UDC 575.
https://doi.org/10.2298/GENSR2001107S
Original scientific paper

GENETIC VARIATION AND MOLECULAR RELATIONSHIPS TAXA OF *Conringia* HEIST. EX FABR. (BRASSICACEAE) BASED ON RAPD MARKERS IN TURKEY

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Sevindik E., M. Y. Paksoy, M. Aydoğan, F. Topseçer (2020). *Genetic variation and molecular relationships taxa of Conringia Heist. ex Fabr. (Brassicaceae) based on RAPD markers in Turkey*.- Genetika, Vol 52, No.1, 107-114.

In this study, genetic variation and phylogenetic analysis of 13 populations of 6 species belonging to *Conringia* genus spreading in Turkey were performed using RAPD markers. Genomic DNA isolation from the leaves of the *Conringia* plant samples was performed via using a commercial kit. Seven RAPD primers were used to identify the genetic diversity between the populations. Polymerase Chain Reaction (PCR) was performed using DNA samples and primers. PCR products were resolved using agarose gel electrophoresis and visualized under UV light. All gel images were analyzed, and the absence and presence of polymorphic bands were scored. The total of 34 DNA bands were detected by seven RAPD primers. PAUP 4.0b10 analysis program was used to calculate phylogenetic tree and genetic distances between the species. The phylogenetic tree was obtained using the UPGMA algorithm and it was composed of two clades. According to the PAUP analysis, the species having the closest distance between each other are *C. planisiliqua* (Ankara-Ayaş) and *C. planisiliqua* (Ankara-Nallıhan) with the value of 0.000 and those having the longest distance are *C. grandiflora* (Akseki Çukurköy) and *C. orientalis* (Elazığ-Baskil) with the value of 0.6000. The results suggest that the RAPD markers are useful tools to demonstrate the genetic relationships between populations of the *Conringia* species.

Keywords: *Conringia*, RAPD-PCR, genetic variation, phylogenetic, Turkey

INTRODUCTION

Turkey, one of the world's unique countries in terms of biological diversity, is a leading country in the world in terms of floristic richness. Particularly high mountains and deep valleys that people cannot reach show rich plant diversity. Turkey hosts a richer flora compared to those of

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other countries with approximately 12000 plant taxa (SEZER *et al.* 2017; AKINCI *et al.*, 2018). Brassicaceae family has members distributed to the world, most of which spread in the temperate regions of the Northern Hemisphere with diversification centre's in the Iranian-Turanian regions (LIU *et al.*, 2012). This family is represented in the world with 49 tribes, 321 genera and around 3660 species (AL-SHEHBAZ, 2012). It has 606 species, including 226 endemics in Turkey, 39 subspecies and 18 varieties (ERDEN and MENEMEN, 2017). It is ranked 4th among the richest families in terms of the number of species in Turkey (MUTLU, 2002). These family members include economically important, edible and industrial oilseeds, spice plants, vegetables and some forage plants (AHMED *et al*., 2012; GIDIK *et al.*, 2016). It also includes the molecular plant model, such as *Arabidopsis thaliana* (WARWICK, 2011). *Conringia* genus belongs to Brassicaceae family. It is represented by 6 species in Turkey (*C grandiflora* Boiss. & Heldr, *C. orientalis* (L.) Dumort., *C. persica* Boiss, *C. austriaca* (Jacq.) Sweet, Hort, *C. perfoliata* (synonym of *C. clavata* C.A. Mey.) Busch, *C. planisiliqua* Fisch. & Mey), one of which (C. *grandiflora*) is endemic with approximately 17% endemism rate (SELVI *et al.*, 2019). Many molecular marker techniques have been developed and widely used in plant systematic studies, measurement of variation to establish phylogenetic relationships within or between species, and population genetic research (HWANG and HUH, 2016). One of these techniques, RAPD-PCR (random amplified polymorphic DNA) technique, it is easy to use and features random primers with a length of 9–10 bases (SESLI and YEGENOĞLU, 2017). RAPD markers technique can be used in population genetics, genetic diversity among crop species, conservation genetics and in taxonomical classification studies (WANG *et al*., 2008; ÁBRAHÁM *et al*., 2010; COŞKUN and PARLAK, 2016; IQBAL *et al.*, 2018). In this study, genetic variation and phylogenetic analysis of species belonging to *Conringia* genus spreading in Turkey were performed using RAPD markers.

