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RESEARCH ARTICLE

Genetic diversity analysis of fennel (*Foeniculum vulgare* L.) genotypes using molecular profiling techniques

Neha[1], Yogendra Singh[2], Keerti Tantwai[1], Reena Nair[3] & Vinod K Sahu[1*]

[1]Biotechnology Centre, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482 004, Madhya Pradesh, India

[2]Department of Genetics and Plant Breeding, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482 004, Madhya Pradesh, India

[3]Department of Horticulture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482 004, Madhya Pradesh, India

*Email: vinodsahu084@gmail.com

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Abstract

This study investigated the genetic diversity and relationships among 33 fennel (*Foeniculum vulgare* L.) genotypes using Simple Sequence Repeat (SSR) markers to support germplasm management and breeding efforts. Fifteen SSR primers generated 37 alleles, averaging 2.47 alleles per primer, with Polymorphic Information Content (PIC) values ranging from 0.210 to 0.499 (mean 0.289), indicating moderate genetic diversity among the studied genotypes. Cluster analysis based on SSR marker data categorized the fennel genotypes into nine distinct clusters, reflecting significant genetic heterogeneity within the population and demonstrating the effectiveness of SSR markers in delineating genetic relationships. Four primers FV-6, FV-290063, FV-30919 and FV-18902- were particularly valuable, producing unique banding patterns that accurately identified individual genotypes. The identified genetic clusters provide a scientific basis for selecting diverse parental lines for hybridization programs, while the distinctive SSR markers offer practical tools for accurate genotype identification and authentication. These findings hold significant implications for germplasm conservation, supporting the development of strategies to preserve genetic resources and enhance sustainable utilization of fennel diversity. By integrating molecular tools into fennel breeding approaches, this research contributes to developing superior cultivars with improved yield, quality and adaptability to changing environmental conditions. The moderate genetic diversity observed suggests both opportunities for improvement and the need for broadening the genetic base in breeding programs to ensure long-term genetic gain in this economically important aromatic and medicinal crop.

Keywords

fennel; genetic diversity; genetic relationships; molecular profiling; polymorphic information content (PIC); primer; simple sequence repeats (SSR)

Introduction

Fennel (*Foeniculum vulgare* Mill.), a flowering plant of the Apiaceae family, is prominent in global agriculture and ethnomedicine. Originating from the Mediterranean region, it has been cultivated worldwide due to its economic and medicinal value. The seeds, leaves and fennel bulbs are known for their culinary use and therapeutic properties. Fennel seeds contain essential oils and bioactive compounds, contributing to their antioxidant, antimicrobial, antitumor and memory-enhancing effects (1, 2). Despite its widespread use, there has been a growing interest in advancing genetic research to improve fennel cultivation and optimize its production potential, especially in regions where it has become a staple crop. Fennel is an often-cross-pollinated seed spice crop with a

chromosome number of 2n=22 (2-4). It is predominantly cultivated in arid and semi-arid regions, with India being one of the largest producers of fennel globally. The crop is extensively grown in Gujarat, Rajasthan, Madhya Pradesh and West Bengal, contributing significantly to Indias' spice economy. In India, fennel is regarded as a high-value, export-oriented seed spice due to its adaptability to low-rainfall areas, short crop cycles and profitability. India produces 63 of the 109 spices recognized by the International Organization for Standardization (ISO), with fennel being a key contributor to this status (5).

Recent studies have highlighted the genetic and biochemical richness of fennel seeds, which contain high amounts of protein, essential oils, dietary fibre and minerals like potassium, calcium and iron, making them nutritionally valuable (6). The essential oil in fennel seeds, dominated by anethole, has been found to possess significant pharmacological activities, such as anti-inflammatory, antimicrobial and anti-cancer effects (7, 8, 9). Furthermore, research has shown that fennel possesses a broad spectrum of secondary metabolites, including phenolic acids, flavonoids and phytoestrogens, contributing to its health benefits (10). The genetic diversity of fennel has garnered attention due to its potential to improve crop quality and yield. Molecular markers, such as Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs) and Single Nucleotide Polymorphisms (SNPs), have been used to assess the genetic variability among fennel genotypes. These markers allow for the precise identification of diverse genotypes, facilitating breeding programs to enhance desirable traits like disease resistance and stress tolerance. SSR markers have gained prominence for their co-dominant inheritance, high reproducibility and capacity to generate many alleles (11-15).

