Original Research Article

Morphological and molecular diversity analysis in fennel

Abstract

A study was conducted to assess the morphological and molecular diversity of fennel using 20 genotypes. Multivariate analysis based on morphological traits grouped all genotypes into five major clusters. Cluster II was having nine genotypes, cluster I was having seven genotypes and cluster III was having 2 genotypes out of total genotypes used in this study. The other clusters were mono-genotypic. The highest inter-cluster distance was found between Clusters III and IV. Moreover, cluster IV was superior based on the cluster means for maximum yield-related traits. For the molecular analysis, 11 of the 20 SSR markers were found to be polymorphic. PIC values varied from 0.322 to 0.740. Molecular characterization revealed three major clusters comprising one, eight, and eleven genotypes. The genotypes GF-1, GF-12, RF-101, AF-1, and RF-145 were found to be in the same cluster in both the multivariate analysis and molecular characterization.

Keywords: Fennel, SSR, PIC, molecular marker

Abbreviations: PIC: Polymorphic Information Content, SSR: Simple Sequence Repeat

1. Introduction

Fennel is one of the major seed spices, botanically referred to as *Foeniculum vulgare*, and belongs to the Apiaceae family. It is native to southern Europe and is widely cultivated in the temperate and subtropical regions of the world. In India, it is grown mainly in Gujarat, Rajasthan, Madhya Pradesh, West Bengal, and Uttar Pradesh. It is a biennial medicinal and aromatic plant, which is a cross-pollinated crop with a diploid chromosome number of 2n=22. It is an erect, branching, perennial herb that can grow up to 2 m in height. The fruit, commonly known as the seed, is a schizocarp of two mericarps attached to a dividing carpophore. The seed contains 0.7-1.2% volatile oil. Trans-anethole, fenchone, and methyl chavicol are the main components of essential oil [1]. It is a highly aromatic and flavoring herb with culinary and medicinal uses. Fennel possesses estrogenic, anticancer, chemopreventive, cytoprotective, antioxidant, and other health benefits [2].

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The analysis of genetic diversity and relatedness between species and genotypes is useful in plant breeding programs because it provides a tool for efficient parent selection. Characterization of genotypes with molecular markers enables researchers to evaluate the relative diversity within and between species, to classify accessions with the greatest accuracy and certainty, and to identify diverse genotypes for breeding purposes. Estimation of diversity or characterization of genotypes based on taxonomical descriptors or morphological methods is common, resulting in overlapping complex results that are highly influenced by the environment, and there is a possibility for the incidence of variation at the phenotypic expression level. Therefore, there is a need to support morphological variability with molecular variability studies to choose better-performing genotypes. Microsatellite or simple sequence repeat (SSR) markers have gained considerable importance due to many desirable attributes like their multi-allelic nature, co-dominant transmission, extensive genome coverage, small amount of starting DNA, and ease of detection by polymerase chain reaction (PCR). SSRs are known to have high heterozygosity values and are more informative than dominant DNA markers. Microsatellite markers provide accurate results with a minimum number of loci and alleles employed in the study and give way to evolutionary studies [3].

2. Materials and Methods

2.1 Field evaluation

The experiment was conducted at Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, University of Horticulture Sciences, Bagalkot during Rabi season of 2022. The number of genotypes were used in this experiment were 20. Seeds were sown at a spacing of 45 cm between rows and 20 cm between plants. The umbels were harvested at the light-yellow stage. Five plants were selected for measurements after discarding the border plants. Observations were recorded on days to fifty per cent flowering, days to maturity, umbels per plant, umbellate per umbel, flower per umbellate, seeds per umbel, seed yield per plant (g), seed yield per plot (g), harvest index (%), and test weight (g).

2.2 DNA extraction and PCR amplification

The fennel plant DNA was extracted using the CTAB method [4].The resolved DNA fragments were visualized using a gel documentation unit under UV light.

2.3 Data analysis

The data were subjected to analysis of variance [5]. Genetic diversity was analyzed by Mahalanobis D2 analysis, and genotypes were grouped into various clusters following Tocher's method. Genetic diversity among the collected varieties was assessed using genotypic scores generated from SSR markers. A dendrogram was developed using the neighbor-joining (NJ) clustering methodology based on Nei's genetic distance. The data were analyzed using DARWIN software to estimate basic diversity parameters such as polymorphic information content (PIC). The polymorphic information content was calculated for each primer using the formula PIC= 1 - Σpi2, where pij is the frequency of the ith allele of the jth marker [6].

