Universal Rice Primer (URP) and Start Codon Target (SCoT) Markers in Studying Population Structures and Genetic Variation in *Ferula Assafoetida* L. Accessions

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Abstract

Ferula assafoetida is an herbaceous, annual and monocarpic genus of the Apiaceae family. So far, there has been common usage of Ferula oleo resinous gum in food and herbal medicines. The origin of *F. assafoetida* is can be traced back into the steppes of Iran and some reigns of Afghanistan with an extended distribution. Despite the economic value and therapeutic importance of *F. assafoetida*, only a few studies have reported on the genetic capacity of this herb. The present study was carried out on a set of 90 individual plants belonging to different populations of *Ferula assafoetida* L. via the start codon target marker (SCoT) and the universal rice primer (URP) markers. Twelve SCoT and twelve URP primers generated 192 and 149 polymorphic fragments, while having 16 and 12.41 fragments respectively on average per primer. The Polymorphism information content (PIC) for URP primers and ScoT ranged from 0.31 to 0.43 and 0.34 to 0.44 respectively, which indicated a good efficiency for both markers. The diversity indices including heterozygosity (He), percentage of polymorphic bands (PPB), Shannon's information index (I) and marker index (MI) were calculated based on the SCoT and URP data. The results revealed that SCoT primers were more efficient than URP primers in identifying genetic diversity within populations. Neighbor joining (Nj), as a base for clustering, classified 90 accessions into 5 and 6 groups using SCoT and URP data respectively. Moreover, the combined data (SCoT+URP) succeeded in classifying all accessions into 6 groups, although this did not correspond with the geographical distribution of accessions. Structure analysis divided 90 genotypes into 5 subpopulations using SCoT and URP markers, whereas the combined data (SCoT+URP) divided the accessions into 6 subpopulations, which confirmed the classification achieved by the Nj method. Principal coordinate analysis (PCoA) corroborated these conclusions. According to the analysis of molecular variance) AMOVA(, a high percentage of genetic diversity was found within the species, suggesting a rich diversity of germplasm for breeding plans. The assessment of population structure demonstrated a high rate of gene flow and the mixture of populations. Taken together, our findings suggest SCoT markers are more efficient than URP markers in assessing genetic diversity among *F. assafoetida* genotypes. All in all, genetic diversity could help the selection of appropriate markers and special genotypes for breeding plans.

Highlights

1. The results demonstrated that the major portion of genetic variation occurred within populations (91% based on SCoT markers, 88% based on URP markers and 90% based on pooled data. The findings revealed that molecular variance was much higher within populations than among populations.

2. SCoT, URP and SCoT+URP cluster analysis suggesting, genotypes that are related to different geographical areas and sometimes far apart are located in close branches. These results indicated a very high degree of gene migration among populations.

3. The analysis demonstrated substantial repeatability and high polymorphism among SCoT markers.

4. SCoT and URP mechanisms were found to be the most effective methods for assessing genetic variation among F.assafoetida genotypes

Introduction

Asafoetida (Heeng) is a well-known herb in the traditions of India, Pakistan and Iran. It is sometimes used in foods as a condiment.The genus Ferula occurs in the Umbelifera family and consists of 130 species worldwide. Thirty of these occur in Iran, 15 of which are endemic and the rest are from the Mediterranean region and central Asia (Bahramia et al. 2013). *F.assafoetida* is endemic to the Iranian steppes in particular and to a few parts of Afghanistan (Ross 2007; Leaman 2006). In Iran, two bitter and sweet varieties have been recognized. The sweet type occurs in the highlands and the bitter in the lowlands. *Ferula assafoetida* grows wildly in the central and southern mountains of Iran (Amalraj and Gopi 2017). The main habitats of this plant are the central Iranian plateau and desert regions, encompassing the Zagros Mountains and southern Alborz, while alsooccurring in Fars, Kerman, Khorasan, Yazd, Semnan, Hormozgan, Sistan and Baluchestan, Isfahan, Lorestan, Kohkiluyeh Boyer Ahmad and Bushehr provinces. The only way to regenerate Asafoetida is through the production and distribution of seeds. Each flowering stem of Asafoetida produces a lot of seeds in umbrella-like clusters. Asafoetida seeds are light and can be scattered well by the wind. Asafoetida seed length is 9 to 12 mm and its width is 5 to 7.5 mm. Its 1000-seed weight is 12.5 grams, and its hectoliter weight is 196.4 grams per cubic centimeter (Zare et al. 2011). Since this plant is a valuable source of income for a large number of villagers and farmers in Iran, local people collect the seeds and sprinkle it on land in pastures. This act is supported by organizations which safeguard biodiversity not only to avoid its extinction but also to allow sensible harvests from its populations (Zare et al. 2011).

The plant Asafoetida (*Ferula assafoetida L*.) is used to make dried latex (gum oleo resin), which is exuded from the rhizome and stems of this plant. In Iran, Asafoetidaoleo gum resin is locally called Khorakoma, Anghouzeh or Anguzakoma (Iranshahy and Iranshahi 2011). Asafetida has 3 primary parts, i.e. gum (25%), resin (40–64%) and essential oil (10–17%) (Takeoka 2001). Incisions on the roots or the cutting of the plant stems are the most common methods to obtain oleo-gum-resin. Exudates (oleo-gum-resin) are usually left to dry, are processed and then prepared for export. Assafoetida occurs in two principal forms, mass and tears. The mass type is the most popular form on the market, however (Upadhyay 2017). As a rangeland plant,it is important because of the substances that are extracted from the roots of this plant, i.e. compounds which have medicinal and pharmaceutical properties (Iranshahy and Iranshahi 2011(. For millennia, Assafoetida has been used to cure a wide variety of ailments, including urinary, gastrointestinal, and respiratory diseases, epilepsy, asthma, stomachache, flatulence, intestinal parasite, poor metabolism, and influenza, as well as an aphrodisiac, an emmenagogue and to treat snake and insect bites, although the best documented folk usage has been on intestinal worm infections. Antiviral (HSV, HRV, H1N1, HIV), antispasmodic, hypotensive and anti-diabetic behaviors of Assafoetida have resulted from several trials (Bahramia et al. 2013; Iranshahy and Iranshahi 2011). In Iranian traditional medicine, *F.assafoetida* is considered to be sedative, analgesic, carminative, antispasmodic, diuretic, anthelmintic, expectorant digestive, expectorant, laxative and aphrodisiac (Bagheri et al. 2011; Sadraei et al. 2003; Khajeh et al. 2004). Iran has a wide variety of natural ecosystems that make it one of the world's most diverse sources of plant genetic resources. Its wide range of diversity illustrates its significant capacity in selection, conservation and utilization of genetic resources. While this herb is widely spread in

Iran, the extent of its genetic diversity remains a question. Relevant findings in this area can pave the way for germplasm management initiatives and changes in plant characteristics (Kalia et al. 2014). Molecular markers that are DNA-based happen to be the most useful means of describing genetic variance. Different parameters exist for molecular marker methods and assist in structural analysis as well as in the study of genetic variation. In this context, molecular marker approaches have improved through time and developed further capacities to better determine genetic diversity, population composition and phylogenetic relationships. Advances in genomic tools have produced a wide variety of new marker approaches in recent years, including several new DNA-based marker systems and gene-targeted markers (Poczai et al. 2013). DNA markers are also commonly used as a valid way of measuring genetic variation (Rahimmalek et al. 2009).

