Turkish Journal of Agriculture - Food Science and Technology, 10(4): 678-681, 2022
DOI: https://doi.org/10.24925/turjaf.v10i4.678-681.4867

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology

Genetic Relationship of Seven Endemic *Inula* L. (Asteraceae) Species Grown in Turkey

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ARTICLE INFO

Research Article

Received : 18/11/2021
Accepted : 22/02/2022

Keywords:
Inula
ISSR-PCR
Genetic
Endemic
Turkey

ABSTRACT

In this study, genetic relationship of ISSR markers of seven endemic *Inula* species distributed in Turkey was carried out. Plant samples were collected from different regions of Turkey in 2013 and gDNA was obtained by DNA isolation from green leaves. Genetic relationship between species was determined using 12 ISSR primers. PCR products were run on agarose gel electrophoresis and visualized under UV light. All gel images were examined and the presence and absence of polymorphic bands were scored as 0 and 1. A total of 85 bands were obtained from the primers. Of these, 74 polymorphic and 11 monomorphic bands were obtained. The total polymorphism rate was found to be approximately 87.05%. The phylogenetic tree and genetic distances between species were calculated using the PAUP 0 4.0b10 analysis program. According to the distance matrix, the genetic distance was found between tthe closest *Inula fragilis* and *Inula sarana* (0.28571), while the farthest between *Inula sarana* and *Inula macrocephala* (0.56000) species. The phylogenetic tree was obtained using the UPGMA algorithm, and the tree consisted of two groups. The results were compared with the morphological, palynological nrDNA and cpDNA results of the past. Our findings supported previous studies.

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Introduction

Turkey hosts a very rich floristic structure thanks to its geographical location (Başköse and Dural, 2011). It has 3 different types of flora with the Euro-Siberian flora region in the north, the Mediterranean flora region in the west and south, and the Iran-Turan flora region including the Central, Eastern and Southeastern Anatolia. Plant richness of Turkey manifests itself through species diversity and high number of endemic species (Akgöz, 2013). Asteraceae, among the largest families of flowering plants, consists of more than 24.000-30.000 species and 1600-1700 genera. In the flora of Turkey, this family is represented by 1209 species, 447 being endemic, with an endemism rate of 37% (Tekin and Akdere, 2021; Zardi-Bergaoui et al., 2020). This family includes medical oil crops, horticultural materials and economically important species (Tekin and Akdere, 2021; Zardi-Bergaoui et al., 2020; Nie et al., 2014). Members of this family include important phytochemical compounds such as polyphenols, flavonoids and diterpenoids (Koç et al., 2015). Therefore, species in the family usually stand out with their antioxidant, anti-inflammatory, analgesic and antipyretic activities (Dewan et al., 2013). The genus *Inula* L. belongs to the tribe Inuleae of the Asteraceae family, consists of approximately 120 species and generally spreads in Europe, Africa and Asia (Karlıoğlu-Kılıç et al., 2021; Akcın and Akcın, 2017). It was determined that some species belonging to the genus *Inula* have antibacterial, antifungal, diuretic, antispasmodic, antihemorrhoidal, anti-inflammatory, antitussive, bactericidal, antiproliferative, antidiabetic and hepatoprotective activities, and the well-known chemical components of the genus include mono-, sesqui- and diterpenes, flavonoids and glycolipids (Akcın and Akcın, 2017; Paliwal et al., 2017; Gökbulut et al., 2013). In the chemical analysis of rhizomes and roots of *I. helenium* in particular, also known as elecampane among these species, many bioactive compounds, including polysaccharide inulin, eudesmane type essential oil, sesquiterpene lactones with various biological activities, thymol derivatives, terpenes and sterols were detected (Zheng et al., 2021; Diguță et al., 2014). Traditionally, *I. helenium* species is used in the treatment of arthritis,

diabetes, rheumatism, pulmonary tuberculosis, and acute respiratory diseases as expectorant, antitussive and diaphoretic (Zhao et al., 2006; Zlatic et al., 2019). Comprehensive research on DNA-based molecular markers is being conducted all over the world (Koçak et al., 2020). Molecular markers such as RFLP, RAPD, ISSR, AFLP, SSR, EST, and SNP have been used as genetic markers to quantify genetic differences found in genomes (El Rabey et al., 2015). A popular marker technique, ISSR (Inter-Simple Sequence Repeats) is a dominant marker and widely used for molecular fingerprinting, genetic diversity, taxonomic and phylogenetic relationships, genetic linkage mapping and population structure analysis thanks to its reproducibility and easy detection by PCR (Houmanat et al., 2021; Gogoi et al., 2021; Paul et al., 2020; Rameshkumar et al., 2019; Yousefi et al., 2015; Kurane et al., 2009). The aim of this study is to make genetic relationship using ISSR markers of seven endemic species belonging to the genus *Inula* of the Asteraceae family in Turkey.

