Biochem Genet (2017) 55:87–102
DOI 10.1007/s10528-016-9770-9

ORIGINAL ARTICLE

Molecular Characterization of *Cyclamen* Species Collected from Different Parts of Turkey by RAPD and SRAP Markers

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Received: 23 May 2016 / Accepted: 18 August 2016 / Published online: 7 September 2016

Abstract The genus *Cyclamen* (family Myrsinaceae) contains about 20 species, most of which occur in the Mediterranean region. Turkey has critically important *Cyclamen* genetic resources. Molecular characterization of plant materials collected from different regions of Turkey in which *Cyclamen* species grow naturally, namely Adana, Antalya, Aydın, Muğla, İzmir, Denizli, Kahramanmaraş, Osmaniye, Eskişehir, Trabzon, and Rize provinces, was performed using RAPD and SRAP markers. DNA was successfully amplified by 30 RAPD primers and 14 SRAP primer pairs. Among the 470 bands generated by the RAPD primers, 467 were polymorphic. The number of bands detected by a single primer set ranged from 11 to 22 (average of 15.6). The percentage polymorphism was 99.3 % based on the RAPD data. In the SRAP analysis, a total of 216 bands were generated, showing 100 % polymorphism. The number of bands detected by a single primer set ranged from 9 to 22 (average of 15.4). All data were scored and UPGMA dendrograms were constructed with similar results in both marker systems, i.e., different species from nine provinces of Turkey were separated from each other in the dendrograms with the same species being clustered together.

Keywords DNA · Genetic relationships · Genetic resources · PCR · Polymorphism

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Introduction

Cyclamen (*Cyclamen* spp.; Myrsinaceae) is among the most popular flowering pot plants, and many cultivars derived from *C. persicum* are grown commercially (Kitamura et al. 2012). The 20 cyclamen taxa originate from the Mediterranean region (Aka Kacar et al. 2013) and under natural conditions typically grow under trees and bushes. Ten *Cyclamen* species grow naturally in Turkey, five of which are endemic to Turkey, which is a genetic epicenter of many plant species. The genus *Cyclamen* is a particularly interesting and traceable genus to examine phylogeographic patterns of differentiation. Although traditionally placed in the Primulaceae, molecular evidence suggested that the genus *Cyclamen* may actually be nested in the Myrsinaceae (Kallersjo et al. 2000). There are 20 species in the genus, 17 of which are *circum*-Mediterranean (Grey-Wilson 1997). In the Mediterranean Basin, several species have a widespread distribution with allopatric populations on different islands and continental land masses (Gielly et al. 2001).

There are few studies that have investigated the genetic relationships of *Cyclamen* genotypes by using molecular markers. Naderi et al. (2009) used randomly amplified polymorphic DNA (RAPD) markers to determine inter- and intra-specific genetic diversity among cyclamen accessions collected from different parts of Iran. The genetic diversity of natural Turkish *C. alpinum* populations was investigated by using RAPD markers (Taskin et al. 2012). In another study, in order to make classifications of the genus *Cyclamen*, three regions of DNA were selected for sequencing from two genomic compartments: cpDNA trnL intron, nrDNA ITS1, and ITS2 regions. The resulting molecular data were also combined with published morphological data (Compton et al. 2004). In addition to molecular studies, there are several reports on morphological data on Cyclamen (Anderberg 1994; Debussche and Thompson 2002; Aalaey et al. 2007; Curuk et al. 2015).

RAPD markers, which result from the polymerase chain reaction (PCR) amplification of genomic DNA fragments using short oligonucleotides (usually 10-mers) of arbitrary sequence as primers, provide a fast and easy approach for many purposes in plant genetic analysis (Aka Kacar et al. 2005). Sequence-related amplified polymorphism (SRAP), a PCR-based marker system (Li and Quiros 2001), is a simple and efficient marker system that can be adapted for a variety of purposes in different crops, including map construction, gene tagging, genomic and cDNA fingerprinting, and map-based cloning (Amar et al. 2011). It has several advantages over other systems: it is simple, has a reasonable throughput rate, discloses numerous co-dominant markers, targets open-reading frames (ORFs), and allows easy isolation of bands for sequencing (Uzun et al. 2009).