MATERIALS AND METHODS

Plant samples and DNA isolations

The research material consists of *Conringia grandiflora, C. orientalis, C. persica, C. austriaca, C. perfoliata* and *C. planisiliqua* samples which were collected from different regions of Turkey. The localities of the collected samples are presented in Appendix 1. *Conringia* species were brought to the laboratory and some of them were used for the isolation of genomic DNA while some were made herbarium samples. For the isolation of genomic DNA, a commercial kit (GeneMark Catalog No: DP022) was used.

PCR Amplification

RAPD primers chosen for PCR amplifications were given in Table 1. Ready mix were used as an alternative to PCR reaction. Amplification was carried out by adding 2 μL genomic DNA, 1 μL primers, 5 μL master mix Cat. No: RP02-II-400, RP02-II-2000 (0.75U of Taq DNA polymerase, reaction buffer, 2mM $MgCl_2$, 250μM dNTPs and enzyme stabilizer) and 17 μL dH_2O into PCR tube. The used PCR programs were separately created for each primary based on temperature T_M values of the primers which were used in studies by grounding on reference

articles (Table 2). PCR products were analyzed by electrophoresis on 1% agarose gel and the amplified products were detected after staining with ethidium bromide.

Table 1. Primers used in the RAPD-PCR reactions and their Tm degrees

Primer	DNA Sekansı (5'-3')	Tm (^{o}C)
OPA-15	5'-TTCCGAACCC-3'	32 ^{o}C
OPA-20	5'-GTTGCGATCC-3'	32 ^{o}C
OPA-02	5'-TGCCGAGCTG-3'	34 ^{o}C
OPA-13	5'-CAGCACCCAC-3'	34 ^{o}C
OPE-08	5'-TCACCACGGT-3'	32 ^{o}C
OPA-18	5'-AGGTGACCGT-3'	32 ^{o}C
OPA-05	5'-AGGGGTCTTG-3'	32 ^{o}C

Table 2. Cycles and conditions RAPD-PCR reactions

Step	Heat/Time	Cycles
1. step	94^{o}C/2 min	1Cycle
2. step	94^{o}C/1 min.	35 Cycles
3. step	32-34^{o}C/1 min	35 Cycles
4. step	72^{o}C/1 min	35 Cycles
5. step	72^{o}C/10 min.	1 Cycle

Data and phylogenetic analysis

Following the PCR analysis, DNA bands were scored as "1" in the presence of DNA, "0" in the absence of DNA and a ''?'' for missing data; DNA bands were scored in this way, and monomorphic and polymorphic bands were determined. Genetic relations of *Conringia* species which were used in the research were analysed by using PAUP 4.0b10 (SWOFFORD, 2001) program; UPGMA (unweighted pair group method with arithmetic mean) phylogenetic tree of the same program was drawn based on arithmetic means of family trees, and genetic distance matrix between populations was created.

