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Phylogenetic Analysis of *Chrysochamela* (Fenzl) Boiss. (Brassicaceae) Taxa Growing in Turkey

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ABSTRACT

In this study, we performed a genetic diversity analysis using RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter simple sequence repeat) markers for some *Chrysochamela* species growing in Turkey. PCR was performed using RAPD and ISSR primers and genomic DNA samples from each specimen, and the resulting bands were scored. Phylogenetic relationships and genetic distances between the studied taxa were calculated using the PAUP analysis program. According to the RAPD analysis, the closest genetic distance was found to be 0.20000 between *C. noeana* populations, while the most distant values were found as 0.60000 between *C. noeana* and *C. elliptica*. The ISSR analysis also revealed the closest genetic distance as 0.34043 between *C. noeana* populations, while the most distant values were found to be 0.55319 between *C. noeana* and *C. elliptica*. UPGMA (unweighted pair group method with arithmetic mean) dendrogram generated for both RAPD and ISSR analyses consisted of two groups. As a result, it was found that RAPD data were more appropriate for comparison with the previous anatomical and morphological data of the studied taxa.

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Introduction

Turkey is one of the leading countries in terms of floristic richness. With the 12,000 plant taxa, it contains a flora that is much richer than the flora of many countries. When considering the fact that the amount of floristic richness of the whole continent of Europe is 13,000 plant taxa, the richness of Turkey in terms of plant diversity can be understood more clearly (Sezer et al., 2017). The Brassicaceae (Cruciferae) comprises 49 tribes, 321 genera and 3660 species in the world (Al-Shehbaz, 2012). Southwest Asia, especially Turkey, is generally considered as one of the centers of the greatest diversity, and possible origin, of the family (Yüzbaşıoğlu et al., 2015). This family is an important family of plants, and it includes edible and economic species, vegetables, industrial and edible oil sources, spices and important model organisms (Al-Shehbaz et al., 2006; Ahmed et al., 2012; Filiz et al., 2014). For example, while *Arabidopsis thaliana* is used as a model plant in almost all fields of experimental botany, *Brassica oleracea* complex provides many clues about the genetics of blossoming time (Liu et al., 2012). In Turkey, Brassicaceae is represented by 571 species with 65 subspecies, 24 varieties and 660 taxa belonging to 91 genera (Filiz et al., 2014). The genus *Chrysochamela* (Fenzl) Boiss. is one of the genera of Cruciferae, includes 4 species, and it is distributed across Turkey, Russia, Lebanon and Syria (Appel and Al-Shehbaz 2003). In the flora of Turkey, there are 3 species of *Chrysochamela* genus viz., [(*C. elliptica* (Boiss.) Boiss., *C. velutina* (DC.) Boiss. and *C. noeana* (Boiss.) Boiss.)] (Çakılcıoğlu et al., 2017).

Morphological characteristics used in the classification of plants are also used to determine the diversity and relationships between plant species. However, these characteristics are not reliable enough because of the environmental effects that can readily modify them. In the last decades, the usefulness of molecular markers has been searched for more precise identification and differentiation of different species (Kavalcıoğlu et al., 2010). Markers commonly used in molecular genetic analyses are RFLP, CAPS, STS, SSR, SNP, RAPD, SCAR, AFLP and ISSR markers (Ghasemi et al., 2014). Random amplification of polymorphic DNA (RAPD) markers which are used as a rapid method for screening and identifying variants at DNA level have been widely applied in population genetics (Wang et al., 2008). Inter-simple sequence repeats (ISSR) are very similar to RAPD except ISSR primers are designed to target microsatellite regions. Therefore, ISSR markers have more useful information than that of RAPD (Khorshidi et al., 2017). The use of these markers is easy and quick; and it is an effective technique that can be applied in determining genetic variations, in the formation of genome biology maps, and in phylogenetics (Yorgancılar et al., 2015).

This study aims to conduct a phylogenetic analysis based on RAPD and ISSR markers among 5 *Chrysochamela* taxa of 3 species which are distributed across Turkey and to provide information for phylogenetic studies to be carried out in the future with different markers.

Materials and Methods

Plant Materials, Genomic DNA Isolation, and PCR Reactions

The fresh green leaves from 5 taxa of *Chrysochamela* genus were collected from Sivas, Şanlıurfa and Ankara counties of Turkey (Table 1) and brought to the plant biotechnology laboratory at Aydın Adnan Menderes University. Total genomic DNA isolation from the leaves of *Chrysochamela* was performed using a Plant Genomic DNA Purification Kit (GeneMark). DNA samples were stored at -20°C. In order to visualize gDNA samples, 1% of standard agarose gel electrophoresis procedure was performed. For RAPD-PCR amplifications, eight RAPD primers were used (Table 2). RAPD amplification reactions were carried out in 25 µL volumes containing 5 µL master mix (PCR buffer, $MgCl_2$, dNTPs and Taq DNA polymerase), 1 µL RAPD primers, 2 µL total genomic DNA (about 50 ng), and 17 µL sterilized dH_2O. RAPD-PCR amplifications were performed with an initial denaturation step of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 32-34°C for 45 s and primer extension at 72°C for 2 min, followed by a final elongation at 72°C for 8 min. For ISSR-PCR amplifications, eight ISSR primers were used (Table 3). ISSR amplification reactions were carried out in 25 µL volumes containing 5 µL master mix (PCR buffer, $MgCl_2$, dNTPs, Taq DNA polymerase), 1 µL ISSR primers, 2 µL of total genomic DNA (about 50 ng), and 17 µL of sterilized dH_2O. For ISSR-PCR, amplifications were performed with an initial denaturation step of 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50-52°C for 1 min and primer extension at 72°C for 1 min, followed by a final elongation at 72°C for 10 min. Amplification products were analyzed by electrophoresis on 1% agarose gels buffered with 1X TBE (Tris-Borate-EDTA), stained with ethidium bromide and photographed under ultraviolet light (Figure 1, Figure 2).

