

Application of SCoT Marker to Discriminate Genotypes and Synthetic Cultivars of Fennel (*Foeniculum Vulgare* Mill.)

Azam Akbari (✉ Aakbari91@ut.ac.ir)

Tehran University: University of Tehran

Ali Izadi Darbandi

Tehran University: University of Tehran

Hosein Ramshini

Tehran University: University of Tehran

Keivan Bahmani

Tehran University: University of Tehran

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Abstract

Fennel is a member of the Umbelliferae family and one of the most important and commonly used medicinal herbs. Because plant cultivar registration and protection is now confined to a small number of morphological features, the adoption of molecular approaches as complementing tools is unavoidable. In this investigation, the researchers employed ten start codon targeted (SCoT) markers to identify and distinctiveness three synthetic fennel cultivars and eight parental ecotypes. Ten SCoT primers obtained a total of 54 amplified fragments, of which 44 were polymorphic. SC14 and SC2 primers with 9 bands had the most bands, whereas SC17 primers with 5 bands had the least bands. SC29, SC31, and SC2 primers have the greatest polymorphic information content (PIC), Resolving Power (RP), and Marking Index (MI). The genetic similarity of the genotypes analyzed using the Jaccard similarity coefficient varied from 0.31 to 0.76, with an average similarity of 0.54. Genotypes were distinct from one another and split into five categories using cluster analysis, the results of primary coordinate analysis (PCoA) corroborated these findings. These markers proved to be valuable tools for identifying and distinctiveness fennel cultivars due to their good separation of cultivars and independence from environmental effects. As a result, the markers utilized in this research are appropriate for distinction fennel cultivars.

1. Introduction

According to the World Health Organization, medical plants are responsible for the health of more than 80% of the world's population in developing nations, while the usage of chemical compounds derived from medicinal plants is expanding in industrialized countries (Canter et al., 2005). Fennel is a herbal, perennial, fragrant plant with hermaphrodite flowers and open pollination that is native to the Mediterranean region (Al-Dalam et al., 2012). The seed of the fennel plant is the commercial portion, which is used either directly or to extract essential oils. Food, perfume, and beverage sectors all employ fennel seeds (Barros et al., 2010). Fennel essential oil has been demonstrated to have antioxidant, anti-cancer, antibacterial, antifungal, and analgesic effects in pharmacological investigations (Diao et al., 2014; El-Awadi et al., 2010). The genetic nature of this plant is heterozygous-heterogeneous, and its development is such that it is adversely affected by autogamy. Breeding methods for this plant should be such to minimize autogamy and purity, and in this case, it is possible to produce hybrid or synthetic cultivars (Nemat Zadeh and Kiani, 2005). Synthetic cultivars have various advantages over hybrid approaches, including less requirement for precise pollination control, higher yield of synthetic cultivars under changing environmental circumstances, cheap cost of synthetic seed manufacturing, and high genetic diversity of synthetic cultivars (Dashora et al., 2003). Because pollination in fennel flowers is difficult to manage and each crossbreeding results in the creation of two seeds, the approach of improving synthetic cultivars appears feasible. New plant cultivars must be identified and detected to be used and protected effectively. The DUS test of distinction, uniformity, and stability, performed by the International Union for the Protection of New Varieties of Plants criteria, can be used to identify and register the cultivar (Van Gastel, 1996)

Morphological features are often utilized for the DUS test of plant cultivars, however, they are limited in number and have an environmental impact, which might pose severe challenges in the future when it comes to showing the distinctiveness of new plants cultivars. It is expected that if the morphophysiological

features of the DUS test are unable to detect cultivar distinctiveness, molecular markers will be utilized as supplementary descriptors to verify distinctiveness. The working group on biochemical and molecular techniques and DNA profiling (BMT), which works under the auspices of the technical committee of the International Union for the Protection of New Plant Cultures (UPOV), has studied the use of molecular markers in the type test, based on their benefits. These markers, particularly DNA markers, offer several benefits over morphological features, including the lack of effect of environmental factors, the capacity to be examined at any stage of plant growth, genomic frequency, and random distribution in the genome (UPOV, 2013).