The aim of this study was to determine the genetic diversity of cyclamen genotypes collected from nine provinces of Turkey using RAPD and SRAP markers.

Materials and Methods

This study was carried out at the Department of Horticulture, Faculty of Agriculture, University of Çukurova, Sarıçam, Turkey.

Plant Materials

Plant material was collected from Adana, Antalya, Aydın, Muğla, İzmir, Denizli, Kahramanmaraş, Osmaniye, Eskişehir, Trabzon, and Rize provinces of Turkey (Table 1). A total of 95 cyclamen genotypes were collected from different *Cyclamen* species (*C. pseudibericum*, *C. cilicium*, *C. persicum*, *C. graecum*, *C. mirabile*, *C. hederifolium*, *C. alpinum*, *C. coum*, *C. intaminatum*, and *C. parviflorum*).

DNA Isolation

Leaves from all samples were immediately frozen in liquid nitrogen and stored at −80 °C. High molecular weight genomic DNA was extracted from the leaf of each sample following the CTAB protocol for minipreps (Edwards et al. 1991). DNA concentration was measured using a NanoDrop (ND 100) spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) and gel electrophoresis. DNA was diluted in water to a final concentration of 50 ng/μL and stored at −20 °C.

RAPD Analysis

Fifty RAPD 10-mer primers (Operon Technologies, Almeda, CA, USA) were initially tested. Primers that produced polymorphic bands were used to amplify all the 95 genotypes studied. Thirty primers which were found to be polymorphic (Table 2) were used to generate RAPD markers. Amplification reactions were performed in 9-μL volumes containing 2X PCR Mastermix (Fermentas K0171, Waltham, MA, USA), 1 U *Taq* DNA polymerase (Fermentas EP0402), 25 mM $MgCl_2$, 30 ng of the primer, and 15 ng of cyclamen DNA. Mixtures were assembled at 0 °C, transferred to a thermal cycler, and then precooled to 4 °C. The amplification was carried out in a model Master Gradient thermal cycler (Eppendorf, Hauppauge, NY, USA) using an optimized in-house program consisting of an initial denaturation step of 2 min at 94 °C, and then 45 cycles of 2 min at 94 °C, 1 min at 37 °C, 2 min at 72 °C, followed by a 10-min elongation step at 72 °C. PCR products were stored at 4 °C before analysis. Amplification products were separated by electrophoresis on 1.5 % agarose gels and 0.5 g/mL ethidium bromide in 1X TAE buffer (40 mM Tris–acetate, 1 mM EDTA, pH 8.0) for 3 h at 70 V. The fragment patterns were photographed under UV light for further analysis. A 1-kb DNA ladder (Fermentas) was used to determine the fragment size.

Table 1 Cyclamen genotypes examined in this study and their sampling locations

No	*Cyclamen* species	Sampling location province/City	DNA concentration ng/µl	DNA quality A260/A280
1	*C. pseudibericum*	Aladağ/Adana	165	2.11
2	*C. pseudibericum*	Aladağ/Adana	206	1.85
3	*C. cilicium*	Aladağ/Adana	256	2.09
4	*C. cilicium*	Aladağ/Adana	184	2.11
5	*C. pseudibericum*	Aladağ/Adana	201	2.06
6	*C. pseudibericum*	Aladağ/Adana	432	2.12
7	*C. pseudibericum*	Aladağ/Adana	124	2.11
8	*C. pseudibericum*	Aladağ/Adana	160	2.11
9	*C. pseudibericum*	Feke/Adana	118	2.12
10	*C. pseudibericum*	Feke/Adana	184	1.87
11	*C. pseudibericum*	Feke/Adana	277	2.04
12	*C. pseudibericum*	Feke/Adana	119	2.08
13	*C. cilicium*	Pozantı/Adana	444	2.08
14	*C. cilicium*	Pozantı/Adana	229	2.07
15	*C. cilicium*	Pozantı/Adana	250	2.11
16	*C. cilicium*	Pozantı/Adana	159	2.12
17	*C. cilicium*	Pozantı/Adana	170	1.85
18	*C. cilicium*	Pozantı/Adana	109	2.11
19	*C. cilicium*	Pozantı/Adana	156	1.75
20	*C. cilicium*	Pozantı/Adana	241	2.03
21	*C. cilicium*	Pozantı/Adana	223	2.05
22	*C. cilicium*	Pozantı/Adana	234	2.05
23	*C. cilicium*	Pozantı/Adana	229	2.03
24	*C. cilicium*	Pozantı/Adana	280	1.99
25	*C. persicum*	Merkez/Adana	201	2.08
26	*C. persicum*	Merkez/Adana	544	2.08
27	*C. persicum*	Merkez/Adana	180	2.05
28	*C. persicum*	Merkez/Adana	108	2.07
29	*C. persicum*	Merkez/Adana	186	2.06
30	*C. persicum*	Yumurtalık/Adana	233	2.07
31	*C. persicum*	Yumurtalık/Adana	215	2.07
32	*C. persicum*	Yumurtalık/Adana	214	1.82
33	*C. persicum*	Yumurtalık/Adana	248	1.89
34	*C. persicum*	Yumurtalık/Adana	210	2.08
35	*C. persicum*	Yumurtalık/Adana	187	2.09
36	*C. persicum*	Yumurtalık/Adana	146	1.85
37	*C. persicum*	Yumurtalık/Adana	69	1.99
38	*C. persicum*	Yumurtalık/Adana	204	1.96
39	*C. persicum*	Yumurtalık/Adana	51	1.94
40	*C. graecum*	Antalya	357	1.99
41	*C. graecum*	Antalya	74	1.94