Table 1: List of SSR primers used for PCR amplification in the present study

SI.No.	Primers	Forward primer sequence	Reverse primer sequence	Ann. Temp (□)
1.	ESSR 1	TGATACATGTGCAAGGAGGG	TCAAAGTCGTGAAACAGATCC	52-61
2.	ESSR 3	ACATAACGAGGCACATCTGG	TGAGTGTGATTAAGAGGGAGGG	52-61
3.	ESSR 5	ACAACACCAAGTACCTAGCG	AGATCTGTTCGAAGTCTCGC	52-61
4.	ESSR 7	TGATCCTAGAATGGTTCTATGC	TGATCTTTAACGGTCCTACC	52-61
5.	ESSR 10	GGGCCGTTCATTATTATCGTCG	ATAGAGACGCGTAGTTCCATGG	52-61
6.	FSSR 1	TGATGTTCACTCTCAGTAATAGG	AAGAAGAGAAATGTATTTGACGC	49-61
7.	FSSR 3	TCCTAGGGATTCACGAGTCC	ACATAATCCAGGACCCTCGC	49-61
8.	FSSR 8	TGGGAGTTGAAGAGGGAGGG	CACATCTACATACTGCAGGAAGC	49-61
9.	FSSR 9	GGGTTATGAGGAATCACGTCC	GTGACTCAGCATGTAACTGC	49-61
10.	FSSR 10	GGCGAGTTACAATATTATGCACCC	TTCCTGGACAACTCTGGTGC	49-61
11.	GSSR 11	CGGGCATGTGTGATATGTAAGG	TGTTATATGTGTGCACGCGC	53-62
12.	GSSR 14	ACATACTGTTGAGGACGAGG	TCACTAGTATCACTATCTTCGCC	53-62
13.	GSSR 16	TCCATAGGAACATCCAAGAAGC	TTATCACCCTGTCAAAGCCC	53-62
14.	GSSR 17	CTACAACGTCATCAAACTTTGG	AAGGATTCTTGAATTCAAATCAGG	53-62
15.	GSSR 18	ACATACATACGCACACACCC	GTTGATATACTATTTCAACGCAGC	53-62
16.	GSSR 20	AAGAATGACACTGTGCGACC	AGCGTGTGATTTGATCAAACCC	53-62
17.	GSSR 21	AGTGCTCATGCGAATTGTCC	CACACGATAAGAACGATAAGAAGG	52-61

18.	GSSR 26	CACTTAAGTGGTTCATGGTCCC	CCAGCAGCACTTCATTCTATGC	52-61
19.	GSSR 27	TCCGAAACTATACCGATTATCCG	ACGGTATCGGTATATTTAACATGG	52-61
20.	GSSR 29	CGAGTTACAATATTATGCACCC	TGCATGTAGTCTCTCTGTGG	52-61

3. Result and Discussion

3.1 Morphological analysis

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The mean values of ten quantitative characters recorded for the 20 genotypes of fennel are given in Table 2. Minimum days to fifty per cent flowering was recorded in genotype AF-1 (83) while maximum was obtained in GF-1 (95.5). Among the 20 genotypes, the minimum days to maturity were observed in RF-205 (129.5.) and maximum for HF-33 (148). Umbel per plant was observed maximum in AF-2 (25.9) and minimum in RF-145 (13.6). Highest umbellate per umbel was obtained in AF-2 (31.7) and minimum was observed in GF-12 (16.1). Highest flower per umbellate was obtained in AF-2 (28.6) and minimum was observed in RF-145 (15.6). Highest seeds per umbel were obtained in AF-2 (806.8) and minimum was observed in AF-3 (557.2). Seed yield per plant was obtained highest in AF-2 (30.83) and lowest in AF-3 (13.41). Seed yield per plot was obtained highest in AF-2 (914.80) and lowest in AF-3 (560.55). Maximum harvest index was noticed in AF-2 (22.18 %) and minimum was observed in AF-3 (10.48 %). The test weight was maximum in AF-2 (10.41 g) and minimum in AF-3 (7.45 g).