DNA-based genetic markers, which are widely employed in species identification, genetic mapping, and seed certification, are one of the most commonly utilized molecular techniques. These markers can also be used to find genes or alleles linked to plant economic traits (Grattapaglia et al., 2009). Because of their low environmental impact and high inheritance, DNA markers are effective instruments for measuring genetic diversity and understanding the properties of genetic resources. These markers provide information directly based on changes in the genome and are often more polymorphic than other markers. As a result, they are the most extensively utilized method for calculating genetic distance (Rout and Aparajita, 2010). The SCoT marker, which is based on short protected areas around the start codon, is the most common (ATG) (Collard et al., 2009; Xiong et al., 2011). This indicator's system is dominating, in a polymerase chain reaction, two single-strand primers are connected in opposing directions to opposite strands of template DNA, and the distance between them is exponentially increased by the polymerase enzyme in the PCR reaction. There will be no primer binding and no DNA fragment synthesis if deletion or addition mutations occur at the primer binding site (Davis et al., 1995). SCoT markers are more reproducible than ISSR and RAPD markers, and it's thought that the length and temperature of the primer aren't the only element influencing their banding pattern reproducible (Collard et al., 2009).

The present research aimed to discover the molecular identification of three high-yielding synthetic fennel cultivars (early, medium, and late) and investigate the possibility of the distinctiveness of new fennel cultivars and parental ecotypes

2. Materials And Methods

This research was conducted in 2021 at the College of Aburaihan, University of Tehran.

2.1. Plants material

Genotypes for this study were; the second generation of synthetic cultivars (Syn2) along with some of their elite parents, Early maturity synthetic with two parental populations (Fasa and Hashtgerd), Medium maturity synthetic with three parental populations (Fozve, Khash, Meshkinshahr), and Late maturity synthetic with two parental populations (Hajiabad and Chahestan) (Table 1).

2.2. DNA extraction

Genomic DNA extraction from young fennel leaves was obtained based on the CTAB method with slight modifications (Murray et al., 1980). A spectrophotometer and loading on a 1% agarose gel were used to assess the amount and quality of the isolated DNA. DNA samples were kept at -20°C until the polymerase chain reaction was performed (PCR). For polymerase chain reactions, the final concentration of DNA samples was diluted using Tris-EDTA buffer at a concentration of 20-40 ng/μl.

2.3. PCR and Electrophoresis

The 10 SCoT primers used in this investigation were chosen based on the primers that had the most polymorphism in previous studies (Joshi et al., 1997; Sawant et al., 1999) (Table 2). The amplification reactions were carried out in a final volume of 20 μl using 10 μl of master mix (2X amplicon), 2 μl of primer (10 M), 2 μl of genomic DNA, and 6 μl of deionized water after adjusting the amplification conditions. The following conditions are used to amplify PCR: Initial denaturation at 94°C for 4 minutes, followed by 35 cycles including DNA denaturation at 94°C for 1 minute and DNA annealing 40-60 C for 1 minute, then extension 72 ° C for 2 minutes and a final extension at 72 ° C for five minutes. The PCR product was separated for 1 hour at 80 ° C in a 1.5 percent agarose gel with TAE buffer, then viewed and scored after staining in ethidium bromide under UV light.

2.4. Statistical analysis of data

Only clearly visible bands were scored when amplified bands from primers were recorded. Each band in dominant markers like SCoT reflects a dual-locus phenotype. Each allele amplified with the primers was scored as 1 for the presence or 0 for absence. The number of total bands (TB), the number of polymorphic bands (PB), and the percentage of polymorphism (% PL) were determined. Based on the formula of Powell et al., (1996) marker performance measures such as polymorphic information content (PIC), marker index (MI), and resolving power (RP) were calculated.