Table 1 continued

No	*Cyclamen* species	Sampling location province/City	DNA concentration ng/µl	DNA quality A260/A280
42	*C. graecum*	Antalya	216	2.07
43	*C. graecum*	Antalya	109	1.87
44	*C. mirabile*	Isparta	62	1.89
45	*C. mirabile*	Isparta	155	1.79
46	*C. mirabile*	Isparta	184	1.88
47	*C. mirabile*	Isparta	167	1.96
48	*C. hederifolium*	Aydın	214	1.93
49	*C. hederifolium*	Aydın	191	2.01
50	*C. hederifolium*	Aydın	205	2.13
51	*C. hederifolium*	Aydın	98	1.99
52	*C. mirabile*	Aydın	242	2.02
53	*C. mirabile*	Aydın	110	2.01
54	*C. hederifolium*	Aydın	182	1.92
55	*C. hederifolium*	Aydın	293	1.92
56	*C. hederifolium*	İzmir	68	1.84
57	*C. hederifolium*	İzmir	12	1.89
58	*C. persicum*	İzmir	80	1.81
59	*C. persicum*	İzmir	122	2.01
60	*C. mirabile*	Muğla	132	2.10
61	*C. mirabile*	Muğla	60	1.98
62	*C. mirabile*	Muğla	201	1.91
63	*C. mirabile*	Muğla	352	1.97
64	*C. alpinum*	Muğla	100	1.87
65	*C. alpinum*	Muğla	328	1.81
66	*C. alpinum*	Muğla	182	2.08
67	*C. alpinum*	Muğla	189	2.02
68	*C. pseudibericum*	Osmaniye	83	1.91
69	*C. coum*	Osmaniye	85	1.99
70	*C. hederifolium*	Osmaniye	86	1.86
71	*C. hederifolium*	Osmaniye	84	1.98
72	*C. pseudibericum*	Osmaniye	175	1.82
73	*C. pseudibericum*	Osmaniye	160	1.91
74	*C. alpinum*	Denizli	119	1.81
75	*C. alpinum*	Denizli	53	1.89
76	*C. alpinum*	Denizli	38	1.85
77	*C. alpinum*	Denizli	250	1.91
78	*C. intaminatum*	Eskişehir	358	1.97
79	*C. intaminatum*	Eskişehir	57	2.04
80	*C. intaminatum*	Eskişehir	173	2.11
81	*C. intaminatum*	Eskişehir	161	2.06
82	*C. intaminatum*	Eskişehir	269	1.87

