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Determination of genetic relationships among *Velezia* L. (Caryophyllaceae) species using RAPD markers

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Abstract: Random amplified polymorphic DNA (RAPD) markers were used to determine genetic relationships among *Velezia* L. species from Turkey. A total of 432 amplified bands were obtained using 14 RAPD primers. The polymorphism in RAPD markers was high (98.61%) and was sufficient to distinguish each species. The degree of band-sharing was used for evaluating the genetic similarity between species and for constructing a dendrogram by the unweighted pair group method with arithmetic mean (UPGMA). Genetic relationships among the species were found to be fully consistent with those obtained by the use of morphological characters. Data obtained from our study demonstrated that the RAPD technique could be successfully used for the determination of genetic relationships among *Velezia* species and for species identification. Furthermore, it can be efficiently employed in future studies to provide preliminary data for conservation of endangered *Velezia* species.

Key words: Genetic relationships, RAPD-PCR, *Velezia* L., Turkey

RAPD markörleri kullanılarak *Velezia* L. (Caryophyllaceae) türlerinin genetik akrabalıklarının belirlenmesi

Özet: Bu çalışmada, RAPD (Random Amplified Polymorphic DNA) belirteçleri Türkiye'de yetişen *Velezia* L. türlerinin genetik akrabalık seviyelerini belirlemede kullanılmıştır. 14 RAPD primerinden, toplam 432 bant elde edilmiştir. RAPD belirteçlerindeki polimorfizm oranı yüksek (% 98,6) ve her türü ayırt etmek için yeterlidir. Paylaşılan ortak bant seviyesi türler arasındaki genetik benzerliği değerlendirmek ve UPGMA (unweighted pair group method with arithmetic mean) tabanlı bir dendrogram oluşturmak için kullanılmıştır. Türler arasındaki genetik akrabalıkların, morfolojik karakterlerin kullanımıyla elde edilen verilerle tamamen uyumlu olduğu bulunmuştur. Elde edilen sonuçlar RAPD tekniğinin *Velezia* türleri arasındaki genetik akrabalıkların belirlenmesinde olduğu kadar türlerin tanımlanmasında da başarıyla kullanılabileceğini göstermiştir. Ayrıca bu teknik gelecekteki çalışmalarda, özellikle tehlike altındaki *Velezia* türlerinin korunması için ön verileri sağlamada etkin olarak kullanılabilir.

Anahtar sözcükler: Genetik akrabalık, RAPD-PCR, *Velezia* L., Türkiye

Introduction

In Turkey, the genus *Velezia* L. (Caryophyllaceae) comprises 6 species: *V. hispida* Boiss. & Balansa, *V. quadridentata* Sibth. & Sm., *V. fasciculata* Boiss., *V. rigida* L., *V. pseudorigida* Hub.-Mor., and *V. tunicoides* P.H.Davis. Among these, *V. rigida* is a cosmopolite species that is widespread in Turkey and throughout the world; *V. quadridentata* is mainly distributed over the northwestern parts of the Marmara and Aegean regions; *V. tunicoides*, a narrow, local endemic species, is distributed only in the Antalya-Göynük region (southern Turkey); *V. hispida*, an endemic species, is distributed only in the middle and southeastern parts of the Aegean region; and *V. pseudorigida* is also an endemic species that is widespread from the mid-Aegean region to the southwestern parts of Turkey (1). During the field study, no specimens for *V. fasciculata* could be found in the A5 Amasya and Çorum regions of northern central Turkey. It is possible that the distribution sites and population size of this species decreased with time due to habitat destruction (2). Recently, *V. tunicoides* and *V. pseudorigida* have been categorized as critically endangered and *V. hispida* has been categorized as near threatened (2).

Accurate identification of the species and a precise diagnostic key are very important for floristic studies; precise identification also aids in conservation efforts and the rational use of genetic resources. For this reason, different types of molecular markers were developed for facilitating taxonomic, evolutionary, and phylogenetic studies based solely on morphology (3-5). As DNA markers are unlimited in number, produce high polymorphism, and are independent of environmental interactions, they are considered valuable tools for determining genetic relationships and diversity. Random amplified polymorphic DNA (RAPD) data are known to be generated faster and more easily than other methods such as restriction fragment length polymorphism (RFLP) and microsatellites (6,7). However, the use of RAPD has been criticized for revealing unreliable phylogenies owing to the occasional lack of reproducibility and a possible lack of homology in comigrating bands (8,9). Despite this limitation, the RAPD method has been successfully employed in several plant genera for revealing genetic relationships following the optimization of polymerase chain reaction (PCR) conditions (3,10-17).

