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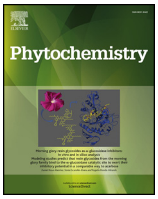

Genetic diversity among and within *Ferula asafoetida* H. Karst. populations using molecular and phytochemical markers

Pooneh Tajbakht[a], Majid Talebi[a], Mehdi Rahimmalek[b,*]

[a] Department of Agricultural Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan, 8415683111, Iran
[b] Department of Agronomy and Plant breeding, College of Agriculture, Isfahan University of Technology, Isfahan, 8415683111, Iran

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ABSTRACT

Ferula asafoetida is an herbaceous, perennial and monocarpic species of the Apiaceae family with medicinal properties. In this study, the genetic diversity of *F. asafetida* populations was assessed using SCoT and SRAP molecular markers. Nine SCoT and nine SRAP primer combinations amplified 211 and 194 bands, showing 96.07% and 92.87% of polymorphism, respectively. The dendrograms generated from Jaccard's similarity matrix and the UPGMA method revealed that genetic relationships among the *F. asafoetida* genotypes were not related to their geographical regions. The analysis also revealed a relatively acceptable differentiation in allele frequencies, illustrating the high genetic diversity and gene flows among the populations. The high percent of diversity among the populations indicated a rich resource of germplasm for breeding programs. Moreover, STRUCTURE analysis showed the high admixture of the studied accessions. According to AMOVA analysis, higher genetic variation occurred within the populations. Total phenolic content was also assessed using the folin-ciocalteu method, observing a relatively extent variety (0.163–0.938 mg TA/g DW) among the populations. In addition, HPLC analysis defined eleven compounds in which p-coumaric and 1,3- caffeoylquinic acids were the most abundant phenolic acids. Finally, Feragheh population possessed the highest TPC (total phenolic content) and gene diversity. Karaj population also exhibited the highest amount of flavonoids and phenolic acids such as kaempferol and p-coumaric acids.

1. Introduction

Ferula is one of the most important genera of the Apiaceae family throughout the world. More than 140 species have been recognized in this genus; they are widespread from the Mediterranean region to central Asia. *F. asafoetida* is one of the most important species of *Ferula*. This species is native to Afghanistan and Iran (Ross, 2007; Leaman, 2006). *F. asafoetida* is useful for various medicinal purposes such as, hysteria, stomach pain (Iranshahy and Iranshahi, 2011) and insect bite (Moharramipour and Nazemi, 2008). The antidiabetic and antispasmodic activity have also been reported for this plant (Fatehi et al., 2004; Abu-Zaiton et al., 2010). It is also used as an antidote for flatulence. The gum is also recommended for pharmaceutical preparations as a local stimulant in the mucous membrane of the alimentary canal (Rahman et al., 2008).

Plants produce a wide range of phenolic metabolites acting mainly as antioxidants (Grace, 2005; Moghaddam and Mehdizadeh, 2015), phytoalexins, attractants for pollinators and contributors to plant pigmentation. Phenolic compounds are considered as excellent oxygen radical scavengers (Bors et al., 1994; Grace, 2005). The chemical constituents of the genus *Ferula* have been studied by many researchers (Iranshahi et al., 2003; Kogure et al., 2004; Motai et al., 2004). *F. asafoetida* has different kinds of phenolic compounds. It has also potent antioxidant capacity, providing protection against free radical–mediated diseases such as carcinogenesis (Mallikarjuna et al., 2003). Some studies have highlighted the presence of different kinds of polyphenols in the *Ferula* genus (Dehghan et al., 2007). In this respect, the phytochemical and antioxidant activity of *F. asafoetida* has been evaluated in relation to the environmental factors and plant parts (Mehrpour et al., 2016). Many studies have also been focused on the chemical composition and pharmaceutical properties of *F. asafoetida* (Ross, 2007; Rahman et al., 2008; Rollinger et al., 2008; Lee et al., 2010). However, most of these studies have been focused on the oleo gum resin of this species and few studies have been performed in relation to the variation of polyphenolic compounds in different populations.