RESULTS AND DISCUSSION

A total of 34 bands were obtained at the end of RAPD-PCR analysis. Of these bands, 4 were monomorphic, 30 were polymorphic bands. Polymorphism rate was determined as approximately 88%. PAUP 4.0b10 phylogenetic analysis program was used for UPGMA tree consisting of 2 large clades. Clade 1 consists of *C. perfoliata* (Sivas-Yıldızeli), *C. perfoliata* (Kayseri-Bunyan), *C. perfoliata* (Ankara-Ayas) and *C. grandiflora* (Akseki-Çukurköy) species. Clade 2 is divided into two groups; Group A, consists of *C. planisiliqua* (Kırşehir-Çamlıkpark), *C. orientalis* (Ankara-Polatlı), *C. orientalis* (Elazığ-Baskil) and *C. planisiliqua* (Sivas-

Tuzluhisar) species while group B consists of *C. planisiliqua* (Kayseri-Bunyan), *C. austriaca* (Adana-Kozan), *C. planisiliqua* (Ankara-Ayas), *C. planisiliqua* (Ankara-Nallıhan) and *C. persica* (Van-Gürpınar) species (Fig.1).

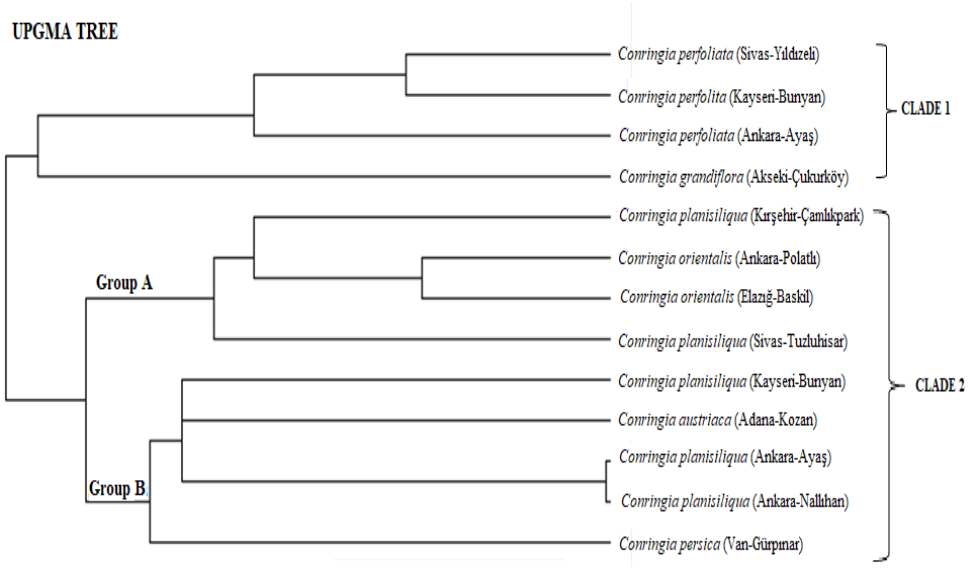

Fig.1. The UPGMA tree generated using RAPD data of *Conringia* species

The genetic distance matrix between the species was performed using the PAUP program. The species having the closest distance between each other are *C. planisiliqua* (Ankara-Ayaş) and *C. planisiliqua* (Ankara-Nallıhan) with the value of 0.000 and those having the longest distance are *C. grandiflora* (Akseki Çukurköy) and *C. orientalis* (Elazığ-Baskil) with the value of 0.6000 (Table 3). WARWICK and SAUDER. (2005) conducted the phylogenetic analysis of Brassicaceae tribe using the chloroplast restriction site polymorphisms and nuclear ribosomal internal transcribed spacer (ITS) and chloroplast *trn*L intron regions. According to the *trn*L intron and chloroplast restriction site polymorphisms results of the study, the species of *C. orientalis* and *C. perfoliata* appeared in the same group. According to the ITS region, *Conringia perfoliata* and *Calepina irregularis* species were found in the same group. LIU *et al*. (2012) conducted the phylogenetic relationships of the species belonging to the Brassicaceae family using chloroplast *mat*K sequences. In the study, only *C. planisiliqua* species were used in *Conringia* genus. *C. planisiliqua* species appeared in the same branch with *Isatis tinctoria* and *Isatis minima* species. SELVI *et al*. (2019) have conducted micromorphological and anatomical studies of *Conringia* species distributed in Turkey. In anatomical studies, they examined the root, stem and leaves in detail. In micromorphological studies, they examined the stem and leaf surfaces of the species. As a result of their studies, leaf mesophile structures, number of root vascular bundles, sclerenchyma, absence or presence of epidermal surface and crystals were