Genetic diversity is a fundamental foundation for evolution, providing crop populations with the ability to endure, adjust to novel conditions and generate new genetic diversities. Some of these variants may be well-suited to meet the long-term environmental changes. Assessing genetic diversity involves various methods within and between populations, such as quantifying the number of distinct organisms and examining individual relationships at different levels, including genus, species, population, individual, genome locus and DNA base sequence. Advancements in molecular marker technology have led to the discovery of numerous markers associated with diverse traits, facilitating the localization and identification of various genes and the characterization of true species and genera. SSR markers have gained prominence among these markers due to their reproducibility and locus-specific nature. Genetic diversity is the overall count of genetic characteristics in a species' genetic composition. It differs from genetic variability, which refers to the inclination of genetic traits to differ. Genetic diversity acts as a mechanism for populations to adapt to shifting environments. When there is more significant variation, it increases the likelihood that specific individuals within a population will carry alleles that are well-suited for the prevailing climate. DNA markers, or molecular markers, are found widely throughout genetic material, existing in vast numbers and being distinct, harmless and inherited according to Mendelian laws. They remain unaffected by the surrounding environment and are free from epistatic interactions.

A genetic marker is any readily measurable characteristic associated with a specific trait of interest aimed at being identified. These markers indicate the presence of a particular allele or the inheritance of a specific trait. The phenotypes used as markers are those in which the observed variation within the target population can be attributed, at least in part, to a single "Mendelian" factor. Three key properties that define a genetic marker are:

◊ The genetic marker must be specific to a particular genetic locus.

◊ It should exhibit polymorphism, showing multiple variations within the studied population.

◊ The phenotyping of the marker should be straightforward and uncomplicated.

◊ The effectiveness of a genetic marker is commonly assessed based on:

◊ It is the level of heterozygosity within the population of interest.

◊ The Polymorphism Information Content (PIC) it carries.

Polymorphism Information Content (PIC) is the likelihood of correctly identifying one of the two homologous alleles from a parent that has passed on an allele to a specific offspring, with the other parents' genotype also being determined. Molecular markers are distinctive segments of DNA that can be detected within the entire genome, occupying specific positions. They are essentially genetic markers, indicating the location of genes or the inheritance of particular traits. Remarkably, molecular markers are phenotypically neutral, not affecting the observable characteristics of an organism. It is essential for a molecular marker to possess specific desirable properties.

i. Polymorphism is a crucial requirement, as the variation in the markers' forms is assessed for genetic diversity studies.

ii. The marker should exhibit co-dominant inheritance, differentiating homozygous and heterozygous states in diploid organisms.

iii. Even widespread distribution throughout the genome is essential for a markers' effectiveness.

iv. Easy, rapid and cost-effective detection methods are preferred for a practical genetic marker.

v. Reproducibility is a key characteristic that ensures consistent and reliable results.

vi. Facilitating a high data exchange between laboratories enhances the markers' utility and collaboration in research.

Simple Sequence Repeats (SSRs), or Microsatellites, offer extensive genomic coverage. They can easily automate, possess locus specificity and exhibit multiple alleles. Numerous agronomic and quality traits demonstrate quantitative inheritance and the underlying genes responsible for these traits have been quantified using Quantitative Trait Locus (QTL) tools. SSR markers find broad applicability in genetic analysis for crop improvement strategies. They are particularly popular in plants due to their abundance, hyper-variability and

suitability for high throughput analysis. The polymorphism at a locus is determined by the varying number of repeats, which can either increase or decrease the length of the locus. This polymorphism is commonly observed when comparing the PCR fragment length using capillary electrophoresis. SSRs are abundantly present in eukaryotic genomes (16). Understanding the genetic diversity within fennel populations is crucial for breeding programs to improve crop traits such as yield, disease resistance and stress tolerance. Simple Sequence Repeats (SSRs), also known as microsatellites, have emerged as powerful tools due to their high polymorphism, co-dominant inheritance and extensive genome coverage. SSRs are repetitive DNA sequences distributed throughout the genome, characterized by their high mutation rate and polymorphic nature. These markers are handy in genetic studies because they provide detailed insights into genetic diversity and population structure. The primary advantages of SSR markers include:

High polymorphism: SSRs exhibit high levels of genetic diversity, making them practical for assessing genetic diversity.

Co-dominant inheritance*:* Unlike dominant markers, SSRs can differentiate between homozygous and heterozygous states.

Locus specificity*:* SSR markers are specific to genomic loci, allowing precise identification of genetic differences.

Reproducibility*:* The markers are highly reproducible across different laboratories, enhancing the results' reliability.

Molecular research on SSRs in fennel: Recent studies have applied SSR markers to assess genetic diversity in fennel, revealing valuable insights into the genetic architecture and relationships among various fennel populations. A detailed survey of the genetic diversity of fennel germplasm using SSR markers. Their study analyzed 20 diverse fennel germplasm lines using 27 SSR primer pairs (17). Out of these, only 4 primer pairs exhibited polymorphism. Based on the SSR data, the study classified the fennel lines into 9 clusters. The limited number of polymorphic markers highlighted the need to develop more SSR markers to resolve genetic diversity within fennel better. The study underscored the importance of selecting diverse primer pairs and expanding the marker repertoire to enhance genetic analysis.

SSR markers to explore genetic diversity in fennel by analyzing 39 SSR primer pairs. The study identified 16 primer pairs that produced amplification products, with 7 primers generating non-specific bands. The successful primers were used to analyze various fennel varieties, including commercial and unknown genotypes. Capillary electrophoresis was employed to detect polymorphisms in the SSR motifs (18). The study demonstrated the utility of SSR markers in distinguishing between fennel varieties and provided a basis for future research on genetic improvement and diversity analysis.