Materials and Methods

Plant Materials and Genomic DNA Isolation

In this study, seven endemic *Inula*: *Inula macrocephala* Boıss. Et Kotschy Ex Boiss, *Inula sarana* Boiss, *Inula fragilis* Boıss. Et Hausskn., *Inula tuzgoluensis* M. Öztürk and Ö. Çetin, *Inula oculus-christi* subsp. auriculata (Boiss. and Balansa) Yildirim and Senol, *Inula helenium* L. subsp. *orgyalis* (Boiss.) Grierson and *Inula helenium* L. subsp. *vanensis* Grierson species used. In the study, the leaves of the Inula plant collected from different regions of Turkey during field studies since the summer of 2013 were used. The place where the endemic species were collected, the herbarium number and date are shown in Table 1. For the genomic DNA isolation from plants, a commercial kit (GeneMark) was used. gDNA samples were stored at 20°C until use.

PCR Amplifications

For ISSR-PCR amplification, into the PCR tube; added 1 µL of genomic DNA (20–100 ng), 10 µM primer, 5 µL of master mix and 18 µL of dH_2O. The PCR program optimized for ISSR primers involved initial denaturation at 94°C for 1 minute, denaturation at 94°C for 1 minute, annealing at 47-53°C for 1 minute and extension at 72°C for 1 minute, and a final elongation at 72°C for 10 min, and the process was completed in 35 cycles. PCR products were analyzed via electrophoresis on a 1.5% agarose gel, and the amplified products were detected after being stained with ethidium bromide.

ISSR Analysis

After the PCR analyses, DNA bands were scored as follows: "1" was given if there is DNA in the DNA bands, "0" was given if there is no DNA, and "?" was given for missing data; and monomorphic bands were discarded and ISSR analyses were performed on polymorphic bands. Genetic relationship of *Inula* species used in the study was analyzed using the PAUP 4.0b10 (Swofford, 2001) program, and genetic matrix between populations was revealed by drawing UPGMA phylogenetic tree of the same program according to the arithmetic means of the pedigree trees.

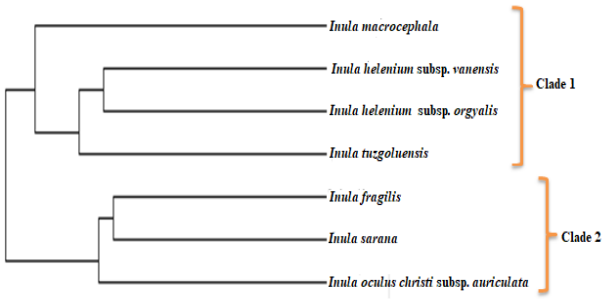

Figure 1. The UPGMA tree generated using ISSR data of seven endemic *Inula* species

Results and Discussion

The studies conducted on *Inula* to date are mostly based on sequence data. In the past years, using *Inula* species, morphological, anatomical and phenetic (Karanović et al., 2016; Paksoy, 2011; Abid and Qaiser, 2006), palynological (Karlıoğlu Kılıç et al., 2021), nrDNA ITS (Sevindik, 2014; Englund et al., 2009; Gutiérrez-Larruscain et al., 2018), cpDNA *trn*L-F, *ndh*F, *psb*A-*trn*H, *rps*16-*trn*Q, *rpl*32-*trn*L, *ndh*F-*rpl*32 (Anderberg et al., 2005; Sevindik, 2014; Englund et al., 2009; Gutiérrez-Larruscain et al., 2018), RAPD (Amin et al., 2018; Shabir et al., 2015), ISSR (Öztürk and Çetin, 2013; Amin et al., 2018) work has been done. In ISSR-PCR analysis 12 primers were used. Among these primers, UBC-810 and UBC-892 results could not be obtained (Table 2). In ISSR analysis a total of 85 bands were obtained. Of these, 74 were polymorphic and 11 were monomorphic, and the polymorphism rate was 87.05 %. According to ISSR dataset, UPGMA phylogenetic tree constructed consists of two clades. Clade 1 consists of *I. macrocephala, I. tuzgoluensis, I. helenium* subsp. *orgyalis* and *I. helenium* subsp. *vanensis* species, Clade 2 consists of *I. sarana, I. fragilis* and *I. oculus-christi* subsp. *auriculata* species (Figure 1). According to the distance matrix, the genetic distance was found the closest between *Inula fragilis* and *Inula sarana* (0.28571), while the farthest between *I. sarana* and *I. macrocephala* (0.56000) species (Table 3). Sevindik (2014) determined the molecular systematic analysis of *Inula* species with nrDNA ITS and cpDNA *trn*L-F and *ndh*F sequences. In the ITS tree created with the maximum parsimony criterion, *I. macrocephala, I. tuzgoluensis, I. helenium* subsp. *orgyalis* and *I. helenium* subsp. *vanensis* originated in a clade. On the other hand, *I. sarana* and *I. fragilis* emerged in a clade while *I. oculus-christi* subsp. *auriculata* emerged in a separate clade. In the cpDNA *trn*L-F analysis, *I. macrocephala, I. tuzgoluensis, I. helenium* subsp. *orgyalis* and *I. helenium* subsp. *vanensis* species emerged in one clade, while *I. sarana, I. fragilis* and *I. oculus-christi* subsp. *auriculata* emerged in a separate clade. According to Sevindik (2014), in cpDNA *ndh*F analysis, *I. tuzgoluensis* and *I. helenium* subsp. *orgyalis* emerged in the same clade, *I. helenium* subsp. *vanensis, I. sarana, I. fragilis, I. oculus-christi subsp. auriculata* and *I. macrocephala* emerged in a seperate clade.