Table 1 continued

No	*Cyclamen* species	Sampling location province/City	DNA concentration ng/µl	DNA quality A260/A280
83	*C. intaminatum*	Eskişehir	372	1.89
84	*C. pseudibericum*	Kahramanmaraş	71	1.81
85	*C. pseudibericum*	Kahramanmaraş	171	1.85
86	*C. pseudibericum*	Kahramanmaraş	121	1.91
87	*C. pseudibericum*	Kahramanmaraş	85	1.91
88	*C. parviflorum*	Trabzon	214	1.87
89	*C. coum*	Trabzon	326	1.81
90	*C. parviflorum*	Trabzon	127	1.91
91	*C. coum*	Trabzon	269	1.97
92	*C. coum*	Rize	118	1.99
93	*C. coum*	Rize	340	2.01
94	*C. coum*	Rize	250	2.11
95	*C. coum*	Rize	184	1.82

SRAP Analysis

All SRAP primer combinations (Table 3) were initially screened with the 95 genotypes. The 14 primer combinations producing scorable polymorphic bands were used to amplify all 95 genotypes (Table 1). Amplification reactions were done in volumes of 22 µL containing 2× PCR Mastermix (Fermentas K0171), 1 U *Taq* DNA polymerase (Fermentas EP0402), $MgCl_2$, 25 mM of each primer, and 125 ng of cyclamen DNA. The mixtures were assembled at 0 °C and then transferred to a thermal cycler, precooled to 4 °C. The amplification was carried out in a model Master Gradient thermal cycler (Eppendorf) using a program consisting of an initial denaturation step of 5 min at 94 °C, and then 5 cycles of 1 min at 94 °C, 1 min at 35 °C, 2 min at 72 °C, and then 35 cycles of 1 min at 94 °C, 1 min at 50 °C, and 2 min at 72 °C, followed by a 10-min elongation step at 72 °C. PCR products were stored at 4 °C before analysis. Amplification products were separated by electrophoresis on 2.5 % agarose gels and 0.5 g/mL ethidium bromide in 1X TAE buffer for 3.5 h at 110 V. The fragment patterns were photographed under UV light for further analysis. A 100-bp DNA ladder was used as the molecular standard in order to confirm the appropriate SRAP markers.

Data Analysis

Reproducible SRAP and RAPD profiles were scored manually in the binary mode with 1 indicating the presence and 0 indicating the absence of a band, and then the data were used to generate a pair-wise similarity matrix using Jaccard's coefficient (Jaccard, 1908). The unweighted pair group method using UPGMA was employed to create the clustering dendrograms using the NTSYS-PC program (version 2.02i) (Rohlf 1998). The principle coordinates (PCoA) analysis was performed based on the same similarity matrix using the PAST software (Hammer et al. 2001). Polymorphism information content (PIC) values were calculated according to Smith

Table 2 Random amplified polymorphic DNA (RAPD) primers with the number of amplified products

RAPD Primer	Sequence	Size range (bp)	Total number of bands	No. of polymorphic bands	Products detecting polymorphism	PIC Value
OPAK19	TCGCAGCGAG	300–2000	15	15	100	0.87
OPG12	CAGCTCACGA	400–2100	18	18	100	0.87
OPI01	ACCTGGACAC	350–1600	17	17	100	0.84
OPS09	ACTTTGGCGG	400–2000	17	17	100	0.84
OPZ20	GGACCCTTAC	350–1800	11	11	100	0.70
S271	ATCCGCGTG	250–2000	19	19	100	0.82
S272	TGGTCACTGT	250–1500	14	14	100	0.84
S274	ATTGCGTCCA	250–2000	15	15	100	0.82
UBC59	TTCCGGGTGC	250–1500	14	14	100	0.90
UBC8	CCTGGCGGTA	250–1500	17	17	100	0.77
UBC20	TCCGGGTTTG	250–1700	14	14	100	0.91
UBC48	TTAACGGGGA	150–1500	14	13	92.9	0.97
OPAE16	TCCGTGCTGA	250–2000	15	15	100	0.88
UBC54	GTCCCAGAGC	250–1800	17	17	100	0.84
OPB07	GGTGACGCAG	300–1500	17	17	100	0.90
OPB20	GGACCCTTAC	250–2000	20	20	100	0.86
OPAK20	TGATGGCGTC	250–1700	21	21	100	0.86
OPD19	TGATGGCGTC	300–2500	22	22	100	0.82
OPAD10	AAGAGGCCAG	250–1500	16	16	100	0.89
OPA11	CAATCGCCGT	350–1900	17	17	100	0.87
UBC16	GGTGGCGGGA	270–1300	14	14	100	0.72
OPZ18	AGGGTCTGTG	250–1700	15	15	100	0.85
OPP01	GTAGCACTCC	300–1600	13	13	100	0.82
OPZ01	TCTGTGCCAC	300–1600	11	10	90.9	0.80
OPZ06	GTGCCGTTCA	350–1500	14	14	100	0.88
OPZ11	CTCAGTCGCA	250–1600	15	15	100	0.81
OPAE11	AAGACCGGGA	250–1500	18	18	100	0.84
OPZ04	AGGCTGTGCT	250–1900	17	16	94.1	0.82
OPZ14	TCGGAGGTTC	350–1500	12	12	100	0.86
UBC24	ACAGGGGTGA	350–1300	11	11	100	0.78
Total			470	467	99.3	0.87