An extensive analysis of SSR markers across several Apiaceae species, including fennel. The study utilized SSR markers to evaluate the genetic diversity and relationships among various canopy plants (19). The results revealed a high degree of polymorphism among the studied plants, including fennel, with an average of 2.8 polymorphic bands per primer. The study highlighted the effectiveness of SSR markers in characterizing genetic relationships within the Apiaceae family and provided insights into the genetic structure of fennel. The transferability of carrot SSR markers to other Apiaceae crops, including fennel. The study found that out of 49 carrot SSR markers tested, 30 were transferable to fennel and other related species. The transferability of SSR markers across genera suggests a conservation of SSR-flanking sequences, which can be advantageous for genetic studies and breeding programs. This study provided valuable information on the applicability of SSR markers in cross-species research. It highlighted the potential for leveraging SSR markers from related crops for genetic analysis in fennel (20).

Applications and future directions

The application of SSR markers in fennel research has significantly contributed to understanding genetic diversity, population structure and the development of improved fennel varieties. The current literature indicates that while SSR markers are effective for genetic analysis, the continued growth of more diverse and informative markers is needed. Future research should focus on:

Expanding marker repertoire: Developing additional SSR markers to enhance the resolution of genetic diversity studies.

Integrating SSR with other techniques: Combining SSR markers with next-generation sequencing and other molecular techniques to gain deeper insights into the genetic architecture of fennel.

Utilizing transferability: Leveraging SSR markers from related species to explore genetic diversity and improve breeding programs.

The use of SSR markers in *Foeniculum vulgare* Mill. research has provided valuable insights into the genetic diversity and relationships among fennel populations. While existing studies have demonstrated the utility of SSR markers, there is room for further development and application to enhance our understanding of fennel genetics. Continued research and marker development will be crucial for improving fennel breeding programs and ensuring the sustainability of this vital crop. Recent advances in molecular biology have also facilitated next-generation sequencing (NGS) technologies, such as Genotyping-by-Sequencing (GBS), in plant genetic research. The power of GBS in uncovering genetic diversity in fennel and other Apiaceae crops, allowing for more efficient marker-assisted selection and crop improvement. These molecular tools are instrumental in addressing challenges such as climate change and emerging diseases that threaten fennel production. This study aimed to build on these advances by analyzing the genetic diversity of fennel genotypes using molecular markers, focusing on identifying valuable alleles that can contribute to crop improvement. By employing SSRs and SNP markers, we seek to provide insights into the genetic structure of fennel populations, thereby supporting future breeding programs aimed at developing high-yielding, disease-resistant and stress-tolerant fennel varieties.

Materials and Methods

The research was conducted at the Vegetable Research Centre, Department of Horticulture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India. The experimental site is in the Kymore Plateau and Satpura Hills region at 411.78 m above mean sea level. Geographically, Jabalpur is situated at 23.9 °N latitude and 79.58 °E longitude. Jabalpur experiences a semi-arid climate with a subtropical influence. The region has hot and dry summers, cold winters and moderate to high relative humidity. The average annual rainfall is approximately 1350 mm between mid-June and October. Temperature variations are significant, with maximum temperatures ranging from 24 °C to 45 °C and minimum temperatures between 4 °C and 22 °C. Relative humidity ranges from 80-90 % during the rainy season, 60-70 % in winter and 30-40 % during summer. The experimental design employed was a Randomized Block Design (RBD) utilized in the study, comprising germplasm collected from All India Coordinated Research Project (AICRIP project) Table 1. SSR (Simple Sequence Repeat) markers were used to evaluate genetic diversity and relationships among the 33 fennel genotypes. The list of SSR markers is in Table 2.

Methods for genomic DNA Extraction and PCR amplification

Collection of leaf samples **:** Fresh, young, tender leaves were collected from field-grown fennel plants Fig. 1 The leaves were thoroughly washed with distilled water to remove any surface contaminants. They were then weighed and immediately frozen in liquid nitrogen. The frozen leaf tissue was ground to a fine powder using a sterilized mortar and pestle, taking care to prevent thawing during the grinding process.