The Jaccard similarity coefficient was used to assess genetic similarities between genotypes, The UPGMA technique was used for cluster analysis, principal component analysis (PCA) was constructed using the NTSYS-PC program, Version 2.02.

3. Results

Eight of the SCoT primers were able to amplify the band in the examined fennel genotypes, but two of the primers, SC5 and SC12 were unable to amplify any alleles. SCoT primers generated a total of 54 amplified fragments, of which 44 were polymorphic. For 11 genotypes, the mean number of bands generated by each primer was 6.75. SCoT primers amplified 391 sites in all, with a mean of 48.78 for eight primers. SC2 and SC14 primers with 9 bands had the most number of bands, whereas SC17 primers with 5 bands had the least number of bands. Among the genotypes investigated, the Meshkinshahr had the greatest band (38) while the Chahestan had the lowest band (27). The banding pattern of 11 genotypes examined using the SC14 primer is shown in Figure 1. Table 2 shows the findings for the primers that were utilized. The mean number of polymorphisms in the primers tested was 81 percent. Two primers demonstrated 100% polymorphism, the lowest percentages of polymorphisms were found in SC32 (67%), SC30 (67%), and SC26 (67%) (Table 3).

The primers SC29 and SC31 have the most polymorphic information content (PIC), the SC26 primer has the lowest polymorphic information content (PIC). The PIC index is one of the most important measures for determining a marking system's success. In reality, this score is based on the number of detectable alleles and their frequency, and it reveals whether or not polymorphisms induced by a primer can be detected between two samples (Powell et al. 1996). The effectiveness of a molecular marker is determined by the quantity of polymorphism induced and the subjects' distinctiveness capacity; in dominant markers, primers with a PIC value of 0.25 to 0.5 are useful, and the data acquired from them is reliable in diversity research (Botstein et al., 1980). The mean of resolving power (RP) was 2.955, with the greatest value being SC2 and SC29 primers and the lowest value being SC26 and SC30 primers. In addition, the mean of the Marker Index (MI) was 1.475. The greatest marker index (MI) was found in SC2 and SC29 primers, while the lowest was found in SC17 and SC26 primers.

Using the Jaccard similarity coefficient, the genetic similarity of the examined genotypes varied from 0.31 to 0.76, the mean similarity across genotypes was 0.54, indicating that there is sufficient variation among fennel genotypes (Table 4). Fasa and Moghan genotypes were the most similar, whereas Hashtgerd and Chahestan genotypes were the least closes.

Cluster analysis and principal coordinate analysis (PcoA) were used to evaluate genetic linkages between the fennel genotypes. Cluster analysis is a powerful and useful way to evaluate the genetic linkages of individuals and genotypes (Randi and Lucchini, 2002). Cluster analysis of fennel genotypes using Jaccard similarity coefficients and the UPGMA technique was able to segregate all genotypes except Moghan and Meshkinshahr, and split synthetic cultivars and parental genotypes into five groups (Figure 2). And the dendrogram that was created has a coefficient of 0.86, a coefficient greater than 0.8 indicates that the drawn dendrogram is well-fit. Only Hashtgerd genotype and Chahestan genotype were found in groups one and five. The second group included late synthetic cultivar and Fasa. The third group included early maturity synthetic and Hajiabad genotype. Medium maturity synthetic and its parental genotypes were put in a fourth group, which separated early and late synthetic cultivars from their parents into different groupings. The enormous variety of this plant, as well as many other variables such as strong gene flow, mutations, and natural selection, might be blamed for this mismatch.

Principal coordinate analysis

the principal coordinate analysis using data from SCoT data showed that a large number of components can be introduced. In this analysis, the first 3 components represent more than 53% of the total variance. The first component, which justified the most variability among the components, had a contribution of 21.96 percent in total variance.

Figure (3) shows a genotype distribution diagram based on PCO1 and PCO2 components, which corresponded to the cluster analysis results, and the genotypes were divided into five groups.