et al. (1997), using the algorithm for RAPD primers and SRAP primer combinations as follows: $PIC = 1-\Sigma fi^2$, where fi^2 is the frequency of the ith allele.

Results

The genetic diversity among the 95 cyclamen genotypes was evaluated by RAPD (Fig. 1a) and SRAP (Fig. 1b) markers. Amplification was successful with 30 RAPD primers and 14 SRAP primer pairs assayed. Ninety-five cyclamen genotypes were

Table 3 The forward and reverse sequence-related amplified polymorphism primer information for this study

Forward primer	Sequence	Reverse primer	Sequence
ME1	TGAGTCCAAACCGGATA	EM1	GACTGCGTACGAATTAAT
ME2	TGAGTCCAAACCGGAGC	EM2	GACTGCGTACGAATTTGC
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTGAC
ME4	TGAGTCCAAACCGGACC	EM4	GACTGCGTACGAATTTGA
ME5	TGAGTCCAAACCGGAAG	EM6	GACTGCGTACGAATTGCA
ME6	TGAGTCCAAACCGGACA	BA1	GTCGAGCTGCCAATTATA

screened for RAPD markers using 51 primers in a PCR-based DNA amplification procedure. Thirty primers produced clear and good amplification. All RAPD primers that produced polymorphic bands were used to generate RAPD markers with all genotypes (Table 2). Among the 470 bands generated by the 30 selected RAPD primers, 467 were polymorphic. The number of bands detected by a single primer set ranged from 11 to 22, with an average of 15.6. The rate of polymorphism was calculated as 99.3 % among the 95 cyclamen genotypes based on RAPD data. PIC values ranged between 0.72 (UBC16) and 0.97 (UBC48) for RAPD data.

In SRAP analysis, 49 SRAP primer combinations were screened. In total, 14 SRAP primer combinations were determined and used to differentiate the 95 cyclamen genotypes. From the SRAP analysis, a total of 216 bands were generated and the rate of polymorphism was calculated as 100 %. The number of bands detected by a single primer set ranged from 9 to 22, with an average of 15.4. PIC values ranged between 0.70 (ME3F X EM2R) and 0.95 (ME6F X EM1R) for SRAP data (Table 4). PIC values were higher than 0.6 for both marker systems. The average level of stable polymorphisms was very good, demonstrating that RAPDs and SRAPs markers were useful to discriminate all *Cyclamen* genotypes.

Discussion

The number of bands detected by a single primer set and the rates of polymorphism for both RAPD and SRAP analysis were higher than those of many previous reports. Taskin et al. (2012) investigated the genetic diversity among six *C. alpinum* populations in Turkey with 15 RAPD primers. They reported 62.16 % polymorphism with a total of 190 bands, averaging 9.5 bands/locus. In another study, a total of 122 RAPD bands and an average of 11.5 bands/locus were obtained for Iranian *C. persicum* and *C. com* (Naderi et al. 2009).

There are only few reports on the molecular characterization of *Cyclamen* genotypes in the world, but there are many reports explaining the genetic relationship of ornamental and other horticultural plants by RAPD and SRAP markers.