SCoT (Start Codon Targeted) and URP (Universal rice Primers) are gene-targeted markers that are starting to replace others among the different PCR-based DNA markers. They are considered to have several advantages such as their degree of durability, informativeness, high polymorphism and low cost. The Start Codon Targeted (SCoT) polymorphism mechanism is known for its accuracy and simplicity. Its translation initiation codononboth DNA strands (ATG) comprises a short conserved region (Collard and Mackill 2009). SCoT markers are typically repeatable, polymorphic, and cost-effective. This method has been successfully used in establishing the genetic association of several species of plants, including cumin, due to its numerous advantages. The SCoT primer has been reported in many molecular studies of various plants such as fennel, orchid, coconut, *F. assafetida* and durum wheat (Yadav and Malik 2016; Bhattacharyya et al. 2013; Feng et al. 2015; Rajesh et al. 2015; Tajbakht et al. 2018; Etminan et al. 2016, 2018a; Pour-Aboughadareh et al. 2017, 2018). Different genomes of plants, animals and microbes can be fingerprinted conveniently by this marker, for instance, in Korean weedy rice, specifically via a universal rice primer (URP) (Kang et al. 2002). The URP marker has also gauged genetic diversity in Vigna species Dikshit et al. (2007) and it could be used on many other species as well. Although Iranian *F.assafoetida* has been studied by RAPD markers Sarhaddipour et al. (2014), there have been no efforts so far to identify the genetic diversity of this species by targeting specific regions of the genome via gene-based markers (Tajbakht et al. 2018). The purpose of this research was to consider the application of URP and SCoT markers in order to identify the genetic composition of naturally-occurring genotypes in different geographical regions of Iran. This research also aimed to analyze the genetic relationship between and within the Iranian *F. assafoetida* populations. To the best of our knowledge, this is the first time that URP and SCoT markers are studied for applications on the genetic diversity of *F. assafoetida*.

Materials And Methods

Plant materials and DNA extraction

Ninety *F. Assafoetida* accessions were collected as representatives of intra-species diversity. These were from 30 populations that originated in different eco-geographical habitats among mountainous regions of Iran. Sample collection occurred in September and December. The sample position and geographical coordinates are shown in Fig. 1. After seed germination and development, a total genomic DNA of 90

ferula genotypes was extracted from young leaves. A modified CTAB protocol was used for isolating genomic DNA. The consistency and quantity of DNA were measured using an electrophoresis of agarose gel (0.8%).

SCoT-PCR amplification

In this study, 12 primers were designed for the SCoT analysis, according to Collard and Machill)Table 1). A total reaction volume of 20µl SCoT-PCR amplifications were performed in a total reaction volume of 20µl, including 6.5 µl double distilled water, 10µl master mix 2XPCR (ready to use PCR master mix 2X; Ampliqon) and2 µlthe template DNA and 1.5 µl of each primer. PCR amplification occurred by having pre denaturation at 94°C for 5 min, followed by 34 cycles of 94°C for 1 min, annealing at 48.9–55°C (different for each primer) for 45 seconds and extension at 72°C for 1.5 min. The final extension occurred for 5 min at 72°C. Eight temperature gradients were used for the amplification process of the PCR(i.e. 48°C, 49°C, 50°C, 50.5°C, 52°C, 53.5°C, 54°C, and 55°C). A specific annealing temperature was identified for each of the 12 primers. PCR products were detected bya 1.5% agarose gel. They were photographed under UV light after being stained. The amplified SCoT fragment was scored as 1 (for the presence) or 0 (absence) of the bands.

URP- PCR amplification

For URP analysis,12 primers were designed for URP-PCR amplifications which involved a total reaction volume of 20µl, including 6.5µl double distilled water,1.5µl of each primer, 2µl of the template DNA and 10µl of the master mix 2XPCR.URP PCR amplification involved predenaturation at 95°C for 10 min, then 34 cycles of denaturation at 95°C for 1 min, annealing at 42–48°C (different for each primer) for 45 seconds and extension at 72°C. The final extension occurred at 72°C for 7 minutes. A gradient of seven temperatures (i.e. 42°C, 43°C, 44°C, 45°C, 46°C, 47°C and 48°C) was considered when amplifying for the PCR. A specific annealing temperature was identified for each of the 12 primers. PCR products were detected by a 1.5% agarose gel. They were photographed under UV light after being stained. The amplified SCoT fragment was scored as 1 (for the presence) or 0 (absence) of the bands)Table 1).

Data analysis

PCR products were scored independently in SCoT and the URP profiles were scored as absent (0) or present (1) in each position. For the assessment of genetic diversity,a binary matrix included six informative indices to evaluate the discriminatory power of the primers. This included total amplified bands (TAB), percentage of polymorphism (PPB), a number of polymorphic bands (NPB), resolving power (Rp), polymorphism information content and marker Index (MI). The SCoT and URP were computed using the formula PIC = $1\sum_{j=1}^{n} \text{Pij}^2$where Pij is the frequency of the jth allele (marker) for the SCoT and URP markers (Botstein et al. 1980). Genetic diversity was evaluated via molecular variance within and among populations (AMOVA). Principal component analysis (PCoA) was performed using GenAlEx 6.41 software (Peakall and Smouse 2006). AMOVA estimated the genetic variance and described the pattern of genetic variation in the natural populations of *F. assafoetida.* PCoA revealed natural genetic clusters among the

populations and individuals (Peakall and Smouse 2012). Several indices were used for identifying genetic variation, i.e.percentage of polymorphic loci (PPL), the observed number of alleles (Na), Nei's gene diversity (H) Nei (1973) and effective number of alleles (Ne), inter-population differentiation (Gst), Shannon's information index (I) Lewonton (1972) and the number of population migrants per generation, as a representation of gene flow level (Nm). Accordingly, the procedure involved using the formula Nm= (0.5 (1− Gst)/ Gst), where Gst is the diversity among populations. The diversity was measured by the POPGENE program, ver. 1.32 (Yeh et al. 1997; McDermott and McDonald 1993).

Genetic dissimilarities were described according to Jaccard's coefficient Jaccard (1908) and using DARwin ver.6 software (Perrier et al. 2003). The Neighbor Joining (NJ) fandendrogram was made to explain the relationship among separate populations using MEGA ver. 10.1.8 (Tamura et al. 2011). Bayesian clustering trends were carried out for the 90 genotypes, using STRUCTURE program version 2.3.4 (Pritchard et al. 2000). A continuous sequence of K was checked from 2 to 12 in 10 different runs. The initial burn-in time operated at 50,000 for each run, and then 50,000 Markov Chain Monte Carlo (MCMC) iterations. Finally, an online accessible program STRUCTURE HARVESTER was used for determining the K for the final population structure (Earl and von Holdt,2012;http://taylor0.biology.ucla.edu).

