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Research Article

Genetic Diversity Analysis of Aydın/Turkey *Dittrichia viscosa* (L). Greuter (Asteraceae) Populations Using RAPD Markers

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Abstract

In this study, we performed a genetic diversity using RAPD markers for some *Dittrichia viscosa* populations grown in the Aydın region of Turkey. Total genomic DNA isolation from the leaves of *Dittrichia viscosa* was performed using commercial kit. eight RAPD primers were used to determine genetic diversity among populations. Polymerase Chain Reaction (PCR) was performed with all DNA samples and primers with ability of scoreing band. PCR products were run in agarose gel and visualized under UV light. They scored as there were bands (1) and no bands (0) at all gel images and their files were created. A total of 50 characters were obtained from the primers. Phylogenetic relationships and genetic distances between the cultivars were calculated by using the PAUP* 4.0b10 analysis program. According to the PAUP data, the closest genetic distance was determined 0.20000 and between Central and İncirliova populations, the most distant genetic distance were determined 0.36842 between İncirliova and Koçarlı populations. In the phylogenetic analysis has been obtained using UPGMA algorithms and phylogenetic tree consist of two clades. The results also propose that RAPD markers are useful tools for indicating genetic relationships among *Dittrichia viscosa* populations.

Key words: *Dittrichia viscosa*, RAPD, genetic diversity, Turkey.

Aydın/Türkiye'de Yayılış Gösteren *Dittrichia viscosa* (L). Greuter (Asteraceae) Popülasyonlarının RAPD Markırları ile Genetik Çeşitliliğinin Tespiti

Özet

Bu çalışmada, Türkiye'nin Aydın bölgesinde yayılış gösteren *Dittrichia viscosa* popülasyonlarının RAPD markırları kullanılarak genetik çeşitliliği gerçekleştirilmiştir. *Dittrichia viscosa* örneklerinin yapraklarından genomik DNA izolasyonu ticari kit kullanılarak gerçekleştirildi. Sekiz adet RAPD primeri popülasyonlar arasındaki genetik çeşitliliği belirlemek için kullanılmıştır Polimeraz Zincir Reaksiyonu, DNA örnekleri ve primerler kullanılarak gerçekleştirildi. PCR ürünleri agaroz jel elektroforezinde yürütülüp, UV ışığı altında görüntülendi. Tüm jel görüntüleri incelenmiş olup polimorfik bantların varlığı ve yokluğu 0 ve 1 olarak skorlandı. Primerlerden toplam 50 karakter elde edildi. Popülasyonlar arasındaki filogenetik ağaç ve genetik uzaklıklar PAUP* 4.0b10 analiz programı kullanılarak hesaplandı. PAUP analizine göre, en yakın genetik mesafe 0.20000 değer ile Merkez ve İncirliova popülasyonları arasında iken, en uzak genetik mesafe 0.36842 değer ile İncirliova ve Koçarlı popülasyonları arasında çıkmıştır. Filogenetik ağaç UPGMA algoritması kullanılarak elde edilmiş olup, ağaç iki kladdan oluşmuştur. Sonuçlar, RAPD markırlarının *Dittrichia viscosa* popülasyonları arasındaki genetik ilişkileri göstermek için yararlı araçlar olduğunu öne sürmektedir.

Anahtar kelimeler: *Dittrichia viscosa*, RAPD, genetik çeşitlilik, Türkiye.

Introduction

Asteraceae is one of the largest plant families and contains many species and genus. This family includes around 25000 species in the world (Kılıç, 2014). Members of this family have an important economic value and they are used in food, medicine, ornamental plant industries as well as pharmacology (Süslü et al., 2010). The genus *Dittrichia* was first defined by Greuter (1973) while it used to be accepted as a section of *Inula* beforehand (Petropoulou et al., 2004). It has a wide Mediterranean spread with a marginal domain in the Middle East and Atlantic Europe regions. *Dittrichia viscosa* [Syn. *Inula viscosa* (L.) Aiton] is a green plant that grows common in the Mediterranean region (Topakçı et al., 2005). *Dittrichia viscosa* is a plant which is used in antiviral, antifungal and antimicrobial studies thanks to its common biologic activity, the lactones involved and components such as sesquiterpenes. In addition, fresh upper parts of the plant is used for wound treatment in public medicine in Turkey (Baytop, 1984; Al-Qudah et al., 2010).