While this plant has extensive distribution in Iran, there is no exhaustive information regarding the genetic diversity of this valuable plant. Nowadays, DNA markers have been used as a robust technique to

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* Corresponding author.
E-mail address: mrahimmalek@cc.iut.ac.ir (M. Rahimmalek).

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evaluate the genetic diversity (Rahimmalek et al., 2009). Among different kinds of PCR-based DNA markers, gene-based markers such as SCoT (Start Codon Targeted) and SRAP (Sequence-Related Amplified Polymorphism) markers are beginning to replace the other DNA markers due to their accurate data. SCoT method has been developed based on the short conserved flanking region of the ATG start codon in plant genes using single 18-mer primers in PCR. SCoT markers are generally reproducible and highly polymorphic markers. SCoT markers have been used to assess the genetic relationships of some plants species such as Cumin (Ebrahimiyan et al., 2016) and fennel (Yadav and Malik, 2016). SRAP marker is a simple marker technique developed based on two 17 or 18 nucleotides long primers and targeted open reading frames (ORFs), where the first 10 or 11 bases starting at the 5′ end are non-specific “filler” sequences followed by the sequence CCGG in the forward primer and AATT in the reverse primer (Li and Quiros, 2001). SRAP marker has been used for the assessment of the genetic diversity in many Apiaceae plants including *Kelussia odoratissima* (Hadian et al., 2014), *Apium graveolens* (Wang et al., 2011), and *Foeniculum vulgare* Mill. (Maghsoudi Kelardashti et al., 2015).

While there is a report regarding the genetic diversity of Iranian *F. asafoetida* based on RAPD markers (Sarhaddipour et al., 2014), no attempt has yet been made to characterize them using gene-based markers to target the functional regions of the genome. Therefore, the aims of this study were to assess the genetic relationships among and within Iranian *F. asafoetida* populations and to determine the genetic structure of genotypes from different geographical regions using SRAP and SCoT markers. Furthermore, another goal of this research was to evaluate the variation of this plant based on major phenolic and flavonoid compounds according to HPLC analysis. To the best of our knowledge, this is the first application of SRAP and SCoT markers for the assessment of genetic diversity among *F. asafoetida* collections.

2. Results

2.1. SCoT data analysis

In the present study, nine primers for each marker were selected for the identification of 64 F. *asafoetida* genotypes based on the number of amplification products, the quality of the profiles, the level of polymorphism, and the reproducibility of the bands. The selected SCoT primers generated 203 amplified fragments, with an average of 23.66 fragments per primer in 64 accessions (Table 1). The size range of the amplified SCoT products was 100–3000 bp, and the number of products per primer varied from 17 in SCoT35 to 27 in SCoT27, SCoT32 and SCoT33. The average percentage of SCoT polymorphic bands was 96.07%. The number of polymorphic bands per primer ranged from 16 to 27, with an average of 22.77 (Table 2). The average PIC value for the amplification products was 0.44 (Table 2). SCoT34 and SCoT35 showed the highest and lowest PIC values, respectively (Table 2). The polymorphic scored SCoT fragments were used for the genetic diversity analysis. Genetic diversity was indicated by the range of Jaccard's similarity coefficients (0.096–0.62). The lowest genetic similarity was observed between accessions 14 and 39, and the highest one was detected between accessions 63 and 2. Another parameter demonstrating the discriminatory power of each marker was the Resolving Power (RP). RP is the ability of each primer to detect the level of variation between individuals calculated according to Prevost and Wilkinson (1999): $Rp = \Sigma Ib$, where Ib (band informativeness) takes the values of: $1-[2|0.5-p|]$, and p is the proportion of individuals containing the band. The estimates of RP were found to be the highest for the primer 33 in SCoT analysis (Table 2) and the primer E_2M_1 in SRAP analysis (Table 3). The lowest amounts of RP belonged to the primer 3 in SCoT analysis, and it was caused by the primer $E_{17}M_1$ in the SRAP data. Overall, the RP value related to SRAP analysis was more than the one belonging to the SCoT data.

