dissimilarity based on Euclidean distance of Kasuri methi genotypes for morphological data

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18
G1	0	3.48	3.49	2.92	4.42	3.09	3.09	3.7	3.58	3.78	3.96	3.88	4.69	4.55	3.92	4.92	5.03	4.52
G2	3.48	0	3.88	3.54	4.41	4	3.22	3.35	3.55	3.13	3.43	4.75	2.94	2.63	3.26	4.88	4.31	3.06
G3	3.49	3.88	0	5.39	4.77	4.04	3.55	2.19	4.42	3.53	2.9	3.62	4.19	4.37	3.9	5.66	3.87	3.87
G4	2.92	3.54	5.39	0	4.8	4.62	4.49	4.78	3.76	4.52	5.4	4.92	4.77	4.76	4.81	5.56	6.18	4.9
G5	4.42	4.41	4.77	4.8	0	5.22	5.12	4.59	5.93	5.22	4.52	6.22	4.91	5.14	5.33	3.84	5.45	4.75
G6	3.09	4	4.04	4.62	5.22	0	4.16	3.68	4.15	4.31	4.97	3.64	4.79	3.28	3.98	4.31	5	4.22
G7	3.09	3.22	3.55	4.49	5.12	4.16	0	3.87	3.7	4.48	2.57	4.03	4.69	3.83	2.51	5.28	4.13	3.98
G8	3.7	3.35	2.19	4.78	4.59	3.68	3.87	0	4.67	3.45	3.92	3.66	4.09	3.41	4.45	5.53	4.77	3.16
G9	3.58	3.55	4.42	3.76	5.93	4.15	3.7	4.67	0	3.97	4.71	2.85	4.15	4.02	2.75	5.42	4.67	4.64
G10	3.78	3.13	3.53	4.52	5.22	4.31	4.48	3.45	3.97	0	4.5	4.46	2.44	4.28	4.51	4.84	4.02	2.99
G11	3.96	3.43	2.9	5.4	4.52	4.97	2.57	3.92	4.71	4.5	0	5.13	4.07	4.31	2.9	5.48	3.05	3.84
G12	3.88	4.75	3.62	4.92	6.22	3.64	4.03	3.66	2.85	4.46	5.13	0	5.08	4.23	3.76	5.8	5.09	4.67
G13	4.69	2.94	4.19	4.77	4.91	4.79	4.69	4.09	4.15	2.44	4.07	5.08	0	3.55	3.81	4.44	2.85	2.1
G14	4.55	2.63	4.37	4.76	5.14	3.28	3.83	3.41	4.02	4.28	4.31	4.23	3.55	0	3.12	4.6	4.29	2.75
G15	3.92	3.26	3.9	4.81	5.33	3.98	2.51	4.45	2.75	4.51	2.9	3.76	3.81	3.12	0	4.79	3	3.78
G16	4.92	4.88	5.66	5.56	3.84	4.31	5.28	5.53	5.42	4.84	5.48	5.8	4.44	4.6	4.79	0	4.6	4.21
G17	5.03	4.31	3.87	6.18	5.45	5	4.13	4.77	4.67	4.02	3.05	5.09	2.85	4.29	3	4.6	0	3.06
G18	4.52	3.06	3.87	4.9	4.75	4.22	3.98	3.16	4.64	2.99	3.84	4.67	2.1	2.75	3.78	4.21	3.06	0