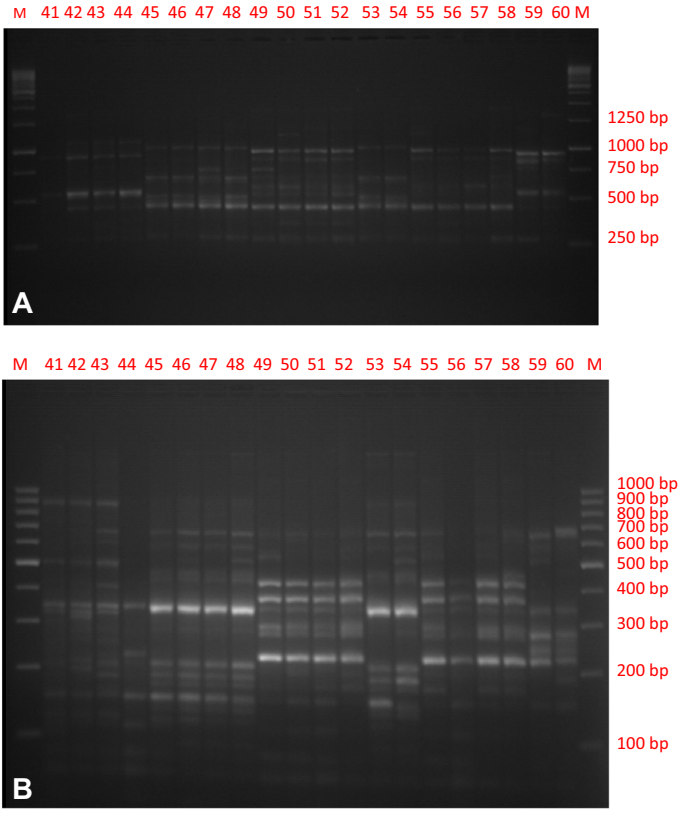

Fig. 1 RAPD and SRAP analyses. **a** RAPD analysis of genotypes 41–60 (see Table 1) of 95 Turkish *Cyclamen* genotypes with primer OPZ01. **b** SRAP analysis of genotypes 41–60 (see Table 1) of 95 Turkish *Cyclamen* genotypes with primer ME4F X EM4R

The similarity coefficient ranged from 0.27 to 0.80 in RAPD analysis and from 0.28 to 0.91 in SRAP analysis. Cluster analysis (UPGMA) employing RAPD and SRAP data resulted in dendrograms and PCoA scatters shown in Figs. 2,3 for RAPD and Figs. 4,5 for SRAP, respectively.

Evaluation of the dendrograms indicates that similar results were obtained for both marker systems. Different cyclamen genotypes collected from different regions of Turkey were separated from each other in dendrograms while cyclamen genotypes belonging to same species were clustered together. *C. persicum* genotypes were clustered together. Similarly, *C. coum* genotypes were clustered in the same branch, but these two groups of genotypes were separated from each other in both marker systems. In another study (Naderi et al. 2009), a total of 26 Iranian *C. persicum* and *C. coum* genotypes were used to investigate the genetic diversity by RAPD markers. The similarity coefficient among the genotypes used in that study was between 0.99 and 0.08. The genotypes were clustered into three groups and the three clusters were 68 % similar. In group A, five wild accessions were separated from other accessions with only 15 % similarity. The lowest

Table 4 Sequence-related amplified polymorphism (SRAP) primers with the number of amplified products

SRAP primer combination	Size range	Total number of bands	Number of polymorphic bands	PIC Value
ME1F X EM2R	110–800	13	13	0.76
ME3F X EM2R	130–850	10	10	0.70
ME1F X EM6R	100–850	9	9	0.71
ME4F X EM 4R	110–900	20	20	0.84
ME2F X EM2R	110–800	16	16	0.80
ME4F X EM6R	140–550	11	11	0.79
ME5F X EM4R	110–950	17	17	0.83
ME3F XEM6R	100–1000	15	15	0.80
ME2F X EM4R	110–1000	21	21	0.86
ME3F X BA1R	100–1000	22	22	0.86
ME 5F X EM 2R	150–950	11	11	0.76
ME 3F X EM 1R	125–780	21	21	0.92
ME 3F X EM 3R	510–820	11	11	0.81
ME 6F X EM 1R	120–750	19	19	0.95
Total		216	216	

similarity in this group was obtained between wild *C. coum* from Lahijan and other wild accessions from different regions of Chaloos. In group B, there were 18 commercial genotypes with divergent flower color. These monotype commercial plants did not show identical profiles, but were separated from three commercial *C. persicum* genotypes (group C) from Chardangeh. The similarity matrix indicated that the lowest genetic similarity (0.08) was between a wild accession WL26 and a white commercial accession, Cw14 and the highest similarity (0.99) was between Cp12 (*C. persicum* cv. 'Laser Orchid') and Cp15 (cv. 'Laser Pink') as well as Cr16 (cv. 'Sierra Deep Rose') and Cr19.