Results

SCoT and URP polymorphism

In the present analysis, 24 SCoT and URP primers were used for estimating the genetic diversity of *F. assafoetida*. Anoverview of the calculation of the URP and SCoT primers information can be outlined according to relevant parameters (Table 1). All of the selected SCoT primers managed to amplify 192 polymorphic fragments among the 90 accessions. Twelve primers were screened while evaluating the genetic variations among and within a small collection of *F.assafoetida* genotypes. The total number of amplified bands (TAB) ranged between 13 (SCoT-10, SCoT-12) and 19 (SCoT-7) with an average of 16 per primer. PIC varied between 0.34 and 0.44 among the 12 primers, while the highest value was attributed to 3 primers (i.e. SCoT-1, SCoT-7, SCoT-9). The resolving power (Rp) occurred between 7.13 (SCoT-11) and 12.22 (SCoT-7) with a mean value of 9.87 per primer. High levels of variation occurred in the marker index (MI) among the primers, whereas the lowest values were recorded for SCoT-7 (7.98) and SCoT-10 (4.32). An average value of 6.18 was calculated for all primers. The12 URP primers amplified 149 fragments among the 90 accessions, all of which were polymorphic. Polymorphic fragments ranged between a minimum of 10 in URP-7, URP-8, URP-9 and a maximum of 18 in URP-6. The PIC ranged between 0.13 and 0.43, with an average value of 0.36 per primer. URP-11 and URP-4 acquired the highest values in the PIC measure. The average value of Rp for the URP was 6.95 and the highest value occurred in the URP-6. The MI parameter varied from 2.79 (URP-1) to 6.84 (URP-11) with an average value of 4.45. The amplification facilitated the analysis of the 90 accessions by SCoT and URP primers. The analysis demonstrated substantial repeatability and high polymorphism among SCoT markers.

Genetic variationand diversity analysis

The results of AMOVA demonstrated that the major portion of genetic variation occurred within populations (91%based on SCoT markers, 88% based on URP markers and 90% based on pooled data (Table 2). The findings revealed thatmolecular variance (%) was much higher within populations than among populations (SCoT = 9%, URP = 12%, pooled data = 10%). Gene flow (Nm) and inter-population differentiation (Gst) confirmed this finding. The genetic differentiation coefficient of (Gst)/gene flow for URP (Nm), SCoTand pooled data were 0.19/2.03, 0.15/2.81 and 0.17/2.38, respectively (Table 2). This indicated that the estimated variance within genotypes was greater than among them. Using URP primers, the highest values of Nei's gene diversity (H = 0.40), Shannon's information (I = 0.58) and polymorphic loci (%) (PPL = 98.66) were recorded in the Kerman population, whereas the lowest of these values (H = 0.20, I = 0.29 and PPL = 50.52) were recorded in the Yazd population and for SCoT primers (Table 3). The effective (Ne), total (Na) and private (PA) number of alleles ranged from 1.35–1.73 to 1.17–1.97 and 0–2, respectively. The Kerman population had private bands in URP and pooled data only (URP + SCoT). Kerman and Yazd populations had the maximum and minimum Na, Ne and PA values, respectively. In SCoT/pooled data (URP + SCoT), the highest values of H (0.33/0.36), I (0.49/0.53), PPL (98.96/98.83), Ne (1.56/1.63) and Na (1.97/1.97) pertained to the Kerman population (Table 3). In URP, SCoT and pooled data analysis, the lowest parametric values of genetic diversity were observed in the Yazd population. Consequently, this also means the lowest values of genetic parameters for SCoT primers in the Yazd population. The findings of this analysis have shown that the populations of Kerman and Yazd in all studied primers have the highest and lowest values in each of the genetic parameters, respectively.

Genetic distance and grouping relationship

To demonstrate the relationships among individuals, Jaccard's genetic distance coefficientwas assessed on the basis of the binary data matrix from SCoT and URP primersfor 90 accessions. In SCoT analysis, Jaccard's coefficient revealed a domain of 0.22–0.91 as the pairwise genetic distance coefficient, with a mean value of (0.63) among all 90 accessions of the 30 population. The highest genetic distance (0.91) was found between accession Kahnoj and Jiroft from the Kerman population, while a minimum distance (0.22) was discovered between the Taft accession of the Yazd population (distance matrix not shown). In addition to the genetic distance coefficient,there was a range of 0.13 and 0.80 for URP data with a mean value of 0.44.The two accessions of 'Haji Abad'from Hormozgan and 'Sarchahan' from Fars population had maximum distance, whereas a minimum distance was discovered among accession'Rodan' from Hormozgan and 'Anar' from Kerman. Using pooled data, a pairwise genetic distance coefficient determined a range of 0.24–0.77 with a mean value of 0.53 among the 90 accessions of the 30 accessions. Relationships were explored among the 90 accessions of the 30 population belonging to 6 distinct populations. This was carried out by cluster analysis, using the NJ procedure and by relying on the matrix coefficient of dissimilarity. The SCoT data generated a fan-dendrogram which divided all accessions into 5 main groups. The trend of clustering indicated aclear pattern of sub divisions. As shown in Fig. 2-A, the first cluster group (A) consisted of 3, 4 and 1 accessions from Esfahan, Fars, and Hormozgan populations, respectively, while 4 populations belonged to group (B) with 13 accessions

(Kerman-2, Fars-5, Esfahan-3,Yazd-3). Six populations belonged to group (C) with 20 accessions (Kerman-4,Yazd-3, Hormozgan-3, Khorasan-3, Fars-4, Esfahan-3), 4 populations belonged to group (E) with 20 accessions (Fars-5, Esfahan-6, Hormozgan-3, Kerman-6) and finally 2 population belonged to group (D) with 28 accessions (Kerman-21, Hormozgan-7) (Fig. 2-A). The results of URP analysis clustered all accessions into 6 main groups. Cluster (A) consisted of two accessions from Esfahan and another two from Fars. While group (B) consisted of 3, 5, 2 and 3 accessions from Esfahan, Fars, Kerman and Yazd, respectively (Fig. 2-B). Four populations belonged to group (C) with 16 accessions (Khorasan-6, Esfahan-1, Hormozgan-4, Fars-5). Three populations belonged to group (D) with 19 accessions (Esfahan-1, Kerman-12, Hormozgan-6) (Fig. 2-B). Three and five populations belonged to group (E) and (F), respectively, while showing 4, 2 and 8 accessions from Hormozgan, Fars and Kerman(Fig. 2-B). Group (E) and (F) had 1, 4, 5, 11 and 3 accessions from Hormozgan, Fars, Esfahan, Kerman and Yazd populations. Group (B) included 13 accessions from 4 different populations. The result of this classification shows 5, 3, 3 and 2 accessions from Fars, Esfahan, Yazd and Kerman populations, respectively (Fig. 2-B). Based on pooled data, the fan-dendrogram showed a high degree of genetic diversity among populations. The result of URP + SCoT analysis clustered all accessions into 6 groups (Fig. 2-C). Group (A) comprised 3, 3 and 1 accessions from Fars, Esfahan and Kerman. Group (B) consisted of 7, 3 and 3 accessions from Fars, Khorasan and Esfahan, respectively. Four populations belonged to group (C) with 20 accessions (Fars-2, Esfahan-3, Kerman-9, Hormozgan-5) (Fig. 2-C). Two populations belonged to group (D) with 18 accessions (Kerman-12, Hormozgan-6). Two populations belonged togroup E and five populations belonged to group (F), referring to 8 and 3 accessions from Kerman and Hormozgan. In these groups, there were 6, 3, 2, 6 and 3 accessions from Fars, Esfahan, Kerman, Yazd and Khorasan populations, respectively (Fig. 2-C).