The taxonomy of *Velezia* is restricted to the morphological observations made by Davis (1), and, to date, no study has been undertaken for the identification and evaluation of genetic relationships of *Velezia* based on molecular methods. In *Flora of Turkey*, the dichotomous diagnostic key for *Velezia* L. was built based on the differences in indumentum, calyx, petal, and inflorescence. It should be mentioned that identification of *Velezia* species solely on the basis of the morphology of reproductive structures is very tedious, especially when accurate identification of 2 morphologically similar species, such as *V. pseudorigida* and *V. rigida*, is undertaken.

Thus, the aim of the present study was to evaluate the usefulness of the RAPD technique in generating DNA markers for identifying morphologically similar *Velezia* species from Turkey and determining the genetic relationships among them.

Materials and methods

Plant materials

Whole plant samples from each species of *Velezia* were collected at different locations in Turkey (Figure 1, Table 1) between June 2003 and July 2007. Since *V. rigida* is a widespread species, 15 populations from different regions were included in the study. *V. hispida* was sampled from 2 localities (B3 Afyon and C3 Isparta), and Isparta was a new record for *V. hispida*. For the other *Velezia* species, plant samples were collected from single localities. Samples were identified based on morphology (Table 1), and voucher specimens were deposited in the herbarium of Eskişehir Osmangazi University (OUFE).

DNA extraction

For DNA analysis, 5 plant samples collected from each locality were used. Total genomic DNA was extracted from herbarium materials by a modified hexadecyltrimethyl ammonium bromide (CTAB) method (18). The quantity and quality of DNA were determined by using a Nanodrop® ND-1000 spectrophotometer (Wilmington, DE, USA).

Table 1. The names and locations of Velezia species used for RAPD analyses.

Species	Accessio no.(OUFE)	Locality and habitat	Altitude
Velezia tunicoides P.H.Davis	13461	C3 Antalya: Göynük, dry stream bed, 12.07.2007, 36°38′849″N, 30°32′962.2″E	2 m
Velezia hispida Boiss.	13440	B3 Afyon: 2 km to Karakaya village, north hillsides, 24.06.2005, 21.07.2005, 29.07.2006, 02.08.2007, 38°53′19.7″N, 30°48′47.8″E	1160 m
Velezia hispida Boiss.	13453	C3 Isparta: Gölcük Lake National Park, roadsides, 23.07.2006, 37°43′60″N, 30°30′E	1200 m
Velezia quadridentata Sibth. & Sm.	13427	B1 İzmir: Emiralem to Menemen, stony places, 21.05.2005, 38°27′48.7″N, 27°12′18.7″E	6 m
Velezia pseudorigida Hub.-Mor.	13449	C2 Muğla: Muğla to Yılanlı Forest Management Planning Department, crossroads of power-plant, southeast, stony-calcareous hillside, 21.07.2006, 37°13′32.1″N, 26°27′33.0″E	1250-1260 m
Velezia rigida L.	10456	A3 Bilecik: Bozüyük to Bilecik, roadsides, 21.06.2003, 39°54′N, 30°2′E	873 m
Velezia rigida L.	13415	A3 Bolu: Nallıhan to Mudurnu, 16 km to Mudurnu, roadsides and under *Juniperus* sp., 29.07.2004, 40°11′1″N, 31°21′2″E	1000 m
Velezia rigida L.	13416	A5 Amasya: Eğerli Mountain, north stony hillsides, 28.06.2005, 40°46′41.4″N, 35°13′11.3″E	910 m
Velezia rigida L.	13419	A5 Amasya: Amasya to Ziyaret, west and east hillsides, roadsides, under *Quercus* sp., 06.07.2007, 40°43′30.8″N, 35°49′0.20″E	720 m
Velezia rigida L.	13420	A5 Çorum: Çorum to Amasya, roadsides, 06.07.2007, 40°35′72.5″N, 35°08′61.1″E	1026 m
Velezia rigida L.	13464	B1 Çanakkale: 11 km to Ayvacık, 20.05.2005, 39°34′17.7″N, 26°32′38.9″E	553 m
Velezia rigida L.	13430	B2 Kütahya: Tavşanlı, 28.06.2006, 39°33′07.8″N, 29°29′52.7″E	938 m
Velezia rigida L.	13431	B2 Kütahya: Tunçbilek, near Ömerler mine, 25.06.2005, 39°40′45.8″N, 29°27′10.4″E	830 m
Velezia rigida L.	13436	B2 Manisa: Sipil Mountain, west hillsides, 21.05.2005, 38°35′31.2″N, 27°26′01.1″E	700 m
Velezia rigida L.	13438	B3 Eskişehir: Türkmen Mountain, 28.06.2007, 39°32′N, 30°15′E	1200 m
Velezia rigida L.	13441	B3 Konya: Akşehir, Tekke village, Sultan Mountains, 13.06.2004, 38°20′N, 31°23′E	1100 m
Velezia rigida L.	13448	C2 Muğla: Fethiye, Babadağ, parasailing area, 23.06.2005, 36°32′19.9″N, 29°10′12.3″E	1816 m
Velezia rigida L.	13459	C3 Antalya: Akseki to Sadıklar, road sides, 15.06.2004, 36°57.663′N, 31°46.266′E	903 m
Velezia rigida L.	13463	C5 Mersin: Gülek-Akçatekir (Tekir), 26.06.2004, 36°55′N, 34°53′E	1250 m
Velezia rigida L.	13462	C5 Adana: Kozan to Feke 20th km, roadsides in rubble, 25.6.2004, 37°49′N, 35°54′E	620 m