Table 1. Names of the fennel genotypes used for the study

S. No.	Name of genotypes	Source of collection
1	Local	Local area of Jabalpur
2	FNL 130	AICRP on Spices
3	FNL 131	AICRP on Spices
4	FNL 132	AICRP on Spices
5	FNL 133	AICRP on Spices
6	FNL 134	AICRP on Spices
7	FNL 135	AICRP on Spices
8	FNL 136	AICRP on Spices
9	FNL 137	AICRP on Spices
10	FNL 138	AICRP on Spices
11	FNL 139	AICRP on Spices
12	FNL 140	AICRP on Spices
13	FNL 141	AICRP on Spices
14	FNL 142	AICRP on Spices
15	JAGUDAN 1	Spice Research Station Jagudan, Gujarat
16	JAGUDAN 2	Spice Research Station Jagudan, Gujarat
17	RF 101	SKNAU, Jobner
18	RF 125	SKNAU, Jobner
19	RF 178	SKNAU, Jobner
20	RF 205	SKNAU, Jobner
21	RF 281	SKNAU, Jobner
22	JF 1	Jaipur Local
23	JF 2	Mandsaur Local
24	JF 3	Mandsaur Local
25	JF 4	Etah (U.P.)
26	JF 5	Khargon Local
27	JF 6	Shajapur Local
28	JF 7	Shajapur Local
29	JF 8	Shajapur Local
30	JF 9	Shajapur Local
31	JF 10	Alwar Local
32	JF 11	Chitrakoot Local
33	JF 12	Chitrakoot Local

Fig. 1. General view of fennel experimental plot.

Genomic DNA isolation and purification **:** Genomic DNA was extracted using a modified CTAB method (21). The ground leaf tissue was mixed with CTAB extraction buffer (see Annexure-I), preheated to 60 °C and incubated at 65 °C for 1 h with intermittent shaking (4-5 times) to ensure thorough mixing and emulsification. Following incubation, the mixture was allowed to cool to room temperature.

An equal volume of chloroform: isoamyl alcohol (25:24:1) was added to the cooled mixture and mixed gently by inversion for 10 min to achieve complete emulsion. The sample was centrifuged at 10000 rpm for 20 min to separate the phases. The aqueous phase (supernatant) was carefully transferred to new tubes. Cold isopropanol (0.5 to 0.6 volumes of the supernatant) was added to precipitate the DNA. The mixture was inverted gently and left undisturbed for 30 min. DNA was spooled out using a sterile glass hook and transferred to a sterile Eppendorf tube. The DNA pellet was washed twice with 70 % ethanol by centrifugation at 10000 rpm for 4-5 min to remove salts and residues. After washing, the ethanol was removed and the pellet was allowed to air dry completely. The dried pellet was dissolved in 300 µL TE buffer and stored at -20 °C (22).

For further purification, 60 µL of RNase solution was added to the DNA sample and incubated at 37 °C for 1 hr to remove RNA contamination. The sample was mixed with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and thoroughly mixed until an emulsion formed. After centrifugation at 10000 rpm for 10 min at room temperature, the aqueous phase was transferred to a fresh Eppendorf tube. DNA was precipitated by adding one-tenth volume of 3M sodium acetate (pH 5.2) and two volumes of ice-cold ethanol and incubated at -20 °C for 1 hr. The DNA was pelleted by centrifugation at 8000 rpm for 10 min at 4°C. The supernatant was removed and the pellet was washed with 70 % ethanol. The tubes were kept open at 37 °C to allow complete evaporation of ethanol. The DNA pellet was then dissolved in an appropriate volume of TE buffer and stored at -20 °C until further use.

Dilution of genomic DNA samples : The stock DNA samples were diluted with TE buffer to an appropriate concentration for use in PCR reactions.