The advancement of DNA marker technology has offered an efficient way to protect plant genetic sources and facilitate their management. This technology can reveal differences between DNA-level heredity compared to morphologic analyses. It is an opportunity to give information about the variations in certain countries in a local region and different countries. Molecular markers help detect distinctive characteristics of species, genetic relativity and phylogenetic relationships between species (Kiani, 2011; Rahman et al., 2007). Random Amplified Polymorphic DNA (RAPD) is a molecular marker that has been widely employed to understand genetic diversity in various plants. RAPD markers can be used in population genetics and conservation genetics and in taxonomical classification studies (Coşkun and Parlak, 2013; Abraham et al., 2010; Kavalcıoğlu et al., 2010; Namkoong and Roberds, 1993). The aim of this study, we performed a genetic diversity using RAPD markers for some *Dittrichia viscosa* populations grown in the Aydın region of Turkey.

Material and Methods

Plant Material and DNA Isolation

D.viscosa plant samples used in this study were collected from different districts of Aydın province in October, 2017. Localities where the samples were collected are presented in Table 1. The samples were brought to the plant biotechnology laboratory in the Department of Agricultural Biotechnology, Faculty of Agriculture at Adnan Menderes University, within silica gels. GenMark DNA isolation kit and related procedure were applied for total genomic DNA isolation. DNA samples were stored -20 °C. In order to visualize gDNA's 0.8% standard agarose gel electrophoresis procedure was performed.

Table 1. Locations of *Dittrichia viscosa* (L). Greuter populations

No	Populations	Locations
1	Koçarlı population	Aydın Koçarlı roadside approximately altitude 290 m
2	Çine population	Aydın Çine roadside approximately altitude 80 m
3	Central population	Adnan Menderes University, Central Campus, approximately altitude 62 m
4	İncirliova population	Aydın İncirliova exit roadside approximately altitude 60 m
5	Çakmar population	Aydın Faculty of Agriculture, Çakmar location, approximately altitude 60 m

RAPD-PCR Amplification

In this study, eight RAPD primers were used. The RAPD primers used are listed in Table 2 for DNA sequences Tm temperatures. The amplification process was performed in 25 µl of PCR reaction volume. Each PCR reaction contained 5 µL master mix (PCR buffer, $MgCl_2$, dNTP, Taq DNA polymerase), 1 µL RAPD primers, 2.0 µl of total genomic DNA (1/5 rates diluted), and 17µL of dH_2O. PCR amplification was performed with an initial denaturation step of 94 °C for 2 min, followed by 35 cycles of strand denaturation at 94 °C for 2 m, annealing at 32-34 °C for 1 m, and primer extension at 72 °C for 1 min, and a final elongation at 72 °C for 10 min. Amplification products were analyzed by electroporesis on 0,8% agarose gel buffered with 0.5X TBE (Tris-Borate-EDTA), stained with ethidium bromide and photographed under ultraviolet light. As a results of RAPD-PCR some of the gel photos were shown in Figure 1, Figure 2.

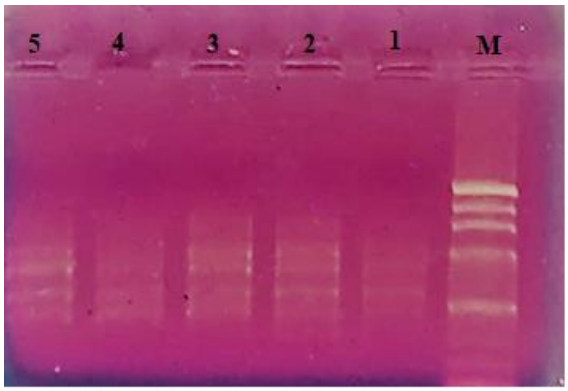

Figure 1: RAPD-PCR gel photo amplified with OPA-02

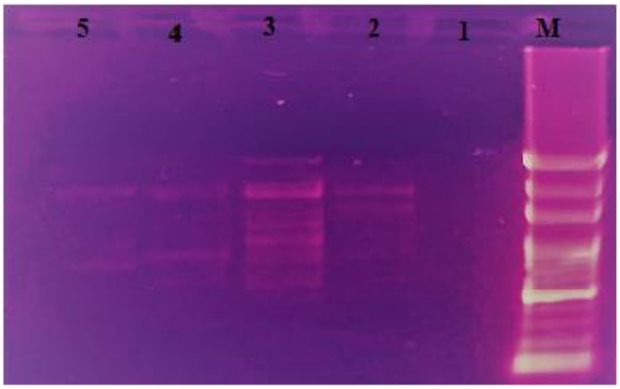

Figure 2: RAPD-PCR gel photo amplified with OPE-08

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

Primer	DNA Sequences	Tm
OPA-15	5'-TTCCGAACCC-3'	32°C
OPA-20	5'-GTTGCGATCC-3'	32°C
OPE-08	5'-TCACCACGGT-3'	32°C
OPA-16	5'-AGCCAGCGAA-3'	32°C
OPA-18	5'-AGGTGACCGT-3'	32°C
OPA-05	5'-AGGGGTCTTG-3'	32°C
OPA-02	5'- TGCCGAGCTG-3'	34°C
OPA-13	5'- CAGCACCCAC-3'	34°C