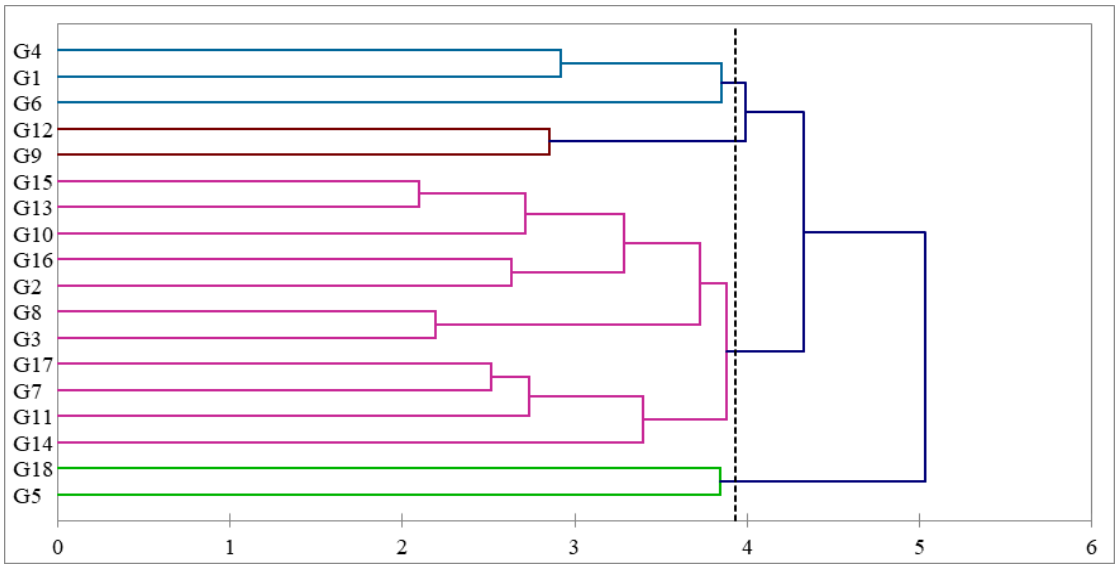

Fig 1. Dendrogram of genotypes of Kasuri methi for morphological data based on Euclidean distance

carotenoid and chlorophyll content had no significant variation.

Relative water content:

The RWC ranged from 50.88% to 69.77%. The maximum Relative water content was recorded in G4 (70.99%), followed by G6 (69.77%) and G18 (65.92%). The minimum RWC was recorded in G8 (50.88%), followed by G1 (51.07%), G13 (52.45%) and G9 (53.02%).

Chlorophyll content:

The chlorophyll content ranged from 1.28 to 2.01 mg g^{-1}. The maximum chlorophyll a was recorded in G9 (2.01 mg g^{-1}), followed by G1 (1.86 mg g^{-1}) and G13 (1.85 mg g^{-1}). The minimum content was recorded in G14 (1.28 mg g^{-1}), followed by G18 (1.29 mg g^{-1}) and G8 (1.31 mg g^{-1}). The maximum chlorophyll b was recorded in G9(1.10 mg g^{-1}), followed by G13 (0.96 mg g^{-1}) and G6 (0.93 mg g^{-1}). The minimum content was recorded in G8 (0.59 mg g^{-1}) and G14 (0.61 mg g^{-1}). The maximum amount of total chlorophyll content was recorded in G9(3.11 mg g^{-1}), followed by G13 (2.81 mg g^{-1}) and G6 (2.75 mg g^{-1}). The minimum amount of chlorophyll was recorded in G14 (1.88 mg g^{-1}), followed by G8 (1.90 mg g^{-1}) and G18 (1.97 mg g^{-1}). A similar result was found by Yadav *et al.,* (2021)

Carotenoid Content:

The carotenoid content ranged from 0.58 to 1.02 mg g^{-1}. The maximum carotenoid content was recorded in G9 (1.02 mg g^{-1}), followed by G13 (0.90 mg g^{-1}) and G6 (0.87 mg g^{-1}). The minimum amount was recorded in G14 (0.58 mg g^{-1}), followed by G8 (0.60 mg g^{-1}) and G10 (0.64 mg g^{-1}).

Membrane Stability Index:

The MSIs ranged from 61.11 to 74.12. The maximum MSI was recorded in G11 (74.12), followed by G18 (73.65) and G17 (72.70). The minimum MSI was recorded in G7 (61.11), followed by G12 (63.33) and G4 (63.77).