4. Discussion

Because morphological and physiological traits are restricted, it is difficult to reliably identify similar varieties in plant species using only morphological features (Giancola et al., 2002; Rongwen et al., 1995). As a result, the inclusion of molecular markers as extra information in the registration of plant cultivars is unavoidable. DNA markers can be used to identify species quickly and easily or to confirm the distinctiveness of a variety (Collard et al., 2008). The SCoT marker was utilized to assess the distinctiveness between three synthetic fennel cultivars and their parental genotypes in the current investigation. As can be shown using the SCoT marker, there was a lot of variation across genotypes, and this marker revealed a lot of beneficial polymorphism. And this marker was able to tell the distinctiveness between three synthetic digits and their parents. Bahmani et al., (2013) stated that 1,043 polymorphic DNA fragments were obtained in research on 25 fennel from all across Iran to evaluate their genetic diversity using 10 RAPD markers. The UPGMA algorithm was used to categorize these accessions based on RAPD primer information, and the accessions were classified into ten groups. according to this study Iranian fennel has high genetic diversity, and the RAPD marker distinguished fennel ecotypes well based on geographical distribution and climatic similarities. Polymorphic information content (PIC) in dominant markers ranged from 0 to 0.5, the higher the number, the more polymorphisms for that site in the genotypes under investigation. The Rsolving Power (RP), which influences both the number of bands and the number of alleles, is the greatest indication for selecting the proper primer. According to the findings, the SC29, SC31, and SC2 primers, which exhibited high rsolving power (RP) and polymorphism index (PIC), should be employed in a future study to investigate the germplasm set of additional fennel genotypes.

5. Conclusion

Advances in molecular marker technology and data analysis have led to identifying and evaluating germplasm, as well as selecting parents in breeding programs. Another application of molecular markers is their use in distinctiveness, uniformity, and stability test. These markers, particularly DNA markers, offer several benefits over morphological features, including the lack of environmental conditions, the capacity to be examined at any stage of plant growth, genomic frequency, and random distribution in the genome. The high mean content of polymorphic information (PIC) and marker index (MI) of the primers employed in this investigation validated the efficiency and distinctiveness capacity of SCoT markers. This marker is an effective and strong technique for exploring genetic links between synthetic cultivars and fennel genotypes due to the high degree of polymorphism. If molecular markers are taken as extra descriptors, the results of this study give adequate data to suggest that they raise DUS test standards. Furthermore, introducing them can have several advantages, Because of the proper separation of cultivars and their independence from the effects of the environment, the markers utilized in the experiment proved to be valuable tools for identifying and distinction fennel cultivars.

Declarations

Acknowledgment

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Conflict of interest

This manuscript has not been published or presented elsewhere in part or entirety and is not under consideration by another journal. There are no conflicts of interest to declare.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors

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Tables

Table 1 Fennel cultivars used in the experiment

Synthetic cultivars	Parents of synthetic cultivars	Genotype	Altitude (m)	Latitude	Longitude
Early maturity synthetic	Parents of Early maturity synthetic	Fasa	1288	28 58 N	53 41 E
		Hashtgerd	1426	35 65 N	50 43 E
Medium maturity synthetic	Parents of Medium maturity synthetic	Moghan	31	39 39 N	47 55 E
		Meshkinshahr	1568	38 23 N	47 40 E
		Fozveh	1612	32 36 N	51 26 E
		khash	1394	28 13 N	61 12 E
Late maturity synthetic	Parents of Late maturity synthetic	Hajiabad	931	28 19 N	55 55 E
		Chahestan	27	27 13 N	56 22 E

Table 2 SCoT primers used for analysis of synthetic varieties and parental

	Primer code	Sequence (5'-3')	GC %
1	SC2	CAA CAA TGG CTA CCA CCC	60%
2	SC14	ACG ACA TGG CGA CCA CGC	70%
3	SC17	ACC ATG GCT ACC ACC GAG	70%
4	SC24	CAC CAT GGC TAC CAC CAT	60%
5	SC27	ACC ATG GCT ACC ACC GTC	70%
6	SC29	CAA TGG CTA CCA CCG GCC	70%
7	SC30	CCA TGG CTA CCA CCG GCG	70%
8	SC31	CCA TGG CTA CCA CCG CCT	60%