Table 2. List of primers used for this experiment

Sr. No.	SSR Primer code	Oligomer sequence (5' to 3') F- Forward base pair R- Reverse Base pair	Tm (°C)
1	FV-253	F: TTGTAGAGATACAGGGTCGAA R: GAGGGGAGTCAGTTAAACAAC	56.5
2	FV-290063	F: TGATTTCTCAAAGGCATTCTA R: TCTTCCTGTCATCTCAAGGTA	56.5
3	FV-6	F: TATGTTCTCAGATTCGGGTTA R: GTTCATCAAACTGTGTCATTGT	56.5
4	FV-9919	F: GCCTATGTATTTGCAAGAATG R: TGCAACATTCAATTGTGTAGA	56.5
5	FV-9919	F: AGTAAAGGCATAATCTGTTGGTGG R: TCATATTATCAACCTCAGGCACAG	56.5
6	FV-11537	F: TTCATGTATCAACTACGCACAC R: CTCTGGGATTGGATTCAAGGAG	56.5
7	FV-15981	F: CTAGCGTTTCCATCTCGTCTC R: AACCCGTAACTTTAACCACCAC	56.5
8	FV-18902	F: GTTTGAACTCGAATGACCACCT R: GGGTCTATCATCACTCTCGC	56.5
9	FV-30720	F: TTCAAATCTCATTAACCACGTA R: TTTCTGATTGGTTAACTGTGTC	56.5
10	FV-30919	F: CGAGTCATGGCAATGTATAAG R: AACATTGATACCTGAACTCCA	56.5
11	FV-144120	F: CTCTTTTCCAAAAATATCACG R: GATGAAAAAGGGTAATTGGTT	56.5
12	FV-155794	F: CAAAGAATGGAAAACATGCTG R: CTTTCCCATTGTCAATTTGC	56.5
13	FV-179837	F: ATTCACCATGACATCACCTC R: ACAGTGTGGGTTTGTATGTGT	56.5
14	FV-181677	F: GCTTACAATTAAGAAGCGAAA R: CATCTTACGGCATATGTTTGT	56.5
15	SSR1015-057F SSR1015-058R	TGACTGTGCCAAGGAAGTTAAT TTCAGACCAAGTATCGCATTTC	55
16	SSR1015-059F SSR1015-060R	TCATTGTTGAATCCTGCCATAG GTTGTGGAGAGTCATTTGGT	55
17	SSR1015-061F SSR1015-062R	TGAAGTCGGGACCTAAAAGATT ACTCAAGTTACCAGCGAAGACC	55
18	SSR1015-063F SSR1015-064R	TAAAGATCAGGGAGGGTGAGAA CTAAAATTCCATCTGCGCTCTT	55
19	SSR1015-065F SSR1015-066R	CCCAGCAAGGAGAAAGATAGA GATCAAACTCGTCCACCTCA	55
20	SSR1015-067F SSR1015-068R	GTTCCCCAGGAGTTCTCTACCT AGGGCAAAACCAAAATACAC	55
21	SSR1015-069F SSR1015-070R	AAAACOATACAATCAATCTCOGG ACTGTCTGCTGAAAACTCAT	55
22	SSR1015-073F SSR1015-074R	GCTGTAGGGCTTGCTTCTATTG TATACCAATTCCTCATCCGCA	55
23	BSSR-8F BSSR-8R	TGAAGCTAATATCCAACAAAGGAAA AGGAGCATGTTATGCTATTAC CAACA	55
24	SSR1015-077F SSR1015-078R	TGGAAGAGATGAAGAAGATGAG AGCGAGTCCAAACATAAGGC	55
25	GSSR-43F GSSR-43R	TTCTTCACCTATGTTGGGGC CGTTCATATGCACAACACTCA	55
26	GSSR-96F GSSR-96R	AGCGTCGTTTTCGCGAGT CGCGGTTAAAGCAAAGCTAAT	55
27	GSSR-113F GSSR-113R	AGTGGTTGTGAGGTTGATTGTG TATGTCGGAAAGGTTCAATGCT	55
28	GSSR-111F GSSR-111R	GAGGAAGGGTAGATCCAGTCA ATGGGATGTCTTTCCCCTCTAT	55
29	GSSR-138F GSSR-138R	CGCTCGAGTTTCGTAGAGT CCTCCCCAACTCAATCCAAT	55

Polymerase chain reaction (PCR) amplification *:* The PCR reactions were conducted following the cycling regime specified, except for the annealing temperatures, which were optimized using a temperature gradient PCR. The primer annealing temperatures were determined to be between 55 °C and 56.5 °C. SSR primers that yielded precise and reproducible results were selected for the study. The reaction mixtures were briefly centrifuged to ensure thorough mixing of the components. PCR samples were stored at 4 °C for short-term storage and at -20 °C for long-term storage. The amplified products were analyzed by loading them onto agarose gels stained with ethidium bromide to visualize polymorphic primers. Agarose gel electrophoresis was performed to separate the PCR-amplified products. A 2.5 % agarose gel was prepared by dissolving 2.5 g of agarose in 100 mL of 1X TBE buffer, which was then boiled until the agarose was completely dissolved. Before completely cooling the gel, 3 µL of ethidium bromide (10 mg/mL) was added. The gel was poured into a gel tray with a comb, avoiding air bubbles and allowed to solidify. The solidified gel was placed in a horizontal electrophoresis

apparatus and submerged in 1X TBE buffer. PCR products were mixed with 3 µL of 6X loading dye and loaded into the gel wells. A 100 bp ladder was included as a reference marker. The gel was electrophoresed at a constant voltage of 75 V for two hr or until the ladder was adequately resolved. The gel was then photographed using a gel documentation system (Fig. 2). Both the PCR and cycle components are represented in Table 3-4.

Molecular data analysis **:** The polymorphism information content (PIC) of SSR markers was computed, which allows for the evaluation of marker utility in detecting genetic diversity (23). Genetic distance was subsequently determined from the similarity values obtained using the Jaccard coefficient, with the genetic distance. The binary data for SSR bands denoted as '1' for present and '0' for absent, were meticulously recorded and organized in Excel spreadsheets and NEXUS format. Agarose gel electrophoresis data were employed to quantify the number of bands generated by each primer, the proportion of bands common to all genotypes and the frequency of bands unique to individual varieties. Genetic similarity coefficients for pair-wise comparisons were derived using Jaccards' coefficient. This similarity matrix was then analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to produce a dendrogram visually representing the genetic relationships and divergence among the fennel genotypes under study. The UPGMA clustering analysis was performed using NTSYS-pc software (version 2.02), which enabled the hierarchical grouping of genotypes based on their genetic similarity coefficients.