found to be important characters for the identification of *Conringia* species. Previously morphological markers were used for individual identification. Morphological markers can be affected by environmental influences. Compared to morphological markers, proteins and DNA-based data are available from genetically based descriptors (KHAN *et al.,* 2000). The RAPD method is popular because of its technical simplicity and speed (RAHMAN *et al.,* 2017). There are many studies in which RAPD markers are used to determine the genetic diversity and phylogenetic relationships of some species and populations. For example; *Beta vulgaris* L. (SACLAIN *et al.* 2016), *Desmodium* Desv. (RAHMAN *et al.,* 2017), *Lolium/Festuca* (STAMMER *et al.*, 1995), *Rosa* (MILLAN *et al.,* 1996), *Chaenomeles* Lindl. (BARTISH *et al.,* 2000), *Cicer* L. (AHMAD, 1999), *Ornithogalum* L. (ANDRIĆ *et al.,* 2015), *Gossypium* L. (KHAN *et al.,* 2000), *Dendroseris* D. Don (ESSELMAN *et al.,* 2000), *Ocimum* L. (SING *et al.,* 2004), *Allium cepa* L. and *Allium fistulosum* L. (PINKY *et al.,* 2017), *Lilium candidum* L. (ÖZEN *et al.,* 2016) etc.

Table 3. Pairwise genetic distance matrix obtained from RAPD primers

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13
C.perfoliata (Yıldızeli)	-	0.28000	0.48000	0.38095	0.32000	0.14286	0.26667	0.52381	0.42857	0.38095	0.33333	0.52381	0.52381
C.perfoliata (Ankara Ayaş)	7	-	0.36667	0.42308	0.33333	0.23077	0.45000	0.50000	0.50000	0.34615	0.53333	0.34615	0.34615
C.grandiflora (Akseki Çukurköy)	12	11	-	0.50000	0.39394	0.38462	0.50000	0.34615	0.50000	0.40741	0.60000	0.42308	0.42308
C.planisiliqua (Kırşehir)	8	11	13	-	0.30769	0.42308	0.30000	0.46154	0.46154	0.30769	0.20000	0.46154	0.46154
C.planisiliqua (Bunyan)	8	10	13	8	-	0.34615	0.30000	0.38462	0.30769	0.23077	0.40000	0.30769	0.30769
C.perfoliata (Kayseri)	3	6	10	11	9	-	0.50000	0.50000	0.50000	0.42308	0.46667	0.42308	0.42308
C.planisiliqua (Sivas)	4	9	10	6	6	10	-	0.35000	0.30000	0.35000	0.20000	0.40000	0.40000
C.persica (Van)	11	13	9	12	10	13	7	-	0.30769	0.30769	0.33333	0.30769	0.30769
C.austriaca (Adana)	9	13	13	12	8	13	6	8	-	0.23077	0.40000	0.30769	0.30769
C.orientalis (Ankara Polatlı)	8	9	11	8	6	11	7	8	6	-	0.13333	0.30769	0.30769
C.orientalis (Elazığ)	5	8	9	3	6	7	3	5	6	2	-	0.53333	0.53333
C.planisiliqua (Ankara Ayaş)	11	9	11	12	8	11	8	8	8	8	8	-	0.00000
C.planisiliqua (Nallıhan-Çayırhan)	11	9	11	12	8	11	8	8	8	8	8	0	-

CONCLUSION

To conclude, the present study was conducted on molecular diversity between 13 populations of 6 species of *Conringia* species which spread in Turkey by using RAPD markers. Genetic distance and phylogenetic relationship between the populations were identified and related groups were formed based on the results of RAPD data. This research will provide some information about the relationships of the different populations spread in Turkey. It is beyond doubt that further data including more taxa or DNA sequencing data will improve this inference and lead to more reliable results.