Table 3 Amplification results of SCoT primers used in of synthetic varieties and parental

Primer	Total bands	Monomorphic bands	Polymorphic bands	%Polymorphism	PIC	RP	MI
SCoT2	9	1	8	89%	0.331	4.727	2.354
SCoT14	9	2	7	78%	0.279	3.818	1.524
SCoT17	5	1	4	80%	0.284	2.000	0.910
SCoT26	6	2	4	67%	0.171	1.273	0.458
SCoT29	7	0	7	100%	0.397	4.364	2.777
SCoT30	6	2	4	67%	0.220	1.818	0.591
SCoT31	6	0	6	100%	0.358	3.273	2.149
SCoT32	6	2	4	67%	0.259	2.364	1.041

Table 4 Similarity matrix of of synthetic varieties and parental based on SCoT markers

		1	2	3	4	5	6	7	8	9	10	11
1	Chahestan	1										
2	Khash	0.67	1									
3	Fasa	0.66	0.65	1								
4	Hashtgerd	0.31	0.41	0.38	1							
5	Meshkinshahr	0.58	0.68	0.63	0.43	1						
6	Hajiabad	0.57	0.61	0.59	0.43	0.67	1					
7	Fozveh	0.42	0.59	0.54	0.50	0.72	0.68	1				
8	Moghan	0.60	0.71	0.55	0.35	0.76	0.62	0.64	1			
9	syn1	0.60	0.60	0.54	0.54	0.66	0.75	0.60	0.61	1		
10	syn2	0.58	0.42	0.52	0.54	0.59	0.61	0.49	0.49	0.61	1	
11	syn3	0.50	0.60	0.70	0.44	0.73	0.71	0.47	0.55	0.65	0.71	1