To determine the overall usefulness of a given marker system, the marker index (MI) was calculated for SCoT and SRAP, obtaining the values of 10.18 and 8.74, respectively.

The dendrograms generated from Jaccard's similarity matrix and the UPGMA method revealed genetic relationships among the *F.asafoetida* genotypes (Fig. 1). The highest cophenetic correlation coefficient ($r = 0.81$) was obtained between Jaccard's similarity data matrix and the cophenetic matrix, indicating a good fit between the dendrogram clusters and the similarity matrices. The SCoT dendrogram revealed three groups which included some subgroups (Fig. 2). This genotype was collected from the Margoon village in Fars province. In most cases, the collected genotypes indicated considerable variation based on their geographic regions. The results showed that the divergence of accessions was based on their region.

PCA was carried out to visualize the association among the accessions in details. The results showed that the first three principal coordinates could account for 38.76% of the total variation, corresponding largely to the results obtained through cluster analysis.

Table 4 presents the six population-level genetic diversity indices of the observed number of alleles (*Na*), the effective number of alleles (*Ne*), Nei's gene diversity (*H*), and Shannon's information index (*I*) related to the SCoT data. Overall, among the six study regions, the Yazd region showed the highest values of Nei's (0.37) genetic diversity in the SCoT data, while the Karaj region had the lowest values of Nei's genetic diversity (0.25). Moreover, Yazd population was recorded as having the highest value for the Shannon index (0.55) in the SCoT analysis. In addition, Yazd population possessed the highest number and percentage of polymorphic loci (99.01) in terms of SCoT markers.

Analysis of molecular variance (AMOVA) was also performed to study the genetic differentiation among 64 genotypes of *F. asafoetida* from different geographical regions of Iran and to estimate the percentage of intra- and inter-population genetic variations (Table 5). The results of AMOVA analysis revealed significant variations in the regions ($P < 0.001$), such that 90.07% of the total genetic variation in the SCoT analysis occurred within the populations. Besides, the total

Table 1
Geographical characteristics of *Ferula asafoetida* populations used in this study.

Herbarium number	Location	genotype code	genotype No.	Geographical region	Latitude	Longitude	Altitude (m)	Precipitation (ml)
12511	Garmab, Alborz, Iran	Algarm	1 to 9	North	35°25′ N	56°45′ E	1420	332.1
12512	Siyahbisheh, Mazandaran, Iran	Mazsiyah	10 to 19	North	36°21′ N	51°31′ E	1840	709.7
12513	Shemshak, Alborz, Iran	Alshem	20 to 26	North	36°01′ N	51°49′ E	2600	270
12514	Karaj, Alborz, Iran	Alkar	27 to 30	North	35°48′ N	51°00′ E	1312	256
12515	Abarkouh, Yazd, Iran	Yazabar	31 to 40	East	31°10′ N	53°20′ E	1550	82..8
12516	Feragheh, Yazd, Iran	Yazfer	41 to 50	East	31°05′ N	53°03′ E	1216	79
12517	Daran, Isfahan, Iran	Isdar	51	Center	32°59′ N	50°24′ E	2390	317.4
12518	Margoon, Fars, Iran	Famar	52	Southwest	30°34′ N	51°53′ E	2000	500
12519	Aalimakan, Isfahan, Iran	Isali	53 & 54	Center	32°94′ N	50°12′ E	2530	285.5
12520	Damaneh, Isfahan, Iran	Isdam	55 & 56	Center	33°01′ N	50°29′ E	2300	250
12521	Khersanak, Isfahan, Iran	Iskher	57 to 59	Center	32°56′ N	50°35′ E	2900	290
12522	Gachsar, Alborz, Iran	Algach	60 to 64	North	36°07′ N	51°19′ E	2230	243