Table 3. Components of PCR reaction

Component	Reaction volume (10 µl)
Taq buffer (10X)	1 µl
MgCl2	0.7 µl
dNTP mix	0.2 µl
Taq DNA polymerase	0.2 µl
Forward primer	0.5 µl
Reverse primer	0.5 µl
Genomic DNA	1.0 µl
Milli-Q water (to make up volume)	5.9 µL

Table 4. PCR Cycle regime

S. no.	Step	Temperature	Time	Cycles
1	Initial denaturation	94°C	3 minutes	1
2	Denaturation	94°C	1 minute	
3	Annealing	54-56.5°C	1 minute	35 cycles
4	Extension	72°C	2 minutes	
5	Final extension	72°C	7 minutes	1
6	4 ° C		∞	

Results

Molecular profiling using SSR markers was employed to characterize fennel genotypes, leveraging this methods' high accuracy and user-friendliness. As PCR-based molecular markers, SSR markers are highly reliable due to their basis in repetitive DNA sequences. The Polymorphic Information Content (PIC) value, which indicates the variability at a specific locus, was used to evaluate the effectiveness of the SSR primers in distinguishing between genotypes' PIC values represented in Table 5. A higher PIC value suggests a greater likelihood of polymorphism among genotypes at a given

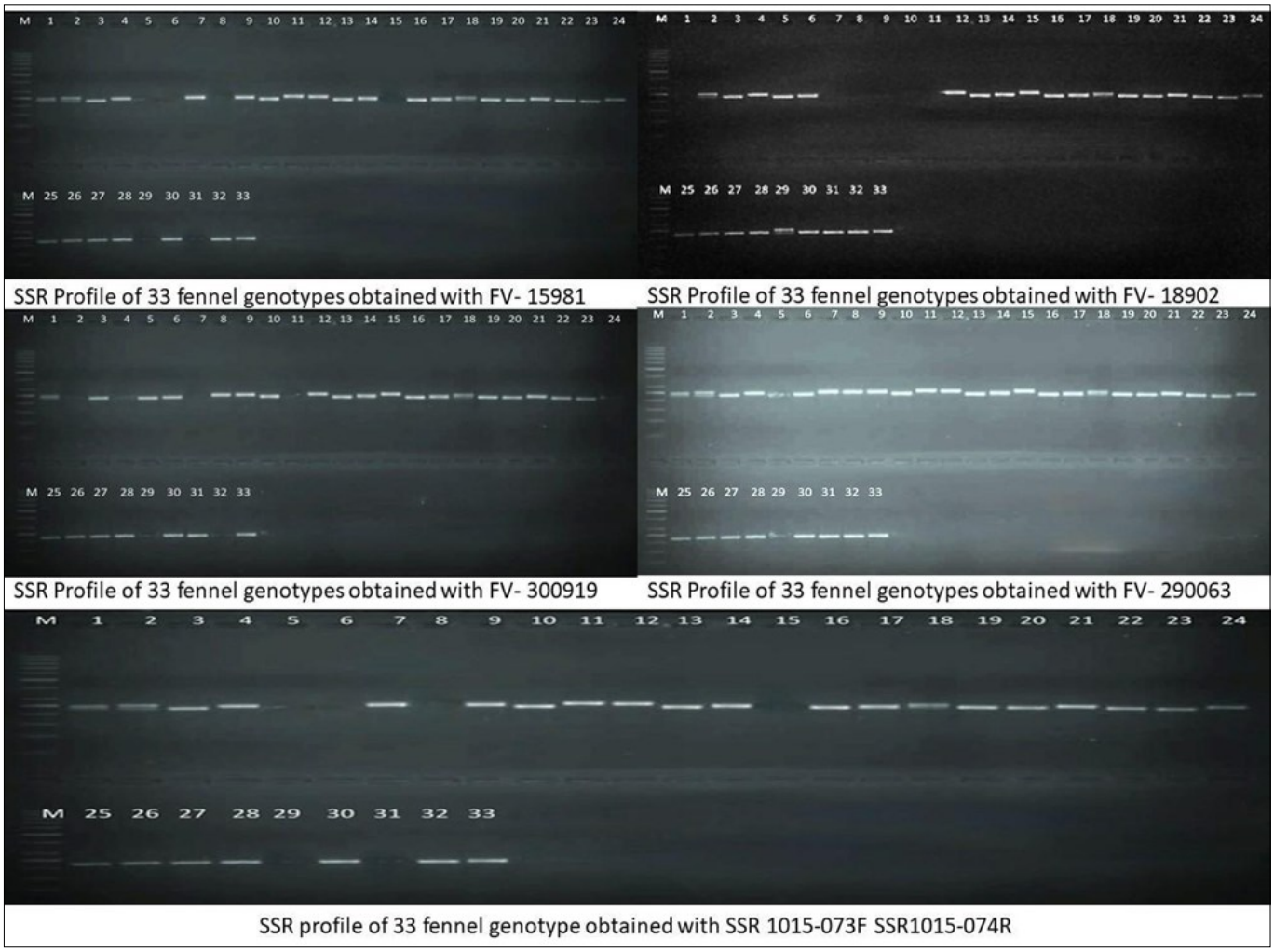

Fig. 2. SSR profiling of 33 fennel genotypes obtained with FV - 290063.

