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ARTICLE

Molecular characterization of Iranian *Dracocephalum* (Lamiaceae) species based on RAPD data

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ABSTRACT Taxonomic relationship and genetic diversity among 17 accessions belonging to six Iranian *Dracocephalum* L. species and one accession of *Lallemantia* as closely related genus was analyzed using RAPD markers. Forty RAPD markers were used and only twelve of them gave reproducible polymorphic bands among the accessions studied. In total 262 bands were produced out of which 10 bands were monomorphic and 252 bands were polymorphic. Among the taxa investigated *Dracocephalum kotschyi* (Damavand population) showed the highest number of RAPD bands (144), while *D. multicaule* (Zanjan population) showed the lowest number (95). UPGMA cluster analysis showed efficacy of RAPD data to differentiate the species at molecular level. *Dracocephalum polychaetum* and *D. surmandinum* as two different species in Flora of Iranica revealed a close relationship with *Dracocephalum kotschyi* and formed a mixed subcluster. The RAPD analysis offered rapid and reliable tools for the estimation of inter- and intra specific variability in *Dracocephalum*. **Acta Biol Szeged 55(2):227-230 (2011)**

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The genus *Dracocephalum* L. (Lamiaceae) consists of around 60 species distributed in the temperate regions of the Northern Hemisphere. In the flora of Iran, the genus is represented by eight species, which are mainly distributed in the northern and central parts of the country, belonging to the Irano- Turanian phytogeographical region (Rechinger 1982). With the exception of the widespread endemic species *D. kotschyi* L. and the cultivated one *D. moldavica*, the rest of the species (namely *D. polychaetum*, *D. surmandinum*, *D. multicaule*, *D. subcapitatum* and *D. aucheri*) exhibit more or less highly restricted distributional patterns in Iran. The first four medicinal and most scented perennial herbs have some morphological characters in common and in some treatments they were considered to be subspecies from *D. multicaule*.

Recently, much attention has been paid to the *Dracocephalum* genus and its chemical constituents because of their diverse activities, such as anticancer, antioxidant, antihypoxic, and immunomodulatory activities (Zeng et al. 2010). Random Amplified Polymorphic DNA (RAPD) is one of the molecular markers widely used to study genetic diversity in plants and to study the species relationships (Bogani et al. 1994; Oxelman 1996; Sanz-Cortés 2001; Çelebi et al. 2008; Mirjalil et al. 2009; Sheidai et al. 2010; İkinci and Oberprieler 2010). As far as our literature could ascertain, little research has been performed within *Dracocephalum* using molecular markers. Therefore, a project was initiated to evaluate the genetic diversity within *Dracocephalum* and ascertain the discriminating potency of RAPDs to distinguish inter- and intra-species relationship.

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Material and Methods

Plant material

The aerial parts of plants were collected during the flowering stage (2004–2010) as shown in Table 1. Leaf samples dried in silica gel and were stored at -20ºC until required.

DNA extraction

Total genomic DNA was extracted from leaves dried in silica gel or taken from herbarium specimens with the DNeasy plant mini kit DNA extraction (Qiagen) following the manufacturer's protocol.

Screening and PCR amplification with RAPD primers

Random primers (decamers) from the Operon series were tested for the amplification of DNA. In total twelve primers were used in this study viz., OPA04, OPA15, OPB03, OPC04, OPC06, OPH07, OPI18, OPM10, OPM11, OPM19, OPR06 and OPR12. The PCR reactions were carried out in 20µl reaction volume containing 20-25ng/µl DNA, 25pM primer, 10µl PCR master mix (Ampliqon) and 8.0 µl of nuclease free

Table 1. Locality and voucher information of the taxa studied.