Non-significant variations were observed in total chlorophyll and carotenoid contents. These revealed that the accessions mainly were identical and less diversified about pigment content. The Relative water content and MSI show significant variation. These showed their variable performance under stress conditions.

Molecular Characterization:

The advent of molecular markers has made them necessary for genetic characterisation. These are frequently used to supplement and enhance the data from morphological markers, especially in genetic diversity investigations. Codominant and dominant marker systems, such as the sequence-tagged microsatellite markers (STMS) or simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) markers, have been made possible by the use of microsatellite repeats in genomes, Tautz (1989);Zietkiewitcz *et al.,* (1994).

Table 5. Mean performance of 18 Kasuri methi genotypes for different physiological characters

	Relative Water Content (%)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total Chlorophyll (mg g^{-1})	Carotenoid (mg g^{-1})	Membrane Stability Index
JKM2	51.07	1.86	0.74	2.6	0.82	70.03
JKM3	61.77	1.58	0.64	2.21	0.71	66.67
JKM4	57.26	1.82	0.81	2.63	0.85	66.92
JKM5	70.99	1.69	0.72	2.42	0.82	63.77
JKM6	57.73	1.56	0.77	2.34	0.82	69.83
JKM7	69.77	1.83	0.93	2.75	0.87	70.98
JKM8	57.03	1.62	0.7	2.31	0.69	61.11
JKM9	50.88	1.31	0.59	1.9	0.6	64.98
JKM10	53.02	2.01	1.1	3.11	1.02	68.67
JKM11	57.75	1.43	0.61	2.04	0.64	69.69
JKM12	56.96	1.55	0.66	2.2	0.7	74.12
JKM13	61.78	1.51	0.69	2.2	0.68	63.33
JKM14	52.45	1.85	0.96	2.81	0.9	70.18
MR	57.05	1.72	0.87	2.59	0.83	72.56
JN	53.61	1.47	0.61	2.08	0.66	72.7
PHA	65.92	1.29	0.68	1.97	0.7	73.65
NP	54.15	1.54	0.66	2.2	0.69	71.08
PK	60.88	1.28	0.61	1.88	0.58	71.04
GM	58.34	1.61	0.74	2.35	0.75	68.96
Range	51.07-70.99	1.29-1.86	0.59-1.1	1.88-3.11	0.58-1.02	61.11-74.12
SEM	4.21	0.2	0.15	0.33	0.1	1.12
CD 5%	12.09	NS	NS	NS	NS	3.23
MSE	53.28	0.12	0.07	0.33	0.03	3.79