3.2 Mahalanobis D^2 analysis

Cluster II was identified as largest cluster having 9 genotypes followed by cluster I having 7 genotypes and cluster IV with 2 genotypes. Cluster III and V contains single genotypes (Figure 1 and Table 3). The highest inter-cluster distance (Table 3) was observed between clusters III and IV (651.35), followed by clusters II and IV (527.49), and clusters I and V (456.42). The intra-cluster distance (Table 4) ranged from 0 to 115.63, with the highest in Cluster II (115.63), followed by Cluster IV (94.75). Cluster IV had high mean values for characteristics such as plant height, days to first flowering, umbels per plant, umbellate per umbel, flowers per umbellate,

seeds per umbel, harvest index, test weight, and seed yield. This indicates that there is an improvement in seed yield traits, such as umbels per plant, umbellate per umbel, flowers per umbellate, seeds per umbel, harvest index, and seed yield. AF-2 and RF-205 were found to be the most suitable parents. Similar results obtained by some of previous study [7]. For characters such as days to fifty per cent flowering, cluster III was found to be superior, cluster II for days to maturity, and cluster I for the number of branches per plant (Table 5).

3.3 Molecular Characterization

A total of 20 SSR primers were used for analyzing genetic diversity present (Table 1). Out of these, eleven primers did not show any amplification, only 11 produced polymorphic bands. Polymorphic primers identified based on preliminary screening were GSSR 11, GSSR 14, GSSR 21, GSSR 26, GSSR 27, GSSR 29, FSSR 8, FSSR 9, ESSR 1, ESSR 5 and ESSR 10with their PIC 0.322, 0.729, 0.740, 0.417, 0.649, 0.680, 0.678, 0.686, 0.416, 0.526 and 0.611 respectively (Table 6) and found contradictory as reported by some of the workers (Grove and Malik, 2017; Choudhary et al., 2018) [8, 9]. The range of PIC value showed the significance of locus specific PCRbased microsatellite markers and confirmed that SSR markers are highly elucidative and would be useful in hybrid breeding. UPGMA based cluster analysis (Figure 2) showed that the grouping of 20 genotypes in three major clusters based on Nei's genetic distance obtained from SSR marker. First major cluster had 11 genotypes like GF-12, GF-11, GF-1, RF-281, RF-143, RF-145, RF-205, Azad Saunf-1, RF-101, AF-1 and AF-2. The second cluster having 8 genotypes in which RF-157, RF-178, UF-290, AF-3, HF-33, GF-2, RF-125 and UF-291 were related. Third major cluster had one genotypes *i.e*, RajendraSourabh. The genotypes that were grouped together showed high similarity while the genotypes which are far away are considered to be divergent.

Tables 2: Mean performance of 20 fennel genotypes

Genotypes	Days to 50 % flowering	Days to maturity	Umbels / plant	Umbellate/ umbel	Flowers/ umbellate	Seeds/ umbel	Seed yield/ plant (g)	Seed yield/ plot (g)	Harvest index (%)	Test weight (g)
AF-1	95.00	143.00	23.70	22.20	21.00	684.20	16.27	809.12	15.86	8.50
AF-2	92.00	141.00	25.90	31.70	28.60	806.80	30.83	914.80	22.18	10.21
GF-1	95.50	132.00	17.00	28.50	25.50	701.40	16.84	792.99	14.82	8.70
GF-11	91.00	135.50	23.50	19.60	18.80	726.80	14.78	645.27	11.67	9.20
GF-12	90.00	146.50	17.30	29.5	26.10	673.00	14.99	806.65	11.21	8.71
RF-101	90.50	132.50	25.80	29.00	26.20	729.80	16.17	780.73	14.56	8.34
RF-205	89.50	129.50	23.20	28.40	25.00	759.00	27.25	832.27	19.97	9.55
RF-145	91.50	139.50	13.60	16.80	15.60	685.90	17.33	743.25	17.69	8.80
RF-143	88.50	143.50	24.70	24.30	21.70	692.50	16.27	625.04	15.43	8.45
RF-281	89.50	138.50	23.80	25.00	24.00	673.00	27.77	649.25	19.18	9.80
RF-125	91.00	139.50	15.40	18.20	20.10	604.20	22.48	811.75	14.41	7.58
RF-178	93.00	135.50	21.10	24.00	23.20	707.70	19.18	688.94	16.29	9.10
RF-157	86.00	130.50	19.50	28.6	27.60	663.40	17.05	671.90	18.16	9.50
UF-290	92.00	147.50	18.10	19.50	18.30	631.80	14.16	701.30	12.88	8.36
UF-291	91.00	145.50	21.60	24.60	23.40	709.40	15.93	809.22	16.99	9.08
HF-33	96.00	148.00	21.20	28.70	25.80	598.00	14.10	646.72	12.83	8.32
AF-3	83.00	140.00	16.40	28.40	25.60	557.20	13.41	560.55	10.48	7.45
GF-2	93.00	136.00	18.60	16.10	18.00	645.10	17.72	567.32	16.17	9.80
Azad Saunf	95.00	143.50	22.80	16.40	17.10	605.60	17.17	607.22	18.28	9.16
Rajendra Sourabh	85.00	139.00	22.00	28.60	27.40	644.00	15.98	723.25	15.96	9.52
Mean	90.90	139.33	20.76	24.41	22.95	674.94	18.28	719.38	15.75	8.91
SEm±	1.96	2.67	1.68	1.49	1.35	20.81	0.71	14.34	0.81	0.27
CD at 5%	5.80	7.91	4.98	4.40	3.99	61.61	2.10	42.46	2.41	0.79