No.	Taxa	Code	Locality	Voucher
1	*Dracocephalum polychaetum*	POL	Kerman, Babini village, Hazar mountain, 3400m., 7 May 2008, *Gholipour, Kanani & Mirtajedini*	1276 (MPH)
2	*D. polychaetum*	POH	Kerman, Chatroud, Horjond-Sodkoh, 2300m., 27 June 2007, *Gholipour*	1229 (MPH)
3	*D. kotschyi*	KLA	Mazandarn, Haraz road, Polur towarsd Lasem, *Sonboli*	1217 (MPH)
4	*D. kotschyi*	KDA	Tehran, Damvand mountain, 3200m., 6 July 2007, *Gholipour*	1218 (MPH)
5	*D. kotschyi*	KDE	Yasuj, Sisakht, Dena,3200 m.,16 June 2007, *Sonboli, Kanani & Gholipour*	1163 (MPH)
6	*D. kotschyi*	KDI	Alborz, Dizin, *Sonboli & Gholipour*	1299 (MPH)
7	*D. kotschyi*	KBO	Khorasan, Bodjnurd, *Joharchi*	37610 (FUMH)
8	*D. surmandinum*	SUR	Esfahan, Semirom, Surmand mountain,2900 m., 18 June 2007, *Sonboli, Kanani & Gholipour.*	1220 (MPH)
9	*D. multicaule*	MUN	Ardabil, Neor, *Gholipour*	1349 (MPH)
10	*D. multicaule*	MUG	West Azarbaiejan, Khoy, Qotour, Mirzagol Valley, 2300, 3 June 2008, *Sonboli, Gholipour & Kazempour*	1307 (MPH)
11	*D. multicaule*	MUK	West Azarbaiejan, Khoy, Firuragh road, Passak, *Moussavi & Tehrani*	22807 (IRAN)
12	*D. multicaule*	MUZ	Zanjan, Soltaniyeh, *Moussavi & Termeh*	22809 (IRAN)
13	*D. subcapitatum*	SUS	Khorasan, Shirvan, Reshvanloo, *Joharchi*	22812 (FUMH)
14	*D. subcapitatum*	SUN	Semnan, Chashm, Nizva mountain, 3200m., 12 July 2007, *Gholipour & Sonboli*	1219 (MPH)
15	*D. subcapitatum*	SUE	Khorasan, Shirvan, Nemanloo, *Joharchi*	22813 (FUMH)
16	*D. subcapitatum*	SUK	Khorasan, Kalat, *Joharchi*	20864 (FUMH)
17	*D. moldavica*	MOL	West Azarbayejen, Urmia,Ashena abad village,1700m., 12 July 2007 , *Sonboli & Mojarrad*	1221 (MPH)
18	*Lallemantia peltata*	LAL	West Azarbayejen, Takab, Maeen Bolagh pass, 1700m., *Sonboli*	273 (MPH)

distilled water. The amplification was conducted in a thermocycler (BioRad Mycycler) and programmed for an initial denaturation step of 1 minute at 94°C, followed by 44 cycles of denaturation at 94°C for 30 seconds, primer annealing at 40°C for 1 minute and extension at 72°C for 2 minutes. Final extension was carried out at 72°C for 7 minutes and a hold at 4°C temperature. PCR products were resolved on 1.0% agarose gel, in 1.0X TAE buffer at 70 V for 3 hours and then stained with ethidium bromide (0.5µg/ml). Gel with amplification fragments was visualized and photographed by using gel documentation system (Vilber Lurmat, Infinity).

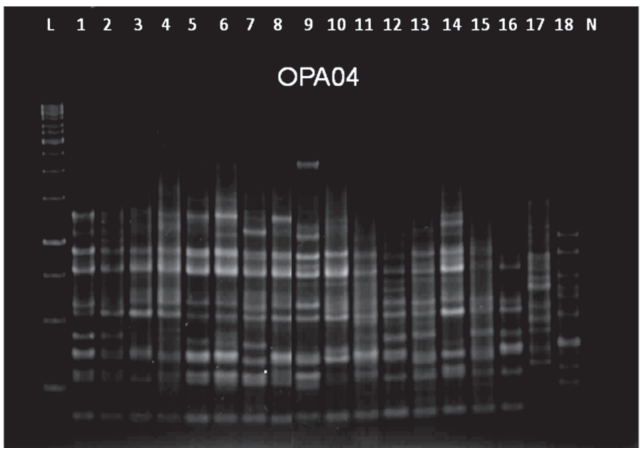

Figure 1. RAPD profiles of 18 accessions of *Dracocephalum* using the primer OPA04. L: represent molecular weight size marker (1 kb ladder). N: negative control. The numbers represent different accessions according to Table 1.

Statistical analysis of RAPD data

For cluster analysis, the correlation coefficient was selected as a measure of similarity among all accessions, and the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) was used for cluster definition. Only distinct, reproducible, well-resolved fragments, in the size range from 250 bp to 3.0 kb, were considered and scored as present (1) or absent (0) for each RAPD reaction. Genetic similarity between pairs was estimated by the Dice coefficient (Sneath and Sokal, 1973). This approach seems to be more appropriate for a genetic character, since the lack of a common band should not imply a similarity in genetic terms. Dendrograms were constructed by cluster analysis based upon the UPGMA of the SPSS ver. 9.0 software.

Results and Discussion

Out of a total of 40 different primers screened, only twelve primers produced reproducible and polymorphic bands for all populations representing the taxa studied (Fig. 1). In total 262 bands were produced out of which 10 bands were monomorphic and 252 bands were polymorphic. Among the taxa investigated *Dracocephalum kotschyi* (Damavand population) showed the highest number of RAPD bands (144), while *D. multicaule* (Zanjan population) showed the lowest number (95). The number of informative polymorphic bands per

Table 2. RAPD polymorphic reproducible bands among the taxa studied.