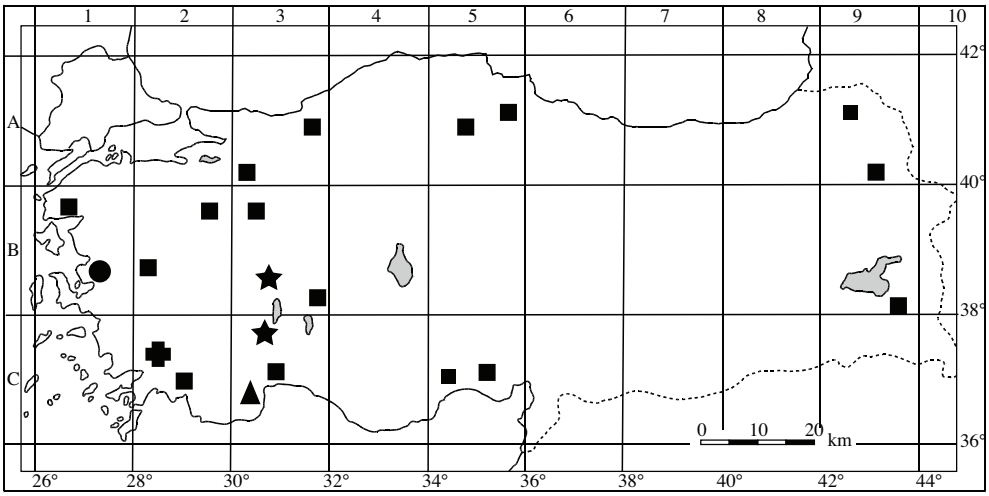

Figure 1. Distribution map of *Velezia* species in Turkey used for RAPD analyses.

DNA samples were diluted to 4 ng/μL, and bulked DNA samples were prepared by mixing equal amounts of the 5 representative DNA samples from each locality. Samples were stored at 4 °C for RAPD amplification.