Cyclamen pseudibericum genotypes collected from southern Turkey were also clustered together, but the differences were determined based on location. *C. pseudibericum* genotypes obtained from Adana districts and Kahramanmaraş genotypes were identified in a separate sub-group. Similarly, *C. hederifolium* genotypes from geographically different locations were clustered together. *C. alpinum* genotypes collected from southwestern Turkey were clustered together in both dendrograms. Taskin et al. (2012) studied six natural *C. alpinum* populations collected from southern and southwestern areas of Turkey by RAPD markers to understand genetic diversity. They determined that *C. alpinum* genotypes have a narrow genetic diversity. Our findings are in agreement with results of that study. In our study, both RAPD and SRAP markers indicated that *C. alpinum* genotypes could not be separated from each other, hence their clustering together. One of the *C. intaminatum* genotypes collected from the central Anatolia region (center) of Turkey was separated from other *C. intaminatum* in SRAP dendrogram. *C.*

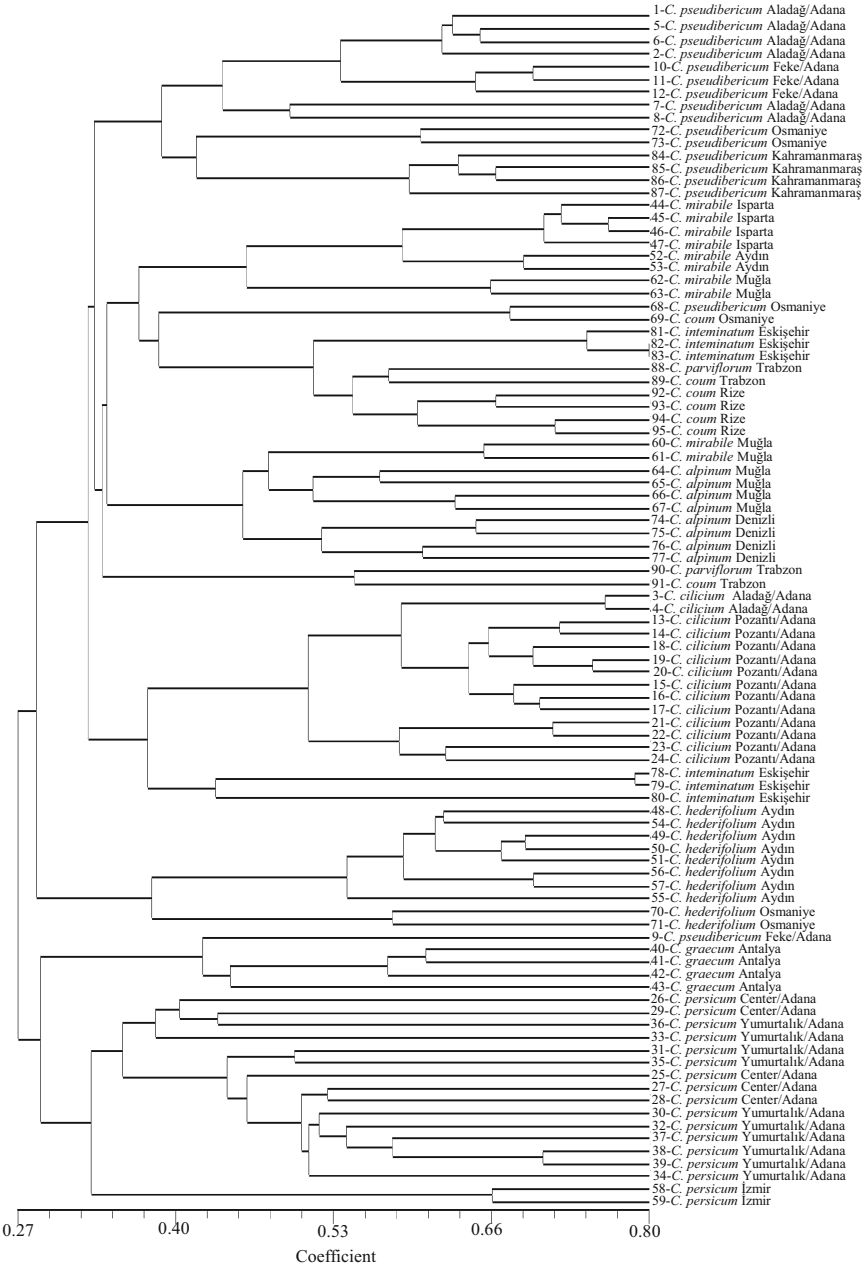

Fig. 2 RAPD dendrogram differentiating 95 Turkish *Cyclamen* genotypes

intaminatum genotypes were divided into two different sub-groups in RAPD dendrogram. *C. intaminatum* genotypes were clustered together with *C. cilicium* genotypes. *C. intaminatum* genotypes are morphologically similar to *C. cilicium*. *C.*

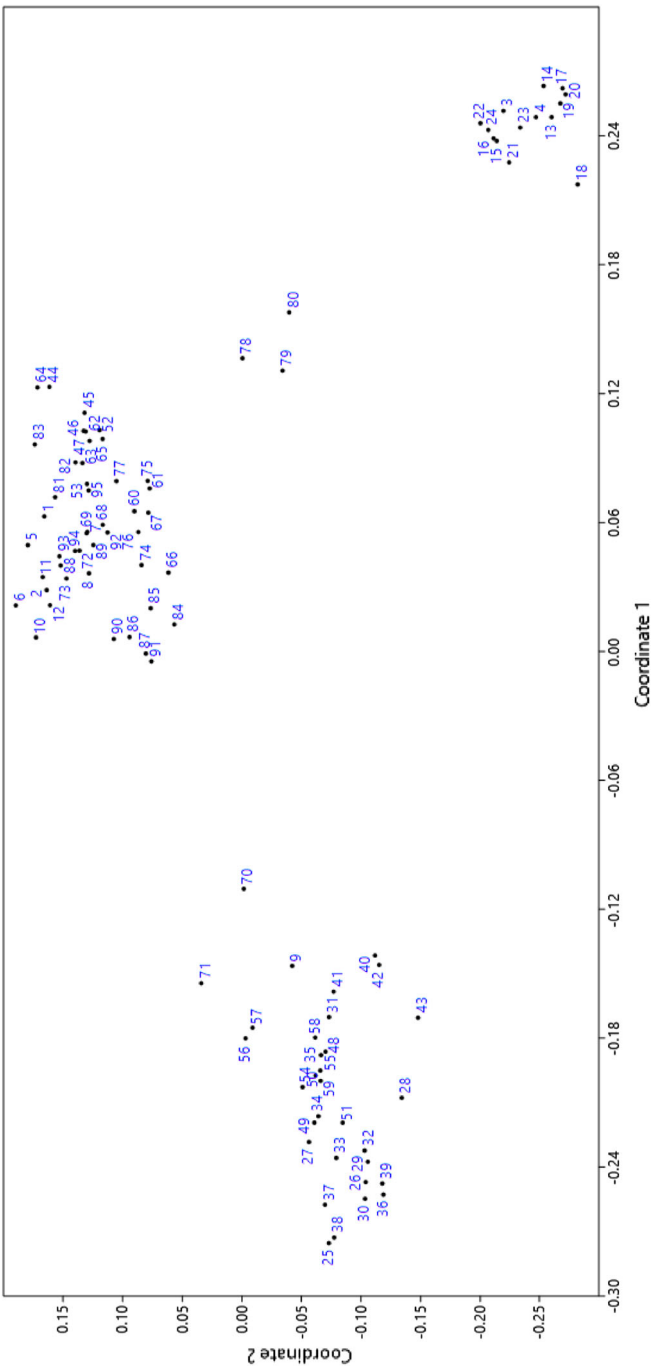

Fig. 3 Biplot (the first two principle coordinates analysis) of 95 cyclamen genotypes generated by the data from RAPD