APPENDIX 1:

Conringia perfoliata (Yıldızeli): Sivas; Yıldızeli, Kümbet village, step, 1500 m, 15.05.2014, *Conringia perfoliata* (Ankara Ayaş): Ankara; Ayaş- Beypazarı, Akkaya village, 650m, 19.05.2014. *Conringia grandiflora* (Akseki Çukurköy): Antalya; Akseki, between Murtiçi and Çukurköy, maquis span, 500 m, 21.03.2014, *Conringia planisiliqua* (Kırşehir): Kırşehir;İnanç village opposite, Üçkuyu back, rocky places, 1450 m, 17.05.2014, *Conringia planisiliqua* (Bunyan): Kayseri; Bunyan, Korumaz mountain, Bölünya locality, field edge, 1500 m, 16.05.2014, *Conringia perfoliata* (Kayseri): Kayseri; Pınarbaşı, Şirvan mountain southern slopes, mountain steppe, 1700 m, 16.05.2014, *Conringia planisiliqua* (Sivas): Sivas; Sivas- Zara road, Tödürge lake edge, 1300 m, 15.05.2014, *Conringia persica* (Van): Van: between Van-Başkale, Çuh passage, steppe, 2500 m, 29.05.2013, *Conringia austriaca* (Adana): Adana: Kozan, Gürümze village upper sections, forest clearance, 1350 m, 18.04.2014, *Conringia orientalis* (Ankara Polatlı): Ankara: Polatlı, Kavuncu bridge near, swamp places, 850 m, 19.05.2014, *Conringia orientalis* (Elazığ): Elazig; Between Keban and Elazig, 5 km, steppe, 850 m, 13.05.2014. *Conringia planisiliqua* (Ankara Ayaş): Ankara; Ayaş- Beypazarı, Akkaya village, 700 m, 19.05.2014. *Conringia planisiliqua* (Nallıhan-Çayırhan): Ankara: Between Çayırhan and Nallıhan, step, 650 m, 19.05.2014.

Received, February07th, 2019
Accepted August 18th, 2019

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GENETIČKA VARIJACIJA I MOLEKULARNI ODNOSI TAKSONA *Conringia* HEIST. EX FABR. (*BRASSICACEAE*) BAZIRANA NA RAPD MARKERIMA U TURSKOJ

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Izvod

U ovom istraživanju, primenom RAPD markera izvedene su genetska raznolikost i filogenetska analiza 13 populacija od 6 vrsta koje pripadaju rodu *Conringia* u Turskoj. Genomska DNK iz lišća uzoraka biljke *Conringia* izolovana je korišćenjem komercijalnog kita. Sedam RAPD prajmera korišćeno je za identifikaciju genetskog diverziteta među populacijama. PCR proizvodi su analizirani elektroforezom na agaroznom gelu i vizuelizovani pod UV svetlošću. Sve gel slike su analizirane, a odsustvo i prisustvo polimorfnih traka ocenjeno je. Ukupno 34 DNK trake su otkrivene sa sedam RAPD prajmera. PAUP 4.0b10 program korišćen je za izračunavanje filogenetskog stabla i genetskih distanci između vrsta. Filogenetsko stablo dobijeno je UPGMA algoritmom i sastojalo se od dva sloja. Prema PAUP analizi, vrste koje su najbliže jedna drugoj su *C. planisilikua* (Ankara-Aias) i *C. planisilikua* (Ankara-Nallıhan) sa vrednostima od 0,000, a one koje imaju najveću udaljenost su *C. grandiflora* (Akseki Cukurkoi) i *C. orientalis* (Elazıg-Baskil) vrednosti 0,6000. Rezultati sugerišu da su RAPD markeri korisni alati za demonstriranje genetskih odnosa između populacija *Conringia* vrsta.

Primljeno 07.II.2019.
Odobreno 18. VIII. 2019