Figures

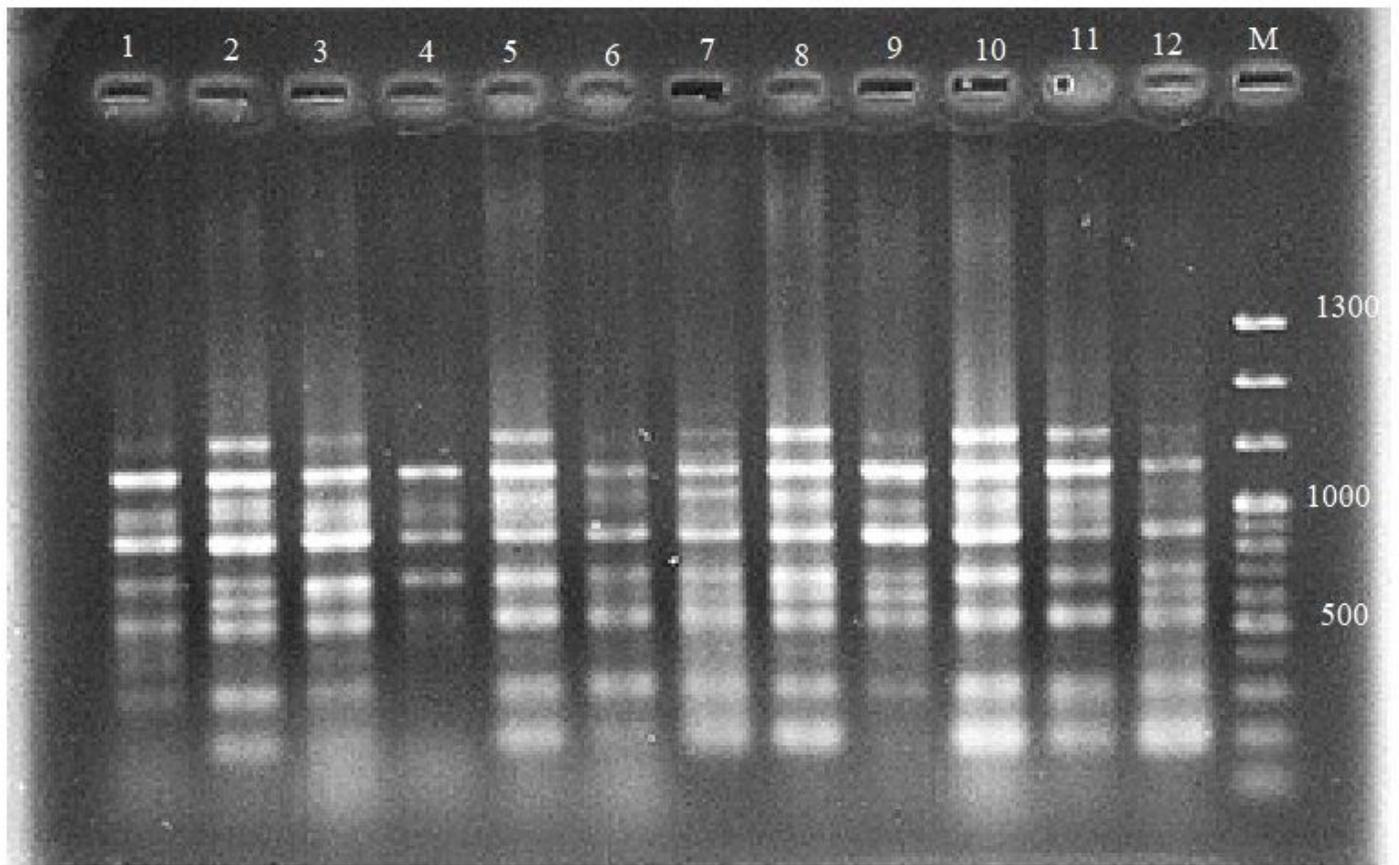


Figure 1

SC14 primer band pattern in fennel genotypes

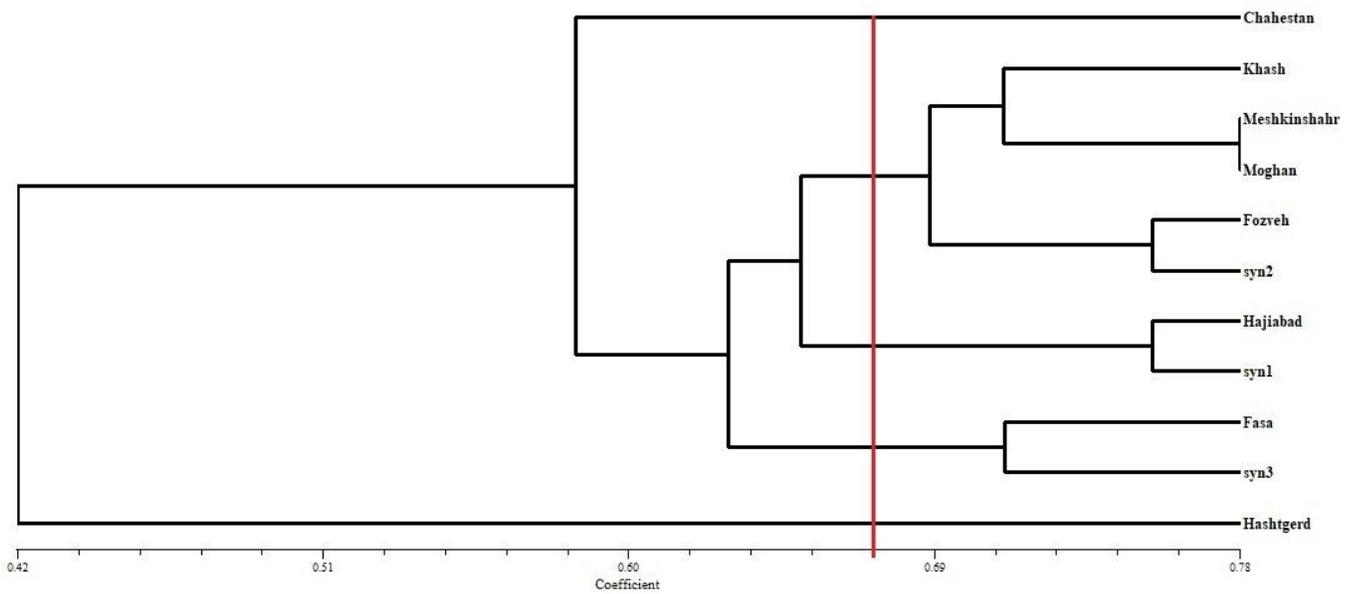


Figure 2