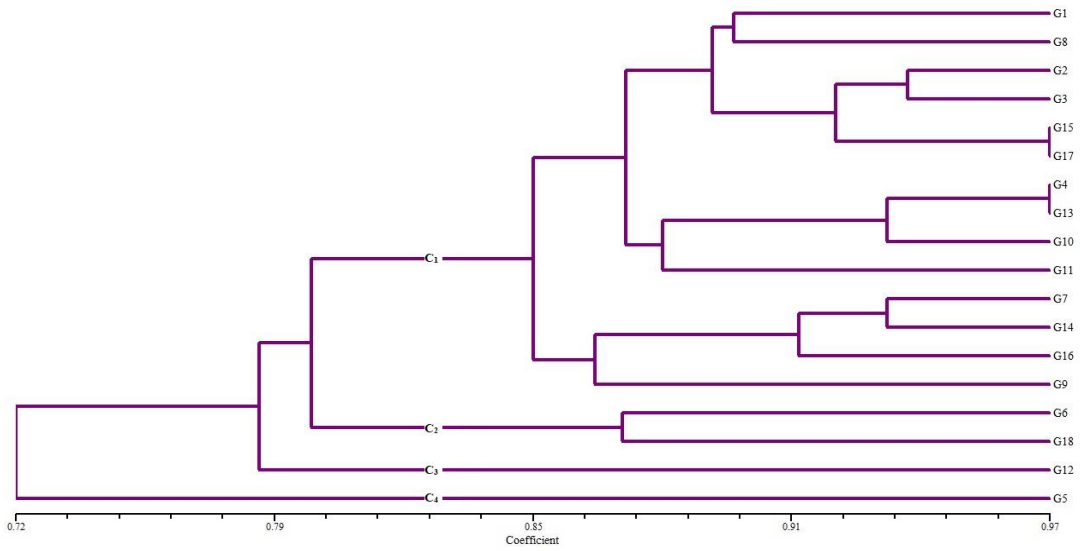

Fig. 2 Dendrogram for Kasuri methi genotypes based on SSR markers

Table 6. Jaccard's similarity coefficient of Kasuri methi genotypes

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18
G1	1																	
G2	0.900	1																
G3	0.900	0.933	1															
G4	0.833	0.867	0.867	1														
G5	0.800	0.767	0.700	0.767	1													
G6	0.833	0.733	0.733	0.733	0.700	1												
G7	0.857	0.893	0.893	0.893	0.714	0.750	1											
G8	0.893	0.857	0.857	0.857	0.714	0.857	0.808	1										
G9	0.800	0.833	0.767	0.833	0.733	0.833	0.857	0.821	1									
G10	0.893	0.857	0.893	0.929	0.750	0.786	0.885	0.923	0.893	1								
G11	0.833	0.800	0.800	0.867	0.700	0.733	0.821	0.857	0.833	0.929	1							
G12	0.833	0.800	0.733	0.800	0.767	0.733	0.821	0.786	0.833	0.857	0.800	1						
G13	0.800	0.833	0.900	0.967	0.733	0.700	0.857	0.821	0.800	0.929	0.833	0.767	1					
G14	0.800	0.833	0.833	0.900	0.733	0.767	0.929	0.857	0.867	0.893	0.833	0.767	0.867	1				
G15	0.867	0.900	0.900	0.900	0.667	0.767	0.857	0.893	0.800	0.893	0.900	0.767	0.867	0.867	1			
G16	0.857	0.893	0.893	0.821	0.679	0.821	0.923	0.821	0.857	0.808	0.750	0.679	0.786	0.893	0.857	1		
G17	0.900	0.933	0.933	0.933	0.700	0.800	0.893	0.929	0.833	0.929	0.867	0.800	0.900	0.900	0.967	0.893	1	
G18	0.833	0.800	0.800	0.800	0.700	0.867	0.750	0.857	0.833	0.857	0.800	0.733	0.767	0.767	0.833	0.821	0.867	1

The similarity indices and consensus dendrogram were developed based on scorable bands observed from the gel electrophoresis of PCR products of 14 SSR primers with 18 genotypes; the presence of the band was scored as 1, and the absence was scored as 0. The SSR data were analysed and a dendrogram (Fig. 2) was produced based on the Jaccard coefficient of similarity using NTSYS 2.01e software (Table 6).

The dendrogram expands from 0.72 to 0.97 distance. The dendrogram revealed five clusters (C_1, C_2, C_3, and C_4) at a genetic distance of 0.84. Cluster C_1 contained 14 genotypes with 2 sub-clusters. Cluster C_2 had two genotypes, G6 and G18. Cluster C3 had one genotype G12, and Cluster C4 had G5.

The genetic similarity matrix based on Jaccard's coefficient in twenty-four genotypes showed similarity values ranging from 0.667 to 0.967. The highest similarity (0.967) was observed between G4 and G13 and G15, with G17. The lowest similarity (0.667) was observed between G5 and G15.

CONCLUSION:

Genetic diversity study provides us with the knowledge for the species existence and status in an ecosystem. This study revealed that morpho-physiological characters, along with molecular markers such as SSR markers, are an efficient method for analysing the biodiversity among Kasuri methi genotypes, and sufficient diversity exists in the genotypes. The maximum dissimilarity was observed between G12 and G5 based on the morphological characters. On the other hand, on the basis of molecular characters, the maximum dissimilarity exists between G5 and G15. Both cases JKM6 stand out from the others in the dendrograms.

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