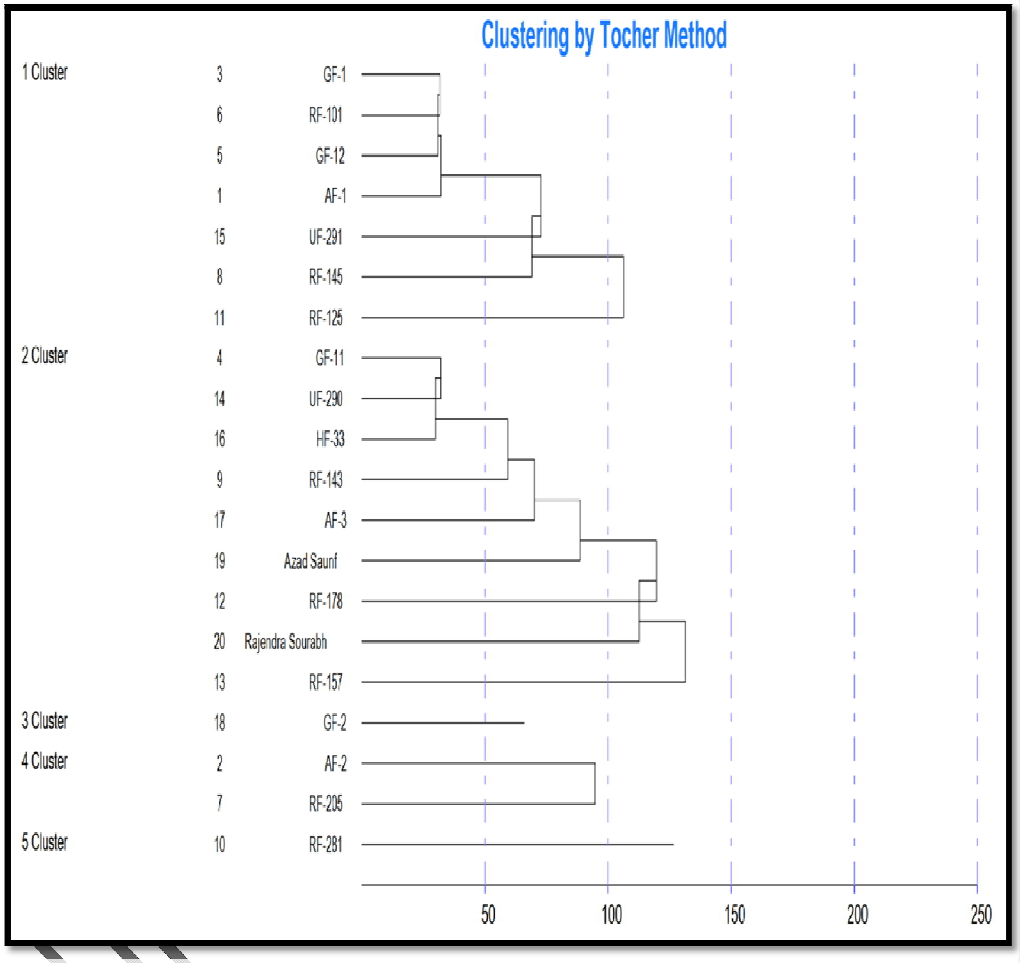


Figure 1: Clustering of fennel genotypes by Tocher's method

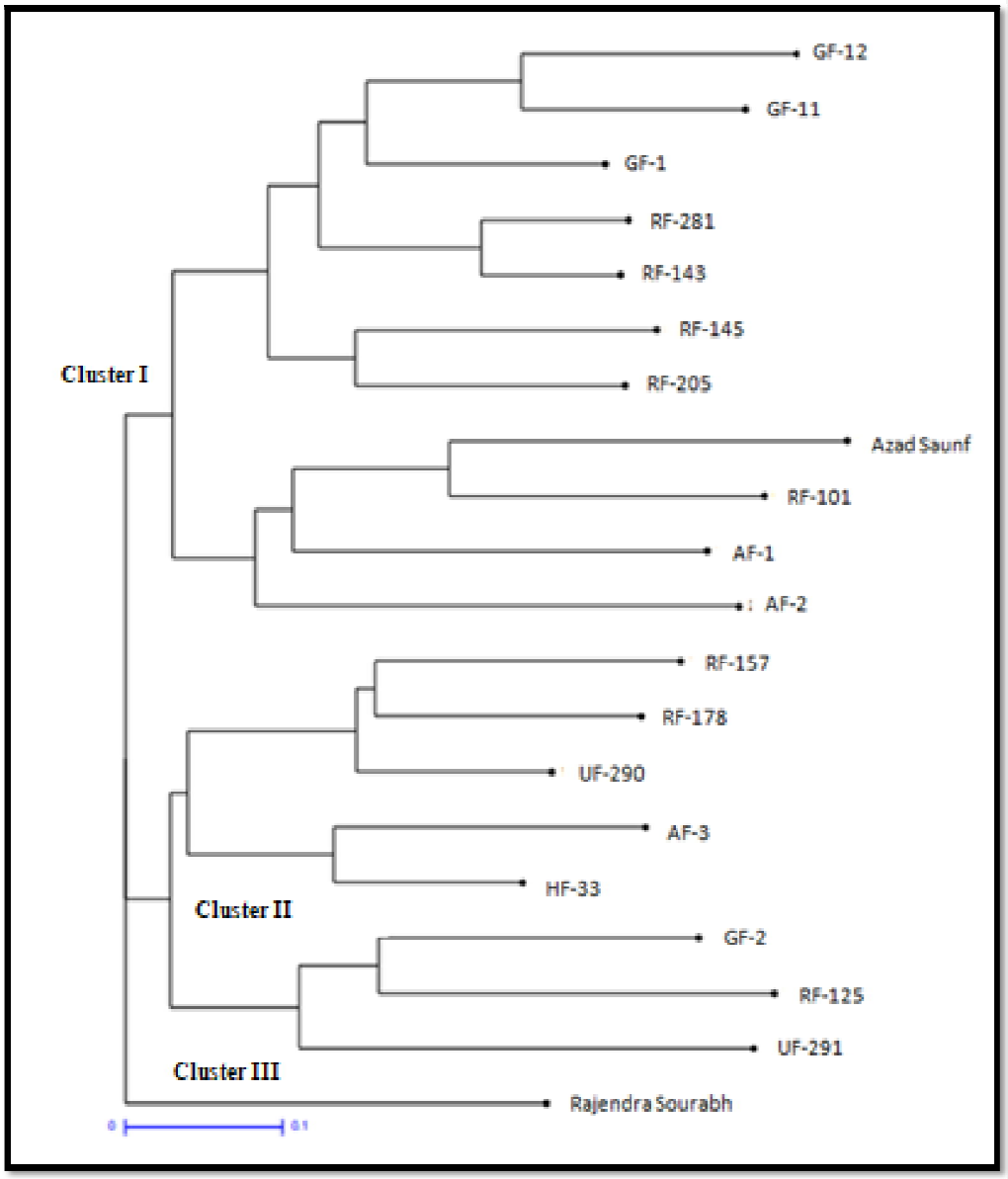


Figure 2: Dendrogram showing genetic relationship among fennel genotypes based on SSR data

Table 3: Composition of clusters in fennel based on D^2 statistics

Clusters	Number of Genotypes	Genotypes included in the clusters
Cluster I	7	GF-1, RF-101, GF-12, AF-12, AF-1, UF-291, RF-145 and RF-125
Cluster II	9	GF-11, UF-290, HF-33, RF-143, AF-3, Azad Saunf, RF-178, Rajendra Sourabh and RF-157
Cluster III	1	GF-2
Cluster IV	2	AF-2 and RF-205
Cluster V	1	RF-281