Principle coordinate analysis

According to SCoT, URP and their integrated results, the Principle Coordinate Analysis (PCoA) was carried out. Through the SCoT, the first two axes described 37.37 (coord1 = 13.08 and coord2 = 24.29) of the overall genetic variation (Fig. 3-A). The fan-dendrogram resembled the results of principal coordinate analysis and cluster analysis. Likewise, the results of URP molecular markers entered into the principal coordinate analysis and indicated that the first to the third principal coordinates accounted for 17.49%, 25.79% and 33.88% of the overall genetic variation (Fig. 3-B). In sum, these results accounted for 77.16% of the total genetic diversity and approved the cluster study. According to the results of the PCoA for pooled data, the first, second and third principal coordinates comprised 11.19, 20.07, 27.52% of total molecular variation, thereby corresponding to 58.78 % of the total genetic variation (Fig. 3-C). When PCoA was based on pooled data, it indicated on eco-geographical segregation for different populations as seen in Kerman and Hormozgan populations are both located in the same origin on the biplot. These observations were validated by cluster analysis (Fig. 3).

Population structure analysis

Based on marker data, the genetic diversity of the 90 individual plants of 30 populations was analyzed based on URP, SCoT and pooled data (URP + SCoT) by using the Bayseian clustering analysis (K = 2 to K = 12) with structure software. Five sub-populations (K = 5) occurred with SCoT primers (Fig. 4-A). Out of the 90 accessions, subpopulation1 included all 21 accessions from Kerman (5), Hormozgan (3), Fars (5) and Esfahan (8), while S2 contained 17 accessions from Kerman (11), and Hormozgan (6) populations (Fig. 4-A). Twelve accessions of Kerman (4), Khorasan (1), Esfahan (3) and Fars (4) were grouped into S3, while S4 included all 21 accessions fromKerman (10), Fars (2), Hormozgan (6), and Esfahan (3). The results of the last group comprised 19 accessions from Khorasan (2), Yazd (6), Fars (5), Kerman (3), and Esfahan (3) populations (Fig. 4-A). However, the maximum ΔK for URP data was reached at K = 5 and the 90 accessions were categorized in sub population S1which comprised 19 accessions from Khorasan (3), Esfahan (6), Fars (9), and Kerman (1), while S2 contained 22 accessions from Fars (6), Khorasan (3), Kerman (3), Esfahan (3), Yazd (6), and Hormozgan (1) (Fig. 4-B). Twenty accessions of Hormozgan (6) and Kerman (14) were grouped into S3, while S4 included all 11 accessions from Kerman (8), and Hormozgan (3). The last group from URP data showed 17 accessions from Kerman (7), Hormozgan (5), Esfahan (3), and Fars (2) populations (Fig. 4-B). The maximumΔK value occurredat K = 6 by using pooled data (Fig. 4-C). All accessions were grouped into 6 clusters, from S1 to S6. Subpopulation 1 included all 12 accessions from Fars (6), Kerman (1), and Esfahan (5), while S2 contained 10 accessions from Kerman (7) and Hormozgan (3) populations (Fig. 4-C). Twenty accessions of Hormozgan (6), Kerman (9), Fars (2) and Esfahan (3) were grouped into S3, while S4 contained all 10 accessions from Esfahan (3), Kerman (2), and Fars populations. Twenty accessions of Khorasan (3), Fars (6), Yazd (5), Kerman (3), and Esfahan (3) were grouped into S5. The results of the last group contained 18 accessions from Kerman (12) and Hormozgan (6) populations (Fig. 4-C).

Discussion

Genetic diversity is an essential element of plant populations in facing environmental stimuli. The frequency of high genetic diversity within the population has been recorded in various species of plantsand theoutcrossing nature of these species contributes to diversity (Sheidai et al. 2013). Studying genetic diversity is a mechanism that describes species or individuals using specific statistical methods or a combination of methods based on morphological characters or molecular properties of individuals and DNA-based marker data that make a more reliable distinction from genotypes (Mohammadi et al. 2003).

The genetic structure of a plant population depends not only on its genetic background, but also on genetic drift, gene flow, natural selection, etc (Hu et al. 2014). Assessing the genetic diversity of germplasm collections may provide accurate classification of accessions and recognition of subsets for potential use for particular breeding purposes (Mohammadi and Prasanna 2003). Molecular characterization is now a preferable method of quantifying variations within plant genetic properties. The efficiency of the molecular marker method depends on the amount of polymorphism which can be seen among the set ofindividuals under examination (Etminan et al. 2017). The literature reviewdemonstrated only a few reports where SCoT and URP markers have been used to characterize the genetic variation,

especially in *F. assafoetida L* relatives. *F. assafoetida* has a particular distribution and it is difficult to find samples.These data could be used for future programs that require genetic variation in ferula species. In this research, 12 SCoT primer and 12 URP primers were used for molecular characterization of 90 individual plantsof the 30 populations .There was a significant genetic variation within the populations.

This research demonstrated that SCoT and URP markers explained 98.37% and 97.91% of polymorphism (Table 1). Such an amount of polymorphism is comparable with that of other studies in different plants, e.g. peanuts with 38.22%, potato with61%, durum wheat with 39% and Triticum species with 85.3% (Xiong et al. 2011; Gorji et al. 2011; Etminan et al. 2016; Guo et al. 2016). With respect to the information marker indices, SCoT markers have shown higher TAB, PIC, Rp, and MI. Nevertheless, the average PIC values for SCoT primers used in this analysis were 0.39, whilst the average of PIC for URP primers is calculated as 0.36. It seems that SCoTprimers are highly able to compare URP Primers in discovering the amount of polymorphism among the populations of Ferula plants. According to this report, high valuesof PIC, MI and Rp are responsible for the sharp efficiency of SCoT primers for genotyping in Ferula accessions. Out of such indicators, PIC, Rp, and MI can grade the ability of markers in studying genetic diversity (Qaderi et al. 2019). As discussed by Vaiman et al. (1994), if the PIC is between 0.25 and 0.5, the primer compounds could recognize moderate polymorphisms between the accessions. As a result, the polymorphism information content (PIC) compares with the ability and power of markers to identify polymorphisms within a population, there by depending on the type of germplasm, the relative frequency of these alleles and the number of alleles. According to the results of this study, MI, PIC, Rp, PPB and TAB in SCoT primers had higher abilities than URP. Earlier, Alikhani et al. (2014) and Sorkheh et al. (2016) demonstrated the high ability of SCOT markers in comparison with CBDP markers in assessing genetic diversity and grouping of *Quercusbrantii* and wild pistacia species populations. Guo et al. (2016) stated that SCoT primer systemsare more informative in terms of polymorphic content than CBDP and SCoT primers. They can better detect inter-specific relationships in wild wheat relatives. These results are consistent with Gholamian et al. (2019); Tiwari et al. (2016); Pour-Aboughadareh et al. (2017, 2018), and Tajbakht et al. (2018) who confirmed that SCoT markers work effectively in describing genetic variation and structure than other molecular marker techniques. Nevertheless, the best marker for a genus such as *F. assafoetida* would require a wide variety of genomes for assessment.Analysis of molecular variance (AMOVA) revealed a wide distribution of genetic variation within the *F. assafoetida* populations (Table 2).