Primer name	Primer sequence	TB	PB	MB	SB	PP (%)
OPA04	5′ AATCGGGCTG 3′	25	25	0	9	100
OPA15	5′ TTCCGAACCC 3′	14	14	0	2	100
OPB03	5′ CATCCCCCTG 3′	25	25	0	6	100
OPC04	5′ CCGCATCTAC 3′	19	17	2	2	89.5
OPC06	5′ GAACGGACTC 3′	14	12	2	1	85.7
OPH07	5′ CTGCATCGTG 3′	24	23	1	5	95.8
OPI18	5′ AATGCGGGAG 3′	27	27	0	2	100
OPM10	5′ TCTGGCGCAC 3′	28	28	0	3	100
OPM11	5′ GTCCACTGTG 3′	29	26	3	5	89.6
OPM19	5′ CCTTCAGGCA 3′	13	12	1	6	92.3
OPR06	5′ GTCTACGGCA 3′	21	20	1	4	95.2
OPR12	5′ ACAGGTGCGT 3′	23	23	0	3	100
Total		262	252	10	49	

TB, Total bands; PB, Polymorphic bands; MB, Monomorphic bands; SB, specific bands; PP, Polymorphism percentage.

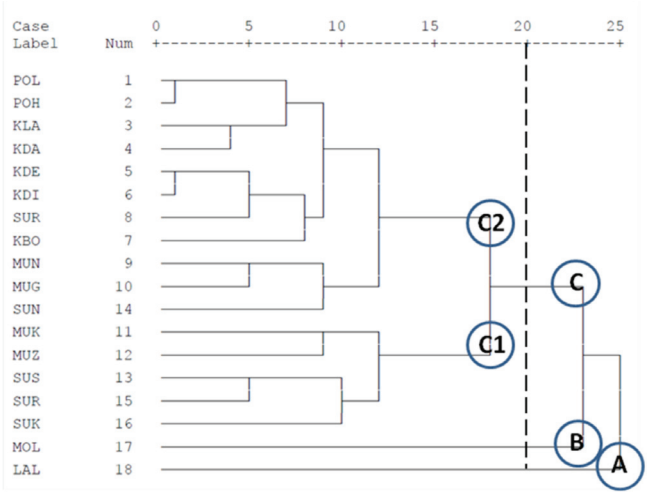

Figure 2. UPGMA dendrogram of 18 accessions of *Dracocephalum* based on 12 RAPD primers. The bar on the bottom represents similarity index based on Jaccard's coefficients. The codes represent different accessions according to Table 1.

primer ranged from 12 for primers OPC06 and OPM19 to 28 for primer OPM10 (Table 2). An average of 21 bands was amplified per sample and primer. The UPGMA dendrogram obtained from cumulative cluster analysis of twelve primers matrix using Jaccard's similarity coefficient clearly delineated all 18 accessions of taxa studied. The combined dendrogram delineated 18 accessions into three main clusters (Clusters A, B and C in Fig. 2).

The first cluster (A) formed by *Lallemantia peltata*, which is considered here as outgroup taxon. The second cluster (B) contained a morphologically distinct species of the genus *Dracocephalum*, i. e. *D. moldavica*. Whereas, the third cluster (C) had two subclusters (C1 and C2), the first subcluster (C1) contained three populations of *D. subcapitatum* (SUS, SUE and SUK) and two populations of *D. multicaule* (MUK and MUZ) while the second subcluster (C2) composed of mixed grouping of accessions belonging to *D. kotschyi*, *D. polychaetum*, *D. surmandinum*, *D. multicaule* (MUN and MUG) and *D. subcapitatum* (SUN).

From the chemotaxonomic point of view, limonene and perilla aldehyde were the main components of *D. polychaetum*, *D. surmandinum*, *D. multicaule*, *D. kotschyi* and *D. subcapitatum* (Sonboli et al. 2010), while citral and geranyl acetate was found to be the principal essential oil constituent of *D. moldavica* (Sonboli et al. 2008). This phytochemical differentiation is in accordance with the results of molecular RAPD data, in which *D. moldavica* is clearly separated from other *Dracocephalum* species (Fig. 2).

DNA-based markers provide precise information on genetic diversity because of the independence of the confounding effects of environmental factors (Powell et al. 1995). RAPD markers are based on random priming, which randomly screen various regions of the genomic DNA. In this study, RAPD marker system revealed high levels of polymorphism among the *Dracocephalum* species indicating its effectiveness for evaluating intra- and inter-specific genetic diversity in the genus *Dracocephalum*. The significance of wild genetic diversity using DNA based markers like RAPD and its efficacy have also been reported for *Satureja hortensis* (Hadian et al. 2008), *Silene* sect. *Auriculatae* (Sheidai et al. 2010) and *Whitania* species (Mirjalili et al. 2009) from Iran.

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