Table 4: Inter cluster and intra cluster D^2 values in fennel genotypes

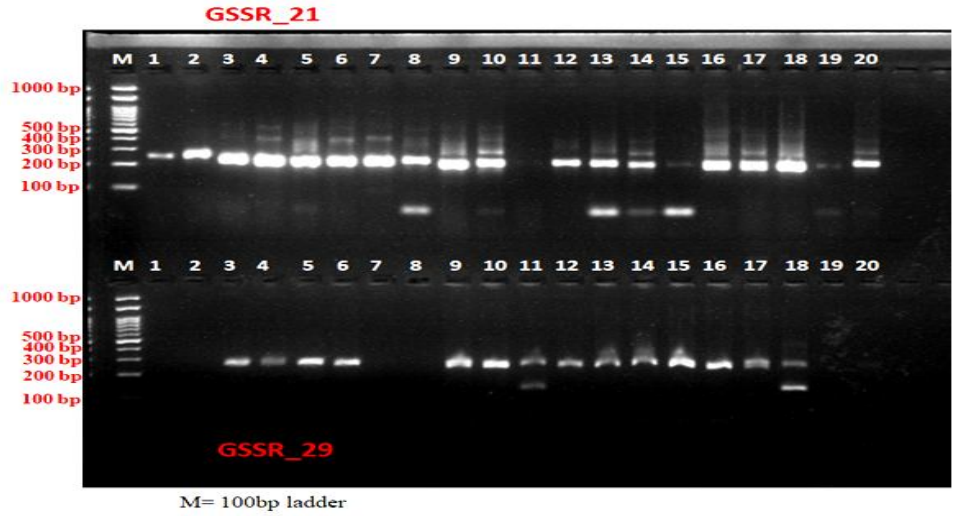
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	**84.55**	185.46	398.76	373.13	456.42
Cluster II		**115.63**	160.43	527.49	341.27
Cluster III			**0**	651.35	247.41
Cluster IV				**94.75**	225.56
Cluster V					**0**

Table 5: Mean value of 12 characters for 5 clusters formed by 20 genotypes in fennel

SI. No	Characters	Clusters				
		I	II	III	IV	V
1	Plant height (cm)	154.66	147.88	148.97	157.10	146.48
2	Branches per plant (No's)	15.01	14.80	14.20	15.00	15.00
3	Days to first flowering (Days)	76.50	76.67	76.00	77.00	74.00
4	Days to fifty per cent flowering (Days)	92.07	89.94	93.00	90.75	89.50
5	Days to maturity (Days)	139.79	140.33	136.00	135.25	138.50
6	Number of umbels per plant	19.20	21.03	18.60	24.55	23.80
7	Number of umbellate per umbel	24.11	24.23	16.10	30.05	25.00
8	Number of flowers per umbellate	22.56	22.83	18.00	26.80	24.00
9	Seeds per umbel	683.99	647.44	645.10	782.90	673.00
10	Harvest index (%)	15.08	14.66	16.17	21.08	19.18
11	Test weight (g)	8.53	8.78	9.80	9.88	9.80
12	Seed yield per plant (g)	17.14	15.79	17.72	29.04	27.77
13	Seed yield per plot (g)	793.39	652.24	567.32	873.54	649.25

Table 6: Particulars of SSR primers used in the study

Locus	Total bands	Number of polymorphic bands	Polymorphism (%)	PIC
GSSR 11	4	3	75	0.322
GSSR 14	14	6	42.85	0.729
GSSR 21	17	4	23.52	0.740
GSSR 26	16	3	18.75	0.417
GSSR 27	18	4	22.22	0.649
GSSR 29	14	4	28.57	0.680
FSSR 8	19	5	26.31	0.678
FSSR 9	16	5	31.25	0.686
ESSR 1	17	4	23.52	0.416
ESSR 5	16	3	18.75	0.526
ESSR 10	10	4	40	0.611
Total	161	45	-	-



M= 100bp ladder

Figure 3: SSR gel profile of fennel genotypes generated by primer GSSR_21 and GSSR_29.

4. Conclusion

Fennel being a cross-pollinated crop is subjected to population improvement strategies following mass selection or recurrent selection in general. Genetic improvement for yield is the basic objective of fennel breeding like all other crops. Genetic diversity analysis helps in identifying diverse genotypes and also to group genotypes showing genotyping similarity for target traits.

SSR markers studied in fennel genotypes showed high genetic variability and these are considered as important molecular tool for estimating genetic diversity and similarities. The genetic relationships presented among the genotypes are helpful for future breeding programs (hybridization) through selection of genetically diverse parents. The results indicated from the present study can be helpful in selection, marker assisted selection (MAS) and crop improvement of fennel genotypes.

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