The results of AMOVA revealed a higher level of molecular variance within populations (URP = 88%, SCoT = 91%, pooleddata = 90%) than among population (URP = 12%, SCoT = 9%, pooled data = 10%). The difference between the populations at the $pcript >$ probability level was significant. The integration of SCoT and URP can be seen as good markers for a more precise evaluation of genetic diversity. The results confirmedthat SCoT primers show significant genetic diversity within medicinal plant populations. These results indicate that the diversity within the populations is high and, in fact, there is no significant difference among the populations. It can be stated that they are a subset of a larger population.The markers nature can partly explain the exact isolation of samples, whereas the high polymorphism of SCoT and URP markers can greatly represent variations. High, medium and low degrees of genetic

distinctionwere observed in populations with Gst values more than 0.15, between 0.05 and 0.15, and less than 0.05, respectively (Hamrick et al. 1991; Nei 1978).

The value of the allele frequency between the population in the case of the SCoT primers is 0.15 in association with URP = 0.19 and pooled data = 0.17. It can be concluded that high genetic variation occurred between populations. Furthermore, the Gst determines the number of migrants per generation (Nm) in calculating gene flow, and the higher its value, the less genetic differentiation there is among populations (Pour-Aboughadareh et al. 2018). However, according to the hypothesis developed by Wright's (1949), if Nm > 1, population sare barred from differentiation because of genetic drift. On the other hand, if Nm < 1, local populations fail to distinguish from one another. According to the results the gene flow, Nm values between populations for SCoT, URP and pooled data were 2.81, 2.03, and 2.38, respectively, and thehigh gene flow (Nm) showed that the genetic variation of *F. assafoetida* is independent of their geographical origin. These findings suggest that gene flow most likely occurred among the wild relatives of *F. assafoetida*. The volume of gene flow has a direct effect on the distribution of genetic variation within and among populations. The level of gene flow depends on the size and degree of segregation among populations. Also, the transport of seeds or pollen among populationsare effective in gene flow and in the directions of the flow (Dumolin-Lapegue et al. 1997). Plant species establish gene flow through pollen grains, their migration and seed transfer to adjacent populations.In fact, these two mechanisms have a significant effect on the degree of genetic diversity and differentiation among plant populations (Pfeifer and Jetschke 2006). Overall, in the case of wild species, the genetic distance is determined by the geographical distance and the gene flow between species. In cross-pollination species, due to a high rate of gene flow, the genetic gap between populations is low and genetic variation is distributed within populations (Pfeifer and Jetschke 2006). Hamrick and Godt (1996) stated that if the genetic flow between habitats is interrupted due to peculiar factors such as habitat destruction or improper harvesting, there will be an increase in genetic distance among populations, and genetic erosion will commence because of homogeneity.

SCoT, URP and SCoT + URP cluster analysis suggesting, genotypes that are related to different geographical areas and sometimes far apart are located in close branches. These results indicated a very high degree of gene migration among populations. In addition, *F. assafoetida* is cross-pollinated in nature and insects serve as its pollinator agents. The plants develop seeds that have large wings, thereby increasing the chances of seed dispersal further (Reddy et al. 2007). The pooled data (SCoT + URP) confirmed the findings of the Beyesian structure. Population structure applies to any of the genetic patterns of individuals within the population. The list of possible subpopulations withina particular population can be distinguished by the frequency of different alleles in each subpopulation, and also by genetic separation between subpopulations (Chakraborty 1993). In addition, this second study analyzes the population structure of *F. assafoetida*. These findings are further confirmed by the study of the Beysian structure. Pritchard et al. (2000) reported that the clustering method is based on the Bayesian statistical index for interpreting population structures.

Although this method can involve a small number of discontinuous markers, it is possible to analyze the population structure, accurately classify individuals into appropriate populations and recognize different individuals. Based on the results of this method, it can be stated that sometimes the classification of genotypes is independent of their geographical origin. In this research, the populations structure analysis was evaluated by structure software and studied populationsfor SCoT primers into 5 categories. URP, SCoT + URP were divided into 6 categories. Accordingly, most of these divisions were based on the geographical areas from which these populations originated, although they were not completely separated from each other. The results of this study demonstrated that the intense mixing between the samples is completely visible and this intense mixing in the germplasm confirms the mixed lineage of the populations under study. These findings indicate that each individual plant might be genetically traced back to offspring fromother populations. It demonstrates that allele frequency is often associated in different populations due to migration or common descent (Evanno et al. 2005). The observed variance in genetic diversity among the populations can stem from the prevalence of allelic variation in these populations, which geographical locations have a substantial role in shaping them (Ni et al. 2018). *F.assafoetida* populations are not segregated based on similar places or names and the studied populations are strongly mixed. These samples may not be related to each other directly, per se, based on lineage.It is possible that mobility, transfer and dispersioncould play significant roles in the rate of migration, thereby giving weight to the hypothesis that populations in one area may have originated in an integrated manner from locations other than their current habitat. It is possible that the physiological structure and easy dispersal of *F.assafoetida* seeds can explain the wide range of geographical habitats for this species, as this canincrease gene flow and exchange.These results are due to the transfer of genotypes from the main regions to other parts of the country (Heidari et al. 2016).

In addition, this finding shows that these regions may be a valuable source of heterogeneity in the discovery of new alleles and candidate genes. Such a rich germplasm makes breeding programs worthwhile, although some populations were not classified on the basis of geographical areas. For instance, the permanent isolation of samples could influence a marker's nature, even as ahigh level of polymorphism of SCoTand URP markers could describe variations inaccurately (Tajbakht 2018). Similar findings were reported in the case of other Apiaceae species, such as *Nigella sativa* Golkar and Nourbakhsh (2019) and *Foeniculum vulgare* (Maghsudi Kelardashti et al. 2018). These characteristics of *F. assafoetida* germplasm may have contributed to an enhanced adaptability, migration and gene flow across regions (Hamrick and Godt 1996). Our findings revealed a high variation in the populations and this was confirmed by AMOVA. In addition, URP markers can be integrated with SCoTmarkers to yield more consistent results on genetic diversity.

Conclusion

Crop improvement is driven by knowledge of the extent and distribution of genetic variation, as well as relationships between breeding materials. This study investigates the population structure and genetic diversity of *F.assafoetida* germplasm. This study discovered a significant differential within populations. Furthermore, the findings showed that URP and SCoT molecular markers were reasonably effective at

evaluating genetic variation among *F.assafoetida* genotypes and separating various individuals from different *F.assafoetida* populations. *F.assafoetida* species had comparatively high admixture and could be categorized into distinct categories depending on their geographical distribution, according to the results of the study. The relatively high degree of gene transfer between species may be due to *F.assafoetida* pollination, seed dispersal, nonnuclear inheritance, and post transcription effects. The findings of this study have indicated that the F.assafoetida species has a high potential for use in the pharmaceutical industry. Based on this knowledge, it is possible to infer that this plant is a natural source of useful phenolic compounds that can be used in breeding programs, industrial processes, and pharmaceuticals. Finally, more population selection for genetic diversity assessment will provide more informative data.