Table 5. PIC value (Polymorphic Information Content)

Primer	Number of alleles	PIC value
FV-290063	3	0.375
FV-9919	2	0.245
FV-6	2	0.355
FV-65	2	0.412
FV18902	2	0.210
FV-30919	2	0.468
FV-253	3	0.375
FV-144120	2	0.499
FV-181677	3	0.252
FV30720	3	0.444
65F, 66 R	3	0.499
73F,74R	2	0.482
96F,96R	3	0.444
111F,111R	2	0.499
138F138R	3	0.252

locus. In this study, PIC values ranged from 0.210 to 0.499, with an average PIC value of 0.289 per primer. The SSR primer FV-144120, 65F, 66R and 111F, 111R exhibited the highest PIC value of 0.499, indicating a strong ability to differentiate between genotypes. This was followed by FV30720 and 96F, 96R, with PIC values of 0.444 and FV-30919 and 73F, 74R, with PIC values of 0.468 and 0.482, respectively. Conversely, the primer FV18902 had the lowest PIC value of 0.210, followed by FV-9919 and FV-181677, each with a PIC value of 0.245 and 138F, 138R with a PIC value of 0.252. Thus, the PIC values for the 15 primers used in the study varied between 0.210 and 0.499, reflecting the range of polymorphic potential across the genotypes.

Clustering analysis using SSR markers

Clustering analysis of 33 fennel genotypes using SSR markers revealed nine distinct clusters, each representing a unique grouping of the genotypes based on their genetic profiles Fig. 3.

Cluster No. 1: This cluster included eight genotypes: FNL-131, FNL-139, FNL-141, FNL-133, FNL-138, FNL-134, FNL-140 and FNL-142.

Cluster No. 2: Comprising eight genotypes, this cluster contained JF-1, JF-2, JF-3, JF-5, JF-10, Jagudan-1, Jagudan-2 and RF-178.

Cluster No. 3: This cluster accommodated three genotypes: FNL-135, FNL-137 and FNL-136.

Cluster No. 4: Included four genotypes: FNL-130, FNL-132, RF-205 and RF-281.

Cluster No. 5: Contained only two genotypes: RF-125 and RF-101.

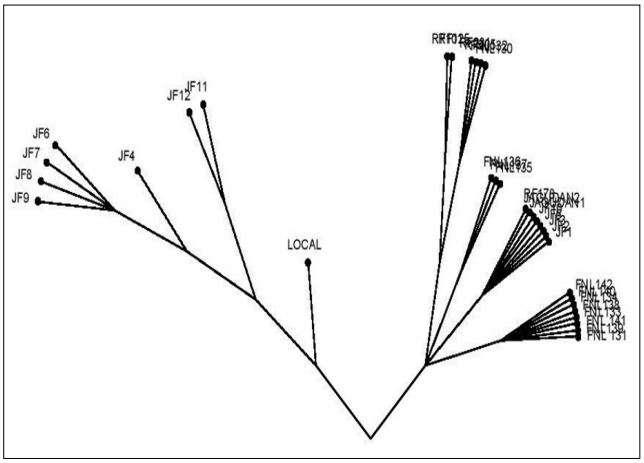

Fig. 3. UPGMA dendrogram for the fennel genotypes based on SSR analysis.

Cluster No. 6: This cluster was represented by a single genotype, LOCAL.

Cluster No. 7: Genotype JF-4 was uniquely placed in this cluster.

Cluster No. 8: Comprising four genotypes: JF-6, JF-7, JF-8 and JF-9.

Cluster No. 9: This cluster contained two genotypes: JF-11 and JF-12.

The use of SSR primers for clustering analysis effectively classified the fennel genotypes into these nine well -defined clusters, highlighting the capability of SSR markers to differentiate among the genotypes irrespective of their lineage or geographic origin. This analysis underscores the genetic diversity within the fennel germplasm, offering valuable insights into the genetic relationships and variability among the different fennel genotypes. This method facilitates detailed investigations into fennels' biochemical, nutritional and genetic aspects, showcasing the utility of linked DNA markers in crop improvement studies.

Identification of distinctive SSR primers

In the present study, several SSR primer sets, including FV-6, FV-290063, FV-30919 and FV-18902, demonstrated distinctive banding patterns across various fennel genotypes. These primers were particularly effective in differentiating genotypes such as RF-125, FNL-134, JF-1, JF-4, JF-9 and FNL-139. The unique banding patterns provided by these primers enabled precise identification of these genotypes, highlighting their utility in genetic characterization.