Table 1. Location of seven endemic *Inula* species in Turkey

Taxa	Location
1. *Inula macrocephala*	Muş: Malazgirt, 1 km north of Kuruca village, 1750 m, 15.08.2013, Paksoy 2123 & Sevindik;
2. *Inula fragilis*	Malatya: Beydağı, above Çamurlu village, 1620 m, 14.08.2013, Paksoy 2121 & Sevindik;
3. *Inula helenium subsp. orgyalis*	Kastamonu: Between Eflani-Daday, 20th km, 1150 m, 01.08.2013, Paksoy 2086 &Sevindik
4. *Inula helenium subsp. vanensis*	Van: Çatak, around Atlıhan village, valley edge, 1300 m, 16.08.2013, Paksoy 2125 & Sevindik
5. *Inula sarana*	Mersin: Anamur, on the Anamur-Kazancı highway, Suollmaz crossing, 1820 m, 28.07.2013, Paksoy 2056 & Sevindik
6. *Inula tuzgoluensis*	Konya; Cihanbeyli, between Gölyazı and Tuzgölü, after Dumanağıl location, 923 m. 28. 07. 2013, Paksoy & Sevindik 2142
7. *Inula oculus christi subsp. auriculata*	Izmir; Ödemiş, Bozdağ, summit road, around the ski slope, 1850 m, 26.07.2013, Paksoy 2051 & Sevindik

Table 2. Primers used in the ISSR-PCR reactions and their Tm degrees

ISSR Primers	DNA Sequences (5'-3')	Tm	Amplification
UBC-831	5'-CTCTCTCTCTCTCTCTT-3'	50°C	+
UBC-830	5'-TGTGTGTGTGTGTGTGG-3'	52°C	+
UBC-807	5'-AGAGAGAGAGAGAGAGT-3'	50°C	+
UBC-808	5'-AGAGAGAGAGAGAGAGC-3'	52°C	+
UBC-836	5'-AGAGAGAGAGAGAGAGYA-3'	52°C	+
UBC-892	5'-TAGATCTGATATCTGAAT-3'	52°C	-
UBC-810	5'-GAGAGAGAGAGAGAGAT-3'	50°C	-
UBC-826	5'-ACACACACACACACACC-3'	52°C	+
UBC-811	5'-GAGAGAGAGAGAGAGAC-3'	53°C	+
UBC-834	5'-AGAGAGAGAGAGAGAYT-3'	52°C	+
UBC-873	5'-GACAGACAGACAGACA-3'	48°C	+
UBC-808	5'-AGAGAGAGAGAGAGAGC-3'	52°C	+

Table 3. Pairwise genetic distance matrix obtained from ISSR primers

Species	1	2	3	4	5	6	7
I. macrocephala	-	0.35065	0.56000	0.37313	0.37143	0.43103	0.49296
I. fragilis	27	-	0.28571	0.41791	0.44776	0.34483	0.30882
I. sarana	42	20	-	0.40000	0.46667	0.47059	0.31148
I. helenium subsp. vanensis	25	28	24	-	0.29851	0.34483	0.43103
I. helenium subsp. orgyalis	26	30	28	20	-	0.32759	0.42623
I. tuzgoluensis	25	20	24	20	19	-	0.39655
I. oculus christi subsp. auriculata	35	21	19	25	26	23	-

The present results are generally compatible with ITS and *trn*L-F results, and incompatible with *ndh*F results. Karlıoğlu Kılıç et al. (2021) investigated the pollen morphology of eight endemic *Inula* species expanded in Turkey. In the UPGMA tree created with morphological data, *I. macrocephala, I. helenium* subsp. *orgyalis* and *I. helenium* subsp. *vanensis* species emerged in one group, *I. fragilis* and *I. sarana* species emerged in another group. These results are compatible with our ISSR results. Paksoy's (2011) taxonomic revision results of *Inula* species in Turkey also supported our ISSR results. Öztürk and Çetin (2013) used 10 ISSR primers in their study and obtained 113 polymorphic bands. In the UPGMA tree created based on the ISSR data set, it was determined the *I. tuzgoluensis* species was found together with *I. aucheriana, I. oculus-christi, I.heterolepis* and *I.britannica* species. As a result, in this study; by using ISSR primers, 85 bands were obtained and the polymorphism rate was 87.05%. The UPGMA phylogenetic tree generated with ISSR data was compatible with the morphological, palynological and molecular results from previous years. Hence, our results suggest that ISSR analyzes are suitable for the differentiation and phylogenetic analysis of endemic *Inula* species.

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