Declarations

Author contributions

Sadeghi Reyhane: Writing - Original Draft, Conceptualization, Data Curation, Formal analysis.

Omidi Mansour: Conceptualization, Validation, Resources, Writing - Review & Editing, Visualization, Supervision, Project administration.

Azizinezhad Reza: Methodology, Software, Formal analysis, Visualization.

Etminan Alireza: Conceptualization, Methodology, Formal analysis, Data Curation, Writing - Review & Editing, Visualization, Project administration.

Naghdi Badi Hassanali: Investigation, Methodology.

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Tables

Table1 Polymorphism of Start Codon Targeted (SCoT) and Universal RicePrimer (URP) primers in the 90 *Ferula assafoetida* accessions

Primer code	Primer Sequence(5´-3´)	Ta (°C)	TAB	NPB	PPB	PIC	Rp	MI
SCoT2	CAA CAATGG CTA CCA CCC	56	15	15	100	0.43	11.06	6.45
SCoT3	CAA CAATGG CTA CCA C CG	56	16	16	100	0.40	10.13	6.4
SCoT5	CAA CAATGG CTA CCA CGA	54	17	17	100	0.41	10.88	6.97
SCoT7	CAA CAATGG CTA CCA CGG	56	17	16	94.11	0.34	8.51	5.44
SCoT8	CAA CAATGG CTA CCA CGT	54	15	15	100	0.37	8.44	5.55
SCoT9	CAA CAATGG CTA CCA GCA	54	17	17	100	0.40	11	6.8
SCoT12	ACG ACA TGG CGA CCA ACG	58	19	19	100	0.42	12.22	7.98
SCoT14	ACG ACA TGG CGA CCA CCG	61	17	16	94.11	0.37	9.62	5.92
SCoT15	ACG ACA TGG CGA CCG CGA	61	16	16	100	0.44	11.57	7.04
SCoT21	CAC CAT GGC TAC CAC CAT	56	13	12	92.3	0.36	7.35	4.32
SCoT24	CCA TGG CTA CCA CCG CCA	61	17	17	100	0.40	10.57	6.8
SCoT26	ACA ATG GCT ACC ACCATC	54	13	13	100	0.35	7.13	4.55
		Mean	16	15.75	98.37	0.39	9.87	6.18
URP 1	ATCCAGGTCCGAGACAACC	48	12	9	75	0.31	5.4	2.79
URP3	AGGACTCGATAACAGGCTCC	48	11	11	100	0.32	4.93	3.52
URP8	CCTCCTCCCTCCT	48	13	13	100	0.38	7.62	4.94
URP9	AGGGCTGGAGGAGGGC	48	14	14	100	0.43	9.84	6.02
URP10	CCTGTGTGTGTGCAT	48	11	11	100	0.32	5.46	3.52
URP11	ATGCACACACACAGG	48	18	18	100	0.38	10.77	6.84
URP12	GGTGAAGCACAGGTG	48	10	10	100	0.41	6.6	4.1
URP13	GGTGTAGAGAGGGGT	48	10	10	100	0.39	5.95	3.9
URP14	CTCTGGGTGGTGCTG	48	10	10	100	0.32	4.66	3.2
URP15	GGCAGGATTGAAGC	48	14	14	100	0.35	7.33	4.9
URP17	AGGAGGAGGGGAAGG	48	13	13	100	0.43	9.24	5.59

MI: marker index; Rp: resolving power; PIC: polymorphism information content; TAB: total amplified bands; NPB: number of polymorphism; PPB: percentage of polymorphism; Ta: annealingtemperature. Y = C or T and R = A or G.

Primer code	Primer Sequence(5´-3´)	Ta (°C)	TAB	NPB	PPB	PIC	Rp	MI
URP18	GAGGGTGGCGGCTCT	48	13	13	100	0.32	5.6	4.16
		Mean	12.41	12.16	97.91	0.36	6.95	4.45

MI: marker index; Rp: resolving power; PIC: polymorphism information content; TAB: total amplified bands; NPB: number of polymorphism; PPB: percentage of polymorphism; Ta: annealingtemperature. Y = C or T and R = A or G.

Table2 Analysis of molecular variance(AMOVA) in F.assafoetida L. populations.

Source of variation	SCoT		URP		SCoT+URP	
	Between Populations	Within Populations	Between Populations	Within Populations	Between Populations	Within Populations
df	5	84	5	84	5	84
SS	417.502	3002.732	363.392	2111.085	780.894	5113.817
MS	83.500	35.747	72.678	25.132	156.179	60.879
Est.Var	3.440	35.747	3.426	25.132	6.866	60.879
Var	9%	91%	12%	88%	10%	90%
PhiPT P=0.001	0.088		0.120		0.101	
G_{ST}	0.1506		0.1972		0.173	
Nm	2.8193		2.0349		2.3871	

Table 3 Summery of genetic variation statistics for all loci of SCoT and URP markers for different Iranian *F.assafoetida* L. populations.

Marker	population	Na	Ne	I	H	PPL
	Esfahan	1.88 ± 0.03	1.55 ± 0.02	0.48 ± 0.01	0.32 ± 0.01	94.27
	Fars	1.86 ± 0.03	1.53 ± 0.02	0.47 ± 0.01	0.31 ± 0.01	93.23
	Khorasan	1.46 ± 0.06	1.44 ± 0.02	0.38 ± 0.02	0.25 ± 0.01	69.27
SCoT	Kerman	1.97 ± 0.01	1.56 ± 0.02	0.49 ± 0.01	0.33 ± 0.01	98.96
	Yazd	1.17 ± 0.06	1.35 ± 0.02	0.29 ± 0.02	0.20 ± 0.01	50.52
	Hormozgan	1.80 ± 0.04	1.55 ± 0.02	0.47 ± 0.01	0.32 ± 0.01	88.54
	Mean	1.69 ± 0.02	1.50 ± 0.01	0.43 ± 0.00	0.29 ± 0.00	82.47 ± 7.66
	Esfahan	1.83 ± 0.03	1.66 ± 0.02	0.52 ± 0.01	0.36 ± 0.01	85.23
	Fars	1.95 ± 0.01	1.70 ± 0.02	0.57 ± 0.01	0.39 ± 0.01	95.97
URP	Khorasan	1.61 ± 0.05	1.49 ± 0.03	0.40 ± 0.02	0.28 ± 0.01	69.13
	Kerman	1.97 ± 0.01	1.73 ± 0.02	0.58 ± 0.01	0.40 ± 0.01	98.66
	Yazd	1.40 ± 0.05	1.39 ± 0.03	0.31 ± 0.02	0.21 ± 0.01	52.35
	Hormozgan	1.81 ± 0.03	1.61 ± 0.02	0.49 ± 0.02	0.34 ± 0.01	83.89
	Mean	1.76 ± 0.01	1.60 ± 0.01	0.48 ± 0.00	0.33 ± 0.01	80.87 ± 7.13
	Esfahan	1.86 ± 0.02	1.60 ± 0.01	0.50 ± 0.01	0.34 ± 0.00	90.32
URP + SCoT	Fars	1.90 ± 0.02	1.61 ± 0.01	0.51 ± 0.01	0.35 ± 0.00	94.43
	Khorasan	1.53 ± 0.04	1.47 ± 0.02	0.39 ± 0.01	0.26 ± 0.01	69.21
	Kerman	1.97 ± 0.01	1.63 ± 0.01	0.53 ± 0.00	0.36 ± 0.00	98.83