RAPD-PCR

RAPD analysis was performed according to the method of Williams et al. (6). A set of 25 random 10-mer primers was purchased from Thermo Inc. (Burlington, MA, USA). After screening, 14 primers that amplified clear, reproducible banding patterns were used for further analysis (Table 2). PCR amplifications were performed in 25 μL of reaction mixture containing 20 ng of template DNA, 1× Taq polymerase buffer and 1 U of Taq polymerase (Fermentas, Glen Burnie, MD, USA), 2.0 mM $MgCl_2$, 1 mM dNTP, and 1 μM of primer. Amplifications were carried out in a Progene Thermal Cycler (Techne Inc., Burlington, NJ, USA) that was programmed for the initial denaturation step at 92 °C for 2 min, followed by 40 cycles of 94 °C for 1 min, 36 °C for 1 min, 72 °C for 2 min, and a final elongation at 72 °C for 7 min. PCR reactions were repeated twice to ensure reproducibility of amplified products. The PCR products were separated on a 1.5% agarose gel containing ethidium bromide (0.5 μg/mL) and photographed with the UVIpro Gel Documentation System (UVItec, Cambridge, UK). Molecular weights of PCR products were estimated using a 100 bp Plus DNA Ladder (Fermentas). A negative control with no DNA template was also included in the PCR reaction to check that there was no contamination.

Data analysis

The amplified bands were scored as present (1) or absent (0). Faintly stained bands that were not clearly resolved were not considered in the data collection. Bands with the same migration distance were considered homologous. The genetic similarity between all accessions was calculated according to the Jaccard coefficient (19). The program FreeTree (20) was used for the construction of an unweighted pair group method with arithmetic mean (UPGMA) dendrogram and bootstrap analysis (500 resampled datasets).

Results

Initially, 25 RAPD primers were screened against total DNA from 5 *Velezia* samples: *V. tunicoides*, *V. hispida*, *V. quadridentata*, *V. pseudorigida*, and *V. rigida*. Based on reproducibility, quality of band profiles, and level of polymorphism, 14 primers were selected for the identification of *Velezia* species and evaluation of their genetic relationships. These primers resulted in the amplification of 432 products of various sizes ranging from 200 bp to 3000 bp (Table 2). RAPD banding patterns using 2 random primers are represented in Figure 2. The maximum number of bands, 46, was amplified with primer 23 and the lowest number, 24, with primer 9. Out of these 432

Table 2. Details of RAPD banding pattern of 5 species of *Velezia* using 14 primers.

Name of the primer	Sequence of the primer	Total number of bands amplified	Range of amplicons (bp)	Percentage of polymorphic bands (PPB %)
P3	5' GAG GCG GCG A 3'	31	400-1700	100
P5	5' CTG CGA CGG T 3'	26	300-2000	100
P6	5' GCG CGG CAG T 3'	33	480-2500	100
P7	5' TTG CGC CCG G 3'	31	200-3000	96.77
P8	5' AGA CGC CGA C 3'	27	400-2700	100
P9	5' GGG AAG AGA G 3'	24	270-2500	100
P11	5' GGC CGA TGA T 3'	26	400-2700	96.15
P13	5' ACC GGC TTG T 3'	27	340-2700	100
P14	5' CAG CAC TGA C 3'	33	300-3000	100
P16	5' TGG TGG CCT T 3'	34	320-3000	94.11
P17	5' GTA GCA CTC C 3'	26	400-3000	100
P21	5' ACG GTG CCT G 3'	27	310-2500	96.26
P23	5' CGC CCA AGC C 3'	46	470-2750	100
P24	5' CGC CCT GGT C 3'	41	380-630	97.56
Total		432	200-3000	98.61

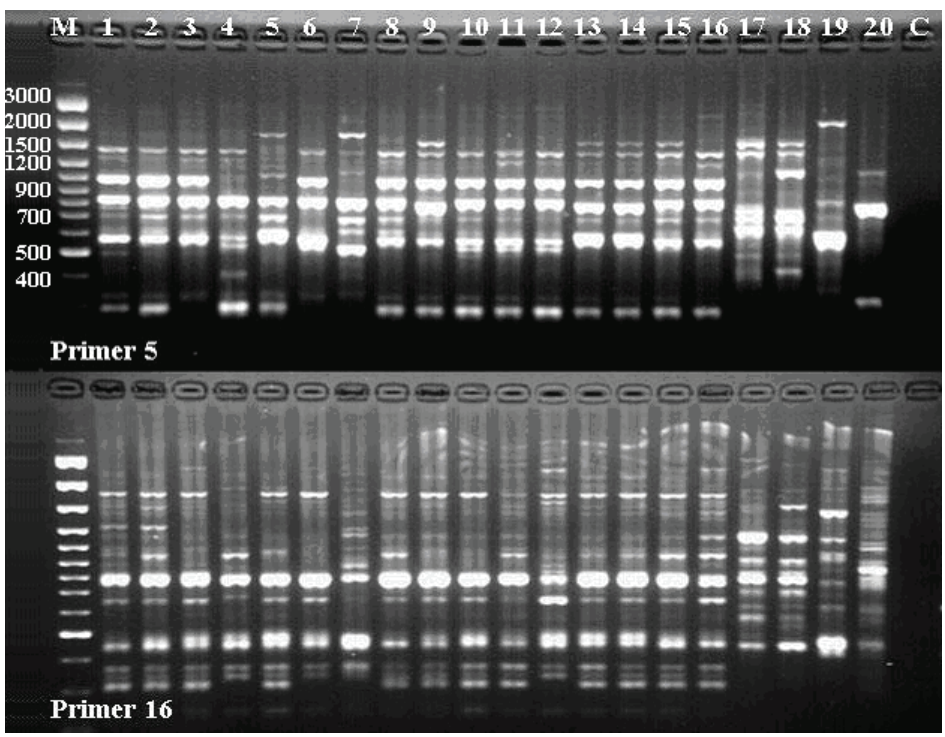

Figure 2. RAPD banding patterns in 5 species of *Velezia* as revealed by primers 5 and 16. M: GeneRuler 100 bp Ladder Plus, 1-15: *Velezia rigida* populations, 16: *V. pseudorigida* population, 17 and 18: *V. hispida* populations, 19: *V. quadridentata* population, 20: *V. tunicoides* population, C: control.

amplified bands, 426 were polymorphic and 6 were monomorphic (Table 2). The highest number of bands, 153, was amplified in *V. rigida*, and the lowest, 128, in *V. hispida*. A unique pattern of amplified fragments was obtained for each *Velezia* species. Some of the fragments were species-specific markers (Table 3). For example, primer 21 amplified specific bands that could distinguish all *Velezia* species.

The similarity matrix was calculated using the Jaccard similarity coefficient (19) (Table 4). *V. pseudorigida* and *V. rigida* (VR15) showed the highest similarity (0.63), and the lowest similarity (0.10) was observed between *V. tunicoides* and *V. pseudorigida*. A wide range of similarity values (0.57-0.89) among different populations of *V. rigida* was also observed.

The UPGMA dendrogram showed 2 distinct clusters (Figure 3). Cluster 1 consisted of 2 subclusters; the first subcluster included species *V. pseudorigida* and *V. rigida*, and the other subcluster contained only *V. quadridentata*. Cluster 2 was also divided into 2 subclusters; the first subcluster consisted of 2 populations of *V. hispida*, and the other subcluster contained *V. tunicoides*.

Discussion

In this study, the RAPD technique was successfully utilized to determine the genetic relationships among the 5 *Velezia* species collected from different regions of Turkey. Numerous molecular studies have been conducted in the family Caryophyllaceae. For example, the phylogeny of Caryophyllaceae was studied by using data from the chloroplast (*matK*) and nuclear (ITS) regions (21). In another study, ribosomal internal transcribed spacer (ITS) sequences and intron sequences of the chloroplast gene *rps16* were used to examine phylogenetic and biogeographical patterns within the genus *Heliosperma* (*Sileneae*) (22). A phylogenetic study of the genus *Polycarpon* was done using DNA sequence data from the chloroplast *rps16* intron and nuclear *RPB2* regions (23). The phylogeny of *Moehringia* and its relationship to *Arenaria* were elucidated using a broad taxon sampling and by combining molecular and morphological data (24). Phylogenetic relationships and the biogeography of the genus *Cerastium* were studied using sequences of 3 noncoding plastid DNA regions: *trnL* intron, *trnL-trnF* spacer, and *psbA-*

Table 3. Selected RAPD markers and their species specific band sizes for 5 *Velezia* L. species.

	V. tunicoides	*V. hispida*	*V. quadridentata*	*V. pseudorigida*	*V. rigida*
Primer P3	2150 bp	-	250 bp	-	-
Primer P5	730 bp	750 bp, 680 bp, 490 bp, 430 bp	480 bp, 2200 bp	-	-
Primer P6	510 bp, 2700 bp	850 bp, 2300 bp	580 bp,630 bp	620 bp	-
PrimerP7	-	2800 bp	2900 bp	610 bp	-
Primer P8	-	690 bp	-	-	-
Primer P9	-	900 bp, 930 bp, 950 bp	550 bp,400 bp	460 bp	-
Primer P11	-	-	-	290 bp, 820 bp, 2100 bp	-
Primer P13	-	-	640 bp	590 bp	-
Primer P14	290 bp, 910 bp, 1190 bp, 2750 bp	740 bp, 830 bp, 1600 bp	530 bp, 820 bp, 990 bp	750 bp	-
Primer P16	610 bp, 730 bp, 1100 bp	580 bp, 710 bp	-	-	250 bp
Primer P17	480 bp	460 bp, 690 bp, 1190 bp	400 bp	-	-
Primer P21	410 bp, 1490 bp, 2900 bp	460 bp, 480 bp	3000 bp	390 bp, 500 bp	490 bp
Primer P23	490 bp, 530 bp, 685 bp, 760 bp, 980 bp	470 bp, 610 bp, 695 bp, 720 bp	860 bp, 890 bp, 2750 bp	-	-
Primer P24	380 bp	-	630 bp, 840 bp	395 bp	415 bp

Table 4. Jaccard similarity coefficients among 5 species of Velezia L. as detected by 14 RAPD primers. VR1: *V. rigida* (Adana), VR2: *V. rigida* (Ziyaret-Amasya), VR3: *V. rigida* (Antalya), VR4: *V. rigida* (Manisa), VR5: *V. rigida* (Bilecik), VR6: *V. rigida* (Bolu), VR7: *V. rigida* (Çanakkale), VR8: *V. rigida* (Çorum-Amasya), VR9: *V. rigida* (Eskişehir), VR10: *V. rigida* (Konya), VR11: *V. rigida* (Mersin), VR12: *V. rigida* (Muğla), VR13: *V. rigida* (Tavşanlı-Kütahya), VR14: *V. rigida* (Tunçbilek-Kütahya), VR15: *V. rigida* (Çitli-Amasya), VPR: *V. pseudorigida* (Muğla), VH1: *V. hispida* (Isparta), VH2: V. *hispida* (Afyon), VQ: *V. quadridentata* (İzmir), VT: *V. tunicoides* (Antalya).

	VR1	VR2	VR3	VR4	VR5	VR6	VR7	VR8	VR9	VR10	VR11	VR12	VR13	VR14	VR15	VPR	VH1	VH2	VQ	VT
VR1	1.0000	--																		
VR2	0.7655	1.0000	--																	
VR3	0.7550	0.7770	1.0000	--																
VR4	0.7123	0.7589	0.7365	1.0000	--															
VR5	0.6500	0.6375	0.6228	0.6516	1.0000	--														
VR6	0.6821	0.7143	0.7400	0.7203	0.6352	1.0000	--													
VR7	0.5818	0.5793	0.5858	0.5714	0.6481	0.5767	1.0000	--												
VR8	0.7351	0.7333	0.7355	0.7162	0.6442	0.7086	0.5964	1.0000	--											
VR9	0.7368	0.7467	0.7712	0.7067	0.6364	0.6883	0.6084	0.7987	1.0000	--										
VR10	0.7006	0.6772	0.6810	0.7267	0.6446	0.6968	0.5976	0.7152	0.7727	1.0000	--									
VR11	0.6987	0.7078	0.7000	0.7020	0.6424	0.6839	0.5858	0.7355	0.7829	0.8146	1.0000	--								
VR12	0.6815	0.6795	0.6937	0.6842	0.6071	0.6993	0.6280	0.6750	0.7089	0.7170	0.7597	1.0000	--							
VR13	0.6708	0.7006	0.6829	0.6624	0.6768	0.6667	0.5906	0.7170	0.7516	0.7821	0.7580	0.7296	1.0000	--						
VR14	0.7215	0.7419	0.7117	0.6604	0.6747	0.6962	0.5988	0.7143	0.7484	0.7785	0.7438	0.7267	0.8993	1.0000	--					
VR15	0.7362	0.7346	0.7576	0.7188	0.6901	0.7222	0.6250	0.7500	0.7730	0.7805	0.7791	0.7515	0.7605	0.80000	1.0000	--				
VPR	0.5786	0.5860	0.5926	0.5478	0.5298	0.5732	0.4514	0.5644	0.5963	0.5951	0.6025	0.5767	0.5783	0.5964	0.6331	1.0000	--			
VH1	0.1379	0.1391	0.1387	0.1378	0.1688	0.1404	0.1659	0.1453	0.1392	0.1519	0.1532	0.1392	0.1506	0.1583	0.1514	0.1577	1.0000	--		
VH2	0.1435	0.1396	0.1441	0.1435	0.1704	0.1514	0.1622	0.1614	0.1498	0.1630	0.1593	0.1498	0.1565	0.1696	0.1570	0.1535	0.7877	1.0000	--	
VQ	01951	0.1854	0.2174	0.2154	0.1896	0.2111	0.2039	0.2146	0.2184	0.2095	0.2057	0.2244	0.1852	0.1881	0.2054	0.2020	0.2010	0.1863	1.0000	--
VT	01142	0.1308	0.1205	0.1346	0.1111	0.1215	0.1382	0.1324	0.1261	0.1295	0.1205	0.1161	0.1333	0.1266	0.1398	0.1075	0.2136	0.2111	0.1969	1.0000

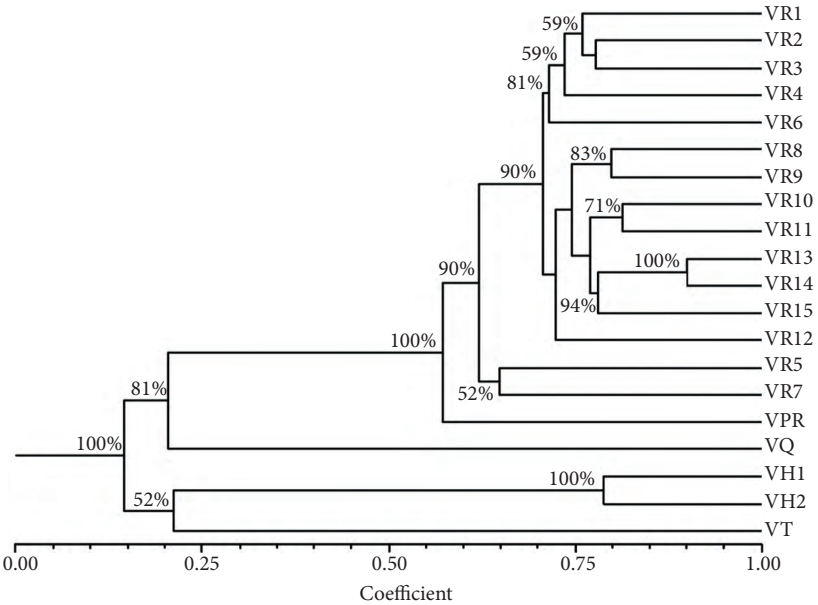

Figure 3. Dendrogram showing the genetic relationship among 5 species of *Velezia* as obtained from UPGMA cluster analysis. VR1: *V. rigida* (Adana), VR2: *V. rigida* (Ziyaret-Amasya), VR3: *V. rigida* (Antalya), VR4: *V. rigida* (Manisa), VR5: *V. rigida* (Bilecik), VR6: *V. rigida* (Bolu), VR7: *V. rigida* (Çanakkale), VR8: *V. rigida* (Çorum-Amasya), VR9: *V. rigida* (Eskişehir), VR10: *V. rigida* (Konya), VR11: *V. rigida* (Mersin), VR12: *V. rigida* (Muğla), VR13: *V. rigida* (Tavşanlı-Kütahya), VR14: *V. rigida* (Tunçbilek-Kütahya), VR15: *V. rigida* (Çitli-Amasya), VPR: *V. pseudorigida* (Muğla), VH1: *V. hispida* (Isparta), VH2: *V. hispida* (Afyon), VQ: *V. quadridentata* (İzmir), VT: *V. tunicoides* (Antalya).

trnH spacer (25). The phylogenetic relationships between the 5 dioecious species in *Silene* section *Melandrium* and their putative hermaphrodite relatives were also investigated based on an extensive geographic and taxonomic sample, using DNA sequence data from the chloroplast genome and the nuclear ribosomal ITS region (26). The genetic relationships of *Dianthus chinensis*, *D. barbatus*, and *D. superbus* were studied using sequence-related amplified polymorphisms (SRAPs) and inter-simple sequence repeats (ISSRs) and morphological trait measurements (27). However, as far as we are aware, no genetic relationship analysis among the *Velezia* species has been carried out. The present study, therefore, is the first molecular research conducted in the *Velezia* genus.

In this study, we tested 25 RAPD primers, and 14 of those primers resulted in satisfactory amplification patterns with a polymorphism rate of 98.94%. The variations in banding patterns as well as the total number of bands amplified in different species (from 128 to 153) indicated that a considerable degree of polymorphism exists among species. High polymorphism rates were also observed among 15 populations of *V. rigida* and 2 populations of *V. hispida*. In addition, some of the amplified fragments were species-specific (Table 3). Most of the primers tested amplified the bands that distinguished the morphologically similar *V. rigida* and *V. pseudorigida*. In addition, identification of 5 *Velezia* species was easily achieved by comparing the amplified products of primer 21. These results imply that RAPD markers can provide valuable information for genetic variation studies undertaken at intra- and interspecific levels in the genus *Velezia*. They may also be useful as taxonomic markers for facilitating the identification of individuals from particular *Velezia* species.

Recently, RAPD markers were effectively used to differentiate 2 *Cephalaria* species, and it was concluded that RAPD-PCR profiles together with morphological data provide taxonomic distinctions at the species level (28). Similarly, among Caryophyllaceae members, RAPD markers were successfully used to study genetic relationships in *Silene* (29) and to assess intra- and interspecific genetic diversity levels in *Cerastium* (30).

The UPGMA dendrogram based on RAPD band profiles formed 2 clusters, and the closely related *Velezia* species shared the same node, justifying their taxonomical statuses. The first cluster contained 2 subclusters; the first subcluster contained *V. pseudorigida* and *V. rigida*, and the second subcluster included *V. quadridentata*. The second cluster contained *V. tunicoides* and *V. hispida*. This grouping pattern, in general, supports the morphological findings. The RAPD banding patterns of *V. rigida* and *V. pseudorigida* were quite close, and the highest genetic similarity (63%) was observed between them. This finding is not surprising because, as indicated earlier, these 2 species are morphologically very similar. The only characteristics that distinguish these 2 species are their petal shapes and the number of flowers in inflorescence. In the first cluster, *V. quadridentata* was placed in the second subcluster. This species has coronal scales, as in *V. pseudorigida* and *V. rigida*; however, it also has a glabrous calyx that serves as the distinctive feature for distinguishing *V. quadridentata* from other *Velezia* species. The second cluster contained 2 species: *V. tunicoides* and *V. hispida*. These 2 species are endemic to Turkey. They can be identified from their petal colors and are morphologically similar in their hispid and tubulate calyces. In addition, shape, number of costa, and indumentum of the calyx are the other important characteristics that distinguish *V. tunicoides* and *V. hispida* from the other *Velezia* species.

The genus *Velezia* is represented by 6 species in *Flora of Turkey*, of which *V. fasciculata* Boiss. was recorded from 2 localities: A5 Amasya and C6 Hatay (1). During our field study, however, no specimen of this species could be found in these localities. Interestingly, several assumed populations were sampled in the A5 Amasya and Çorum regions. Subsequent to the morphological studies and the correction of samples with the voucher specimen obtained from the herbarium of the Hebrew University of Jerusalem (HUJ), it was discovered that the individuals of these populations were *V. rigida*, not *V. fasciculata*. The plant samples taken from these populations were also included in the RAPD analysis, and close genetic relationships with other *V. rigida* populations were determined. Palaestina is identified as the distribution area of *V. fasciculata* according to HUJ records. This species is reported to be at risk of extinction in Israel (31). In Turkey, it can be assumed that since 1967 (last record for the species), distribution sites and population sizes of this species may have decreased as a result of habitat destruction. Hence, intensive excursions in the A5 Amasya and C6 Hatay regions are necessary to determine the current status of this species in Turkey.

In conclusion, the present study demonstrated the usefulness of RAPD markers for the purpose of identifying species and evaluating the genetic relationships among *Velezia* species. These markers could be further employed in pilot studies concerning conservation of the 3 endangered *Velezia* species in Turkey.

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