The ability of these SSR primers to generate distinct banding profiles underscores their value in genetic studies and diversity analyses. By revealing unique genetic markers associated with each genotype, these primers contribute significantly to our understanding of genetic variability within the fennel population. This information is crucial for breeding programs and conservation strategies aimed at maintaining genetic diversity. The effectiveness of these primers in differentiating between genotypes demonstrates their potential as essential tools for further genetic research in fennel and similar crops. Their application can lead to more informed decisions in plant breeding and genetic resource management.

Discussion

Molecular profiling using SSR markers has demonstrated considerable effectiveness in characterizing fennel genotypes, offering valuable insights into genetic diversity and relationships within the crop. Due to their repetitive DNA sequences, SSR markers provide accurate and consistent results, making them a preferred choice for genetic studies. In this study, 37 alleles were amplified across 15 primer sets, with an average of 2.47 alleles per marker. The Polymorphic Information Content (PIC) values ranged from 0.210 to 0.499, with an average PIC of 0.289. These values highlight the efficiency of the SSR primers in detecting genetic diversity and confirm their utility in distinguishing between fennel genotypes.

The clustering analysis revealed nine distinct clusters among the fennel genotypes, a finding that corroborates previous research. Similar genetic diversity patterns underscore SSR markers' efficacy in differentiating closely related fennel genotypes (24). Similarly, the research highlighted the usefulness of SSR markers in revealing genetic structure and diversity within fennel populations. Their studies align with the current findings, demonstrating the ability of SSR markers to provide meaningful insights into genetic diversity (25, 26). These findings, which showed the effectiveness of SSR markers in various plant species, were further supported, including fennel. Their work emphasized the robustness of SSR markers for assessing genetic diversity and relationships, which is consistent with the results of this study (20). Recent studies have reinforced the role of SSR markers in genetic characterization. SSR markers effectively uncovered fennels' genetic diversity and population structure, contributing to a better understanding of its genetic architecture. They highlighted the application of SSR markers in identifying genetic diversity and improving crop breeding programs, demonstrating their importance in fennel genetic studies (27, 28). Further illustrated the utility of SSR markers for discriminating between fennel varieties, aligning with the results obtained in this study.

Identifying distinctive primer sets, such as FV-6, FV-290063, FV-30919 and FV-18902, which produced unique band patterns for specific genotypes, underscores their potential for targeted genetic studies and diversity analyses. These primers facilitate precise genotype identification and offer valuable genetic information that can enhance crop improvement programs. The distinct band patterns associated with these primers enable researchers to identify and characterize fennel genotypes more effectively, contributing to the development of improved varieties (29).

The clustering patterns observed in this study, which align with the genetic variability reported in similar studies, suggest that SSR markers are a robust tool for assessing genetic diversity in fennel. The ability to categorize genotypes into well-defined clusters provides a deeper understanding of fennel germplasm, aiding in identifying genetic relationships and diversity. These insights are crucial for guiding future breeding and conservation strategies, as they allow for the selection of diverse parental lines and the development of varieties with desirable traits. In summary, the study demonstrates that SSR markers are highly effective for molecular profiling and genetic characterization of fennel. The clustering analysis and identification of distinctive primers provide valuable information for understanding genetic diversity and relationships within fennel populations. These findings contribute to the broader knowledge of fennel genetics and have practical implications for crop improvement and conservation strategies.

Conclusion

The study has demonstrated the effectiveness of SSR markers for the molecular profiling and genetic characterization of fennel genotypes. SSR markers, known for their accuracy and reliability due to their repetitive DNA sequences, facilitated the amplification of 37 alleles across 15 primer sets. The Polymorphic information content (PIC) values, ranging from 0.210 to 0.499, with an average of 0.289, underscored the primers' capability to detect genetic diversity and distinguish between fennel genotypes. The clustering analysis revealed nine groups of fennel genotypes, aligning with previous studies and highlighting the genetic diversity within the fennel population. The findings are consistent with earlier research that also noted the utility of SSR markers in identifying genetic diversity and structuring plant populations (19-21). The distinctiveness of primer sets FV-6, FV-290063, FV-30919 and FV -18902, which produced unique band patterns for specific genotypes, demonstrates their potential for precise genotype identification and genetic diversity analysis. SSR markers are a robust tool for assessing genetic diversity and relationships among fennel genotypes. The insights gained from this study are valuable for guiding future breeding and conservation strategies, as they enhance the understanding of fennel germplasm and support the development of improved varieties. This research contributes to the broader field of plant genetics by providing a detailed analysis of fennel genetic diversity and offering practical applications for crop improvement programs.

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Authors' contributions

YS designed the research. Experiments were conducted by NE, who also performed. Data analysis. The manuscript was drafted by RN, VS and KT, with support from scientists. Writing from YS and NE. All authors reviewed and approved the final manuscript.

AI Declaration and Justification*:* The authors declare that AI-based tools were used only for language editing and formatting during the preparation of this manuscript. However, the authors independently performed all scientific content, data analysis and interpretation.

Compliance with ethical standards

Conflict of interest: The authors declare no conflicts of interest.

Ethical issues: None

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