Marker	population	Na	Ne	I	H	PPL
	Yazd	1.27 ± 0.04	1.37 ± 0.02	0.30 ± 0.01	0.20 ± 0.01	51.32
	Hormozgan	1.80 ± 0.02	1.58 ± 0.01	0.48 ± 0.01	0.33 ± 0.00	86.51
	Mean	1.72 ± 0.01	1.54 ± 0.00	0.45 ± 0.00	0.31 ± 0.00	81.77 ± 7.38

PPL: the percentage of polymorphic loci, He:Nei's gene diversity, I: Shannon's information index, Ne: effective number of alleles, Na: total number of alleles

Figures
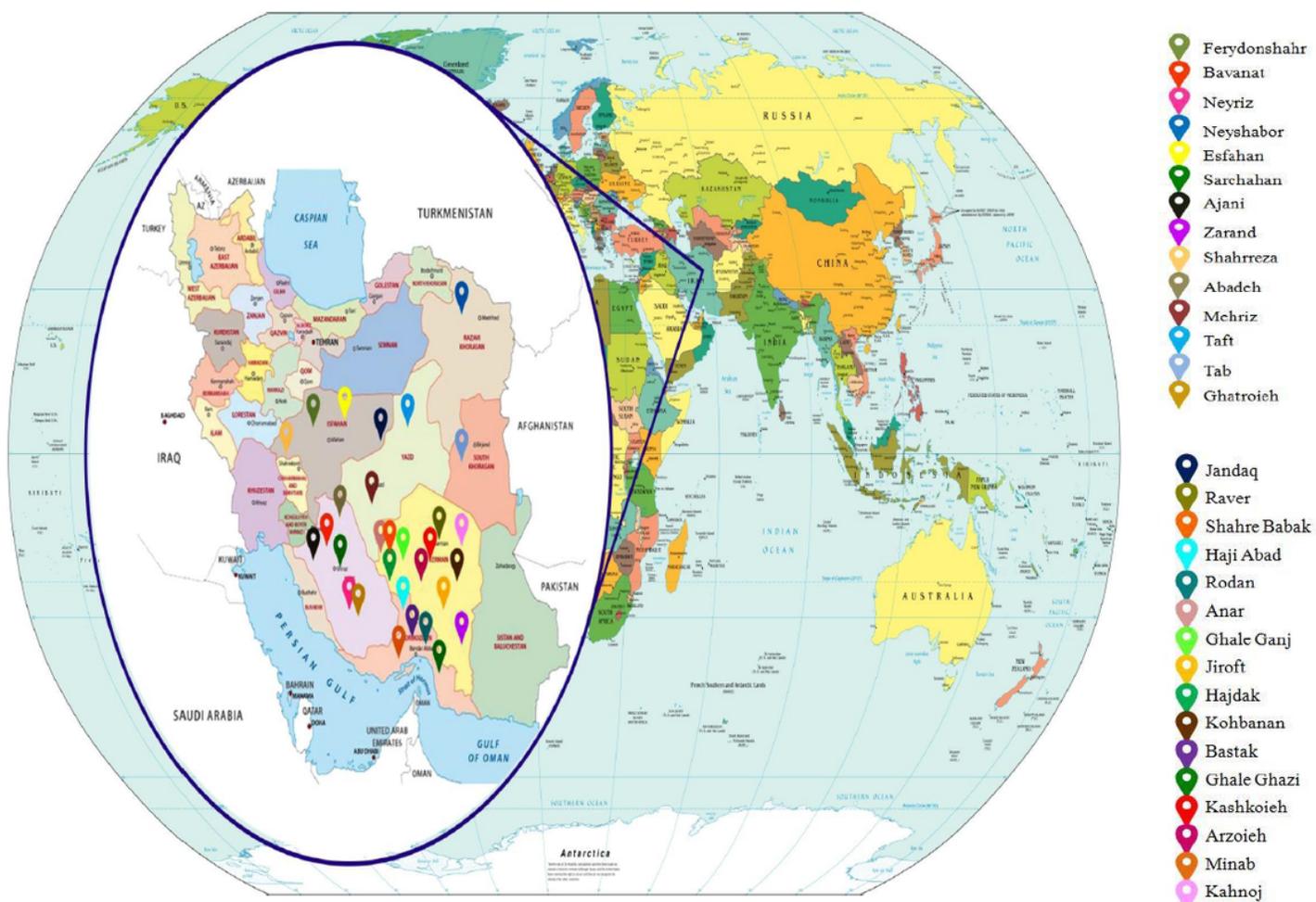
Figure 1

A native distribution map of 30 wild populations of F. assafoetida in Iran.
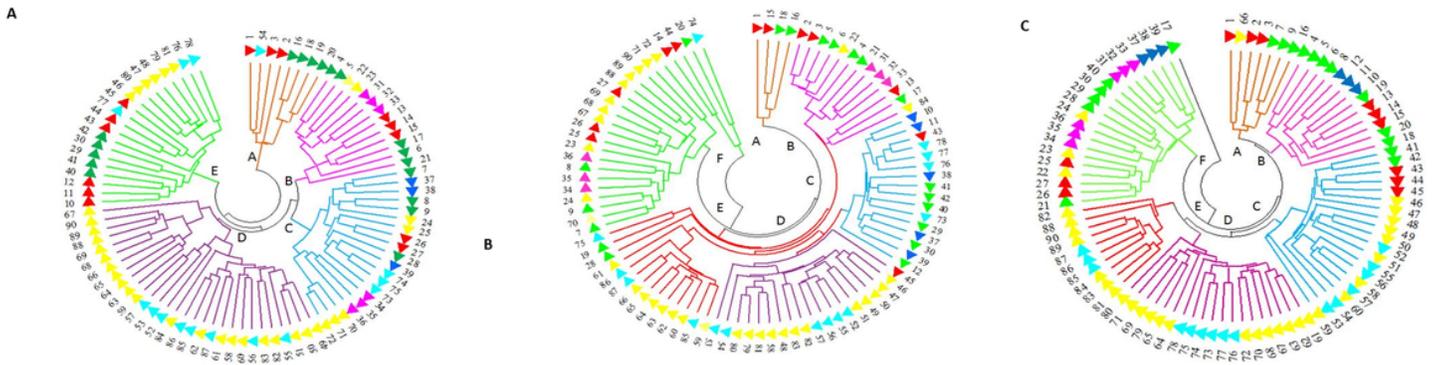
Figure 2