Cluster analysis using UPGMA method for fennel genotypes

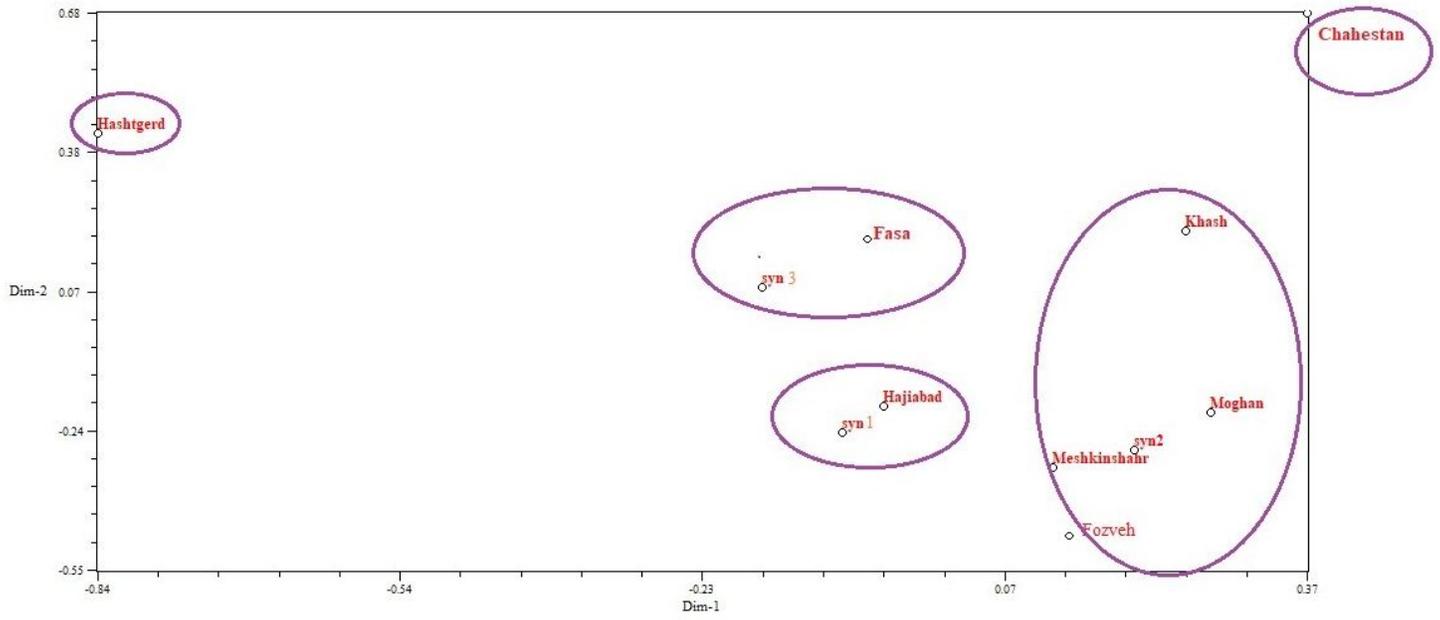


Figure 3

Biplot diagrams of PCoA for fennel genotypes