Table 1 Species used for molecular studies and their collected localities.

Species	Collection areas and collector's number
C. elliptica	B4 Ankara: Between Ayas and Beypazarı, before 5 km to Beypazarı, gypsum slopes, 590 m, Paksoy 1446
C. velutina	C7 Şanlıurfa: Ceylanpinar, Hamitköy vicinity, rock cracks, 400 m, Paksoy 1401
C. noeana	B6 Sivas: Between Düzova and Dedeli village, Rock slopes, 1300 m, Paksoy 1482
C. noeana	B6 Sivas: Hafik, Tuzhisar village, rock slopes, 1000 m, Paksoy 1484
C. noeana	B6 Sivas; Lake Tödürge, around Cumhuriyet University social facilities, 1300 m, Paksoy 1483

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

Primer	DNA Sequences	Tm °C
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

Table 3 Primers used in the ISSR-PCR reactions and their Tm degrees

Primer	DNA Sequences	Tm °C
UBC-831	5'-CTCTCTCTCTCTCTCTT-3'	50°C
UBC-830	5'-TGTGTGTGTGTGTGTGG-3'	52°C
UBC-807	5'-AGAGAGAGAGAGAGAGT-3'	50°C
UBC-826	5'-ACACACACACACACACC-3'	52°C
UBC-808	5'-AGAGAGAGAGAGAGAGC-3'	52°C
UBC-810	5'-GAGAGAGAGAGAGAGAT-3'	50°C
UBC-819	5'- GTGTGTGTGTGTGTGTA -3	50°C
UBC- 873	5'-GACAGACAGACAGACA-3'	52°C

Data Analysis

The photographs were used for the evaluation of the results obtained from the analyses of RAPD and ISSR-PCR. When scoring the amplified bands formed after electrophoresis, only the strong bands were taken into consideration. The presence (1) and absence (0) of the bands were specified to construct the data file. PAUP (Phylogenetic Analysis Using Parsimony and other methods, Version 4.0b10 software was used to perform phylogenetic analyses using RAPD and ISSR data (Swofford, 2001).