Data Analysis

The photographs were used for the evaluation of the results in the analysis of RAPD In the reading of the bands formed at the end of the amplification, only the strong bands were taken into consideration. The presence (1) and absence (0) of bands were specified while forming the data. PAUP (Phylogenetic Analysis Using Parsimony and other methods, Version 4.0b10) software was used to perform phylogenetic analyses using RAPD data (Swofford 2002).

Results and Discussions

In the RAPD- PCR analysis total of 50 bands, and 30 of them were polymorphic. The polymorphic bands rated approximately 60%. PAUP 4.0b10 analysis program was used to calculate the phylogenetic trees and genetic distances between populations. According to the PAUP data, the closest genetic distance was determined 0.20000 and between Merkez and İncirliova populations, the most distant genetic distance were determined 0.36842 between İncirliova and Koçarlı populations (Table 3). The phylogenetic tree was obtained using the UPGMA algorithm, and the tree consisted of two clades (Figure 3). The clade 1 consists of Koçarlı and Çine populations. Clade 2, consisted of, Central population, İncirliova population and Çakmar population. Koçarlı and Çine populations are geographically close to each other. The dendrogram obtained as a result of RAPD-PCR was found in these populations clade 1. Both geographic distribution and RAPD-PCR results are consistent. In clade 2, Central population and İncirliova population were found within the same group. These populations are geographically close to each other. This showed that the geographical distribution of RAPD-PCR and populations was consistent. The RAPD technique has found a wide application area in many fields of biology due to its simplicity and low cost. This technique can be used in a wide range of areas from the detection of genetic relations between populations, to the gene mapping and the plant and animal improvement studies (Bardakçı, 2001). In past studies; such as *Pulicaria* (El-Kamali et al., 2010), *Helianthus annuus* (Raza et al., 2018), *Jatropha* (Ram et al., 2008), *Morus* (Awasthi et al., 2004), *Eriobotrya japonica* (Vilanova et al., 2001), *Allium* (Wilkie et al.,1993), *Oryza sativa* (Suh et al., 1997), *Bellis perennis* (Kavalcıoğlu et al., 2010), *Malus domestica (*Daler et al., 2017), *Pyrus communis* (Gencer et al., 2018), *Hordeum vulgare* (Olgun et al., 2015), *Lactuca sativa* (Sharma et al., 2018) there are studies on RAPD in many plant species.

Table 3. Pairwise genetic distance matrix obtained from RAPD primers

Populations	Koçarlı	Çine	Central	İncirliova	Çakmar
Koçarlı	-	0.28947	0.26316	0.36842	0.31579
Çine	11	-	0.30000	0.30000	0.30000
Central	10	15	-	0.20000	0.36000
İncirliova	14	15	10	-	0.24000
Çakmar	12	15	18	12	-

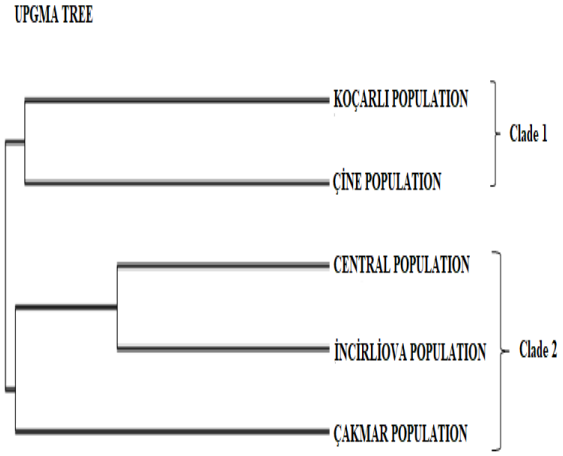

Figure 3: UPGMA dendogram obtained from RAPD data

Conclusion

To conclude, this study reveals the genetic relations in the populations of *Dittrichia viscosa* which spreads in Aydın province by using 8 RAPD primers. Genetic distance and phylogenetic relation between the populations were revealed and the groups were formed through the results based on RAPD data. In addition, this research will offer some information about the relations between different populations located in the region. Undoubtedly, more data including more taxa (e.g. Morphologic and/or DNA sequence data) will improve this conclusion and yield more reliable results.

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