Fan-dendrogram of the 90 genotypes of Ferula assafoetidacreated with neighbor-joining clustering algorithm based on SCoT(A), URP(B) and SCoT+URP(C) data.
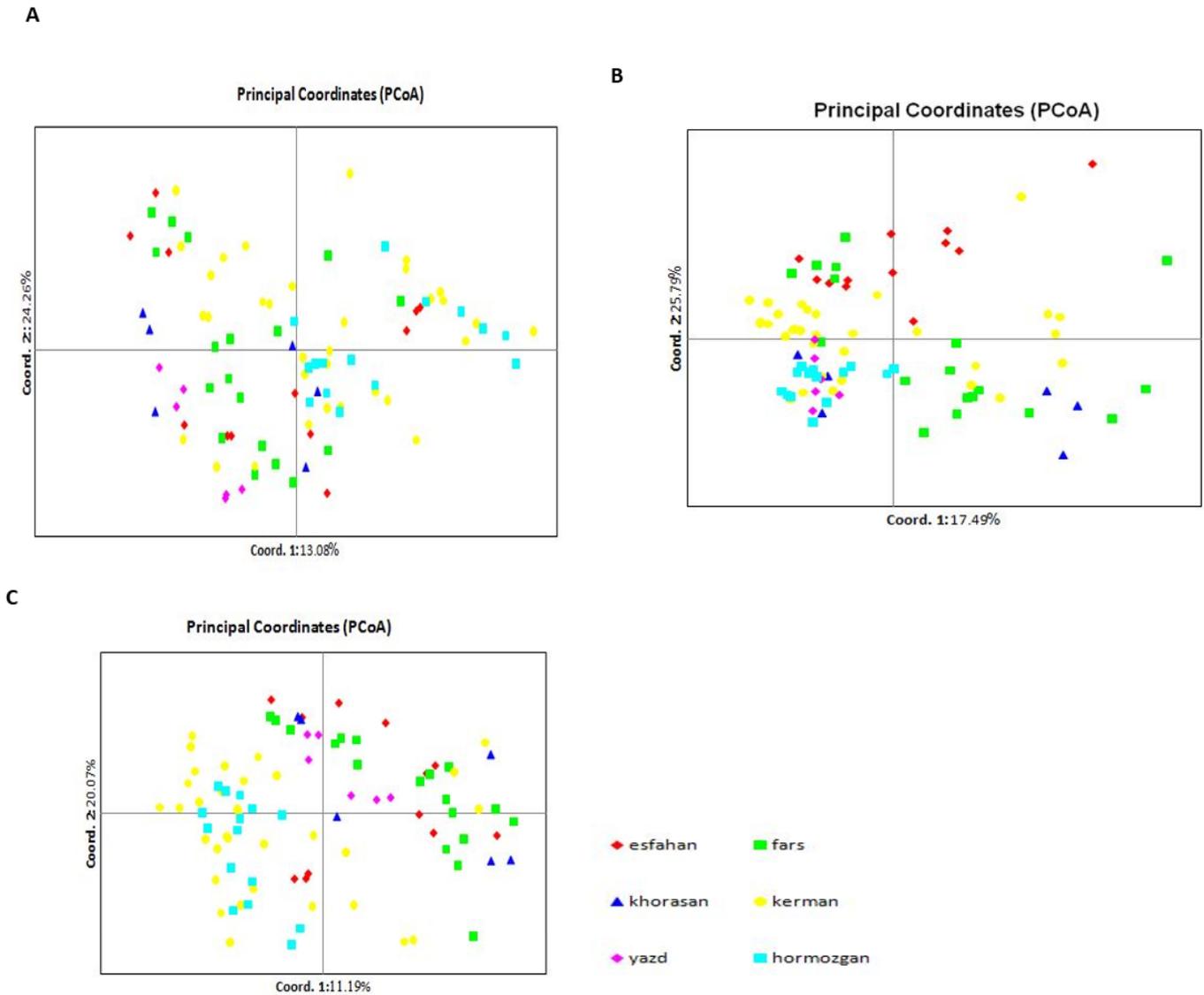
Figure 3

A biplot extracted from the PCoAof 90 F. assafoetida genotypes based on SCoT (A), URP (B) and SCoT+URP (C) results
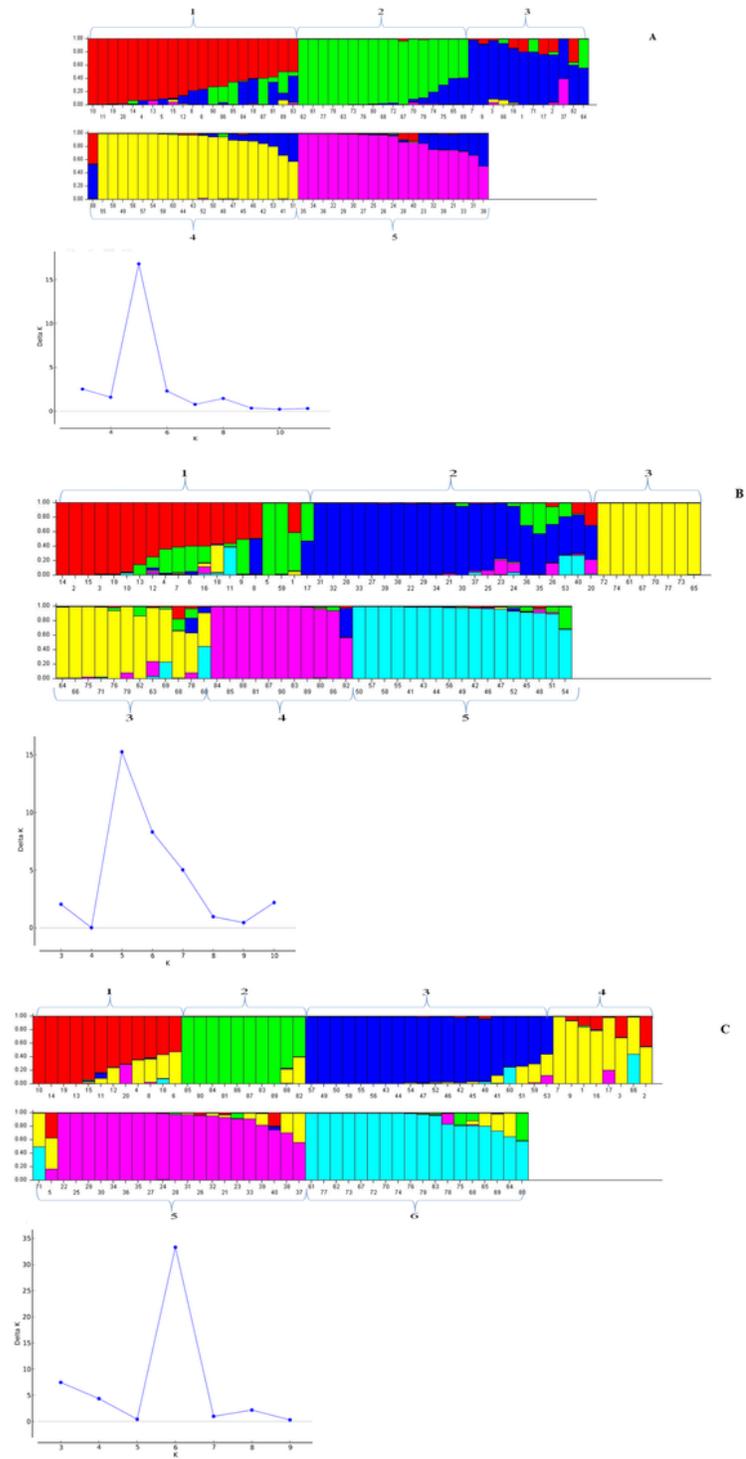
Figure 4

Population structure of 90 F.assafoetida accessions using SCoT (A), URP (B) and integrated data (C).