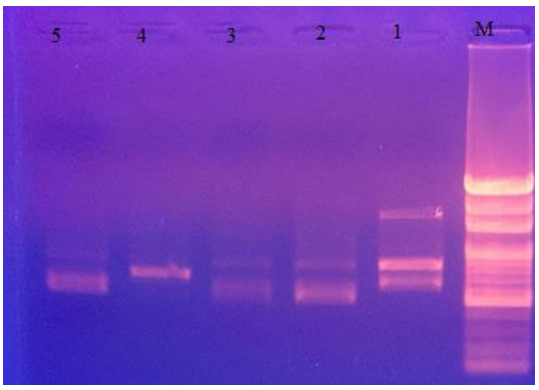

Figure 1 Gel image of the bands amplified using OPA-18 primer in RAPD-PCR

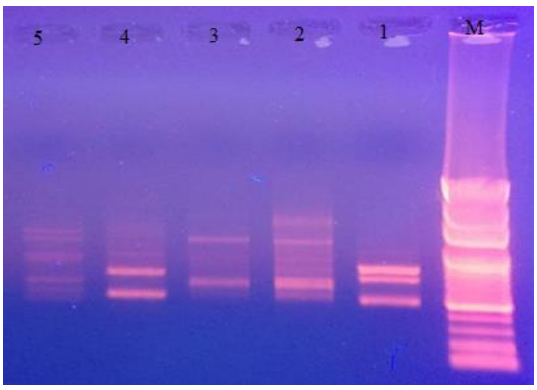

Figure 2 Gel image of the bands amplifies using UBC-831 primer in ISSR-PCR

Results and Discussion

Molecular markers have developed a wide range of applications in the field of molecular biology including phylogenetics, ecology, population genetics and study of complicated genomic traits in both plants and animals (Raza et al., 2019). Both RAPD and ISSR analyses were performed using the PAUP 4.0b10 program. In the RAPD-PCR analysis, a total of 41 characters were detected as 10 constant, 15 variable parsimony-uninformative and 16 variable parsimony-informative characters. PAUP 4.0b10 analysis program was used to construct the phylogenetic trees and calculate the genetic distances between populations. According to the PAUP analysis, the closest genetic distance was found to be 0.20000 between *C. noeana* populations, while the most distant value was found as 0.60000 between *C. noeana* and *C. elliptica* (Table 4). In accordance with the RAPD-PCR data, UPGMA phylogenetic tree consisted of 2 major groups (Figure 3). Group A consisted of only *C. elliptica* species while group B consisted of *C. noeana* populations which were collected from different regions of Sivas province. *C. velutina* was detected as a relative of these populations. In the ISSR- PCR analysis, a total of 58 characters were detected as 6 constant, 32 variable parsimony-uninformative and 20 variable parsimony-informative characters. PAUP 4.0b10 analysis program was used to construct the phylogenetic trees and calculate the genetic distances between populations. According to the PAUP analysis, the closest genetic distance was found to be 0.34043 between *C. noeana* populations, while the most distant value was 0.55319 between *C. noeana* and *C. elliptica* (Table 5). In accordance with ISSR-PCR results, UPGMA phylogenetic tree consisted of two major groups (Figure 4). In Group A, 3 populations of *C. noeana* formed a group and *C. elliptica* was a relative of this group. Group B only consisted of *C. valutina* species. When we consider both RAPD and ISSR results, *C. noeana* populations were observed to be together. However, while *C. valutina* was close to these populations in RAPD results, the ISSR results displayed *C. elliptica* as the close taxa to the same populations. Çakılcıoğlu et al. (2017) carried out a comparative micromorphology and anatomy study on *Chrysochamela* species, and reported that in leaf anatomy, there was collenchyma under phloem in *C. noeana* and *C. velutina*, while there was not in *C. elliptica*. In addition to this, while the existence of collenchyma on xylem was detected in *C. elliptica* and *C. noeana*, it was not detected in *C. velutina*. In trunk anatomy study, while cortex endodermis layer was detected to be 1-2 layer and pericycle was detected to be 1-3 layer in *C. velutina* and C. *noeana*, both layers were detected to be 1 in *C. elliptica*. In seed morphology analysis, while the seed shape was found to be elliptic in *C. velutina* and *C. noeana*, it was found to be oblong in *C. elliptica*; while epidermal cell shape was detected to be irregular in *C. velutina* and *C. noeana*, it was detected to be polygonal isodiametric in *C. elliptica*. When micromorphological and anatomic characters were analyzed, they were found to support RAPD analysis. In RAPD analysis, *C. noeana* populations and *C. velutina* species were found to be in the same group in terms of phylogenetics.

Table 4 Pairwise genetic distance matrix obtained from RAPD primers

Taxa	1	2	3	4	5
*C. elliptica (*Ankara)	-	0.60000	0.52500	0.40000	0.47500
C. noeana (Sivas)	24	-	0.32500	0.35000	0.32500
C. noeana (Sivas)	21	13	-	0.32500	0.20000
C. velutina (Urfa)	16	14	13	-	0.37500
C. noeana (Sivas)	19	13	8	15	-

Table 5 Pairwise genetic distance matrix obtained from ISSR primers

Taxa	1	2	3	4	5
*C. elliptica (*Ankara)	-	0.53846	0.42553	0.51852	0.44828
C. noeana (Sivas)	28	-	0.34146	0.50000	0.40385
C. noeana (Sivas)	20	14	-	0.55319	0.34043
C. velutina (Urfa)	28	24	26	-	0.42593
C. noeana (Sivas)	26	21	16	23	-

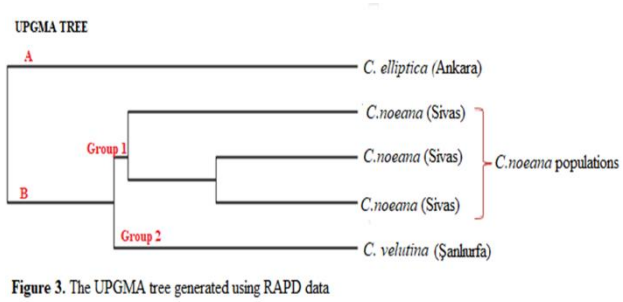

Figure 3 The UPGMA tree generated using RAPD data

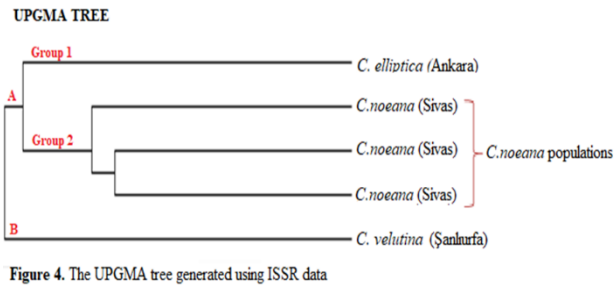

Figure 4 The UPGMA tree generated using ISSR data

Conclusion

In conclusion, the phylogenetic status of the *Chrysochamela* species growing in Turkey was determined by RAPD and ISSR markers. The results suggest that RAPD data were more appropriate for comparison with the previously documented anatomical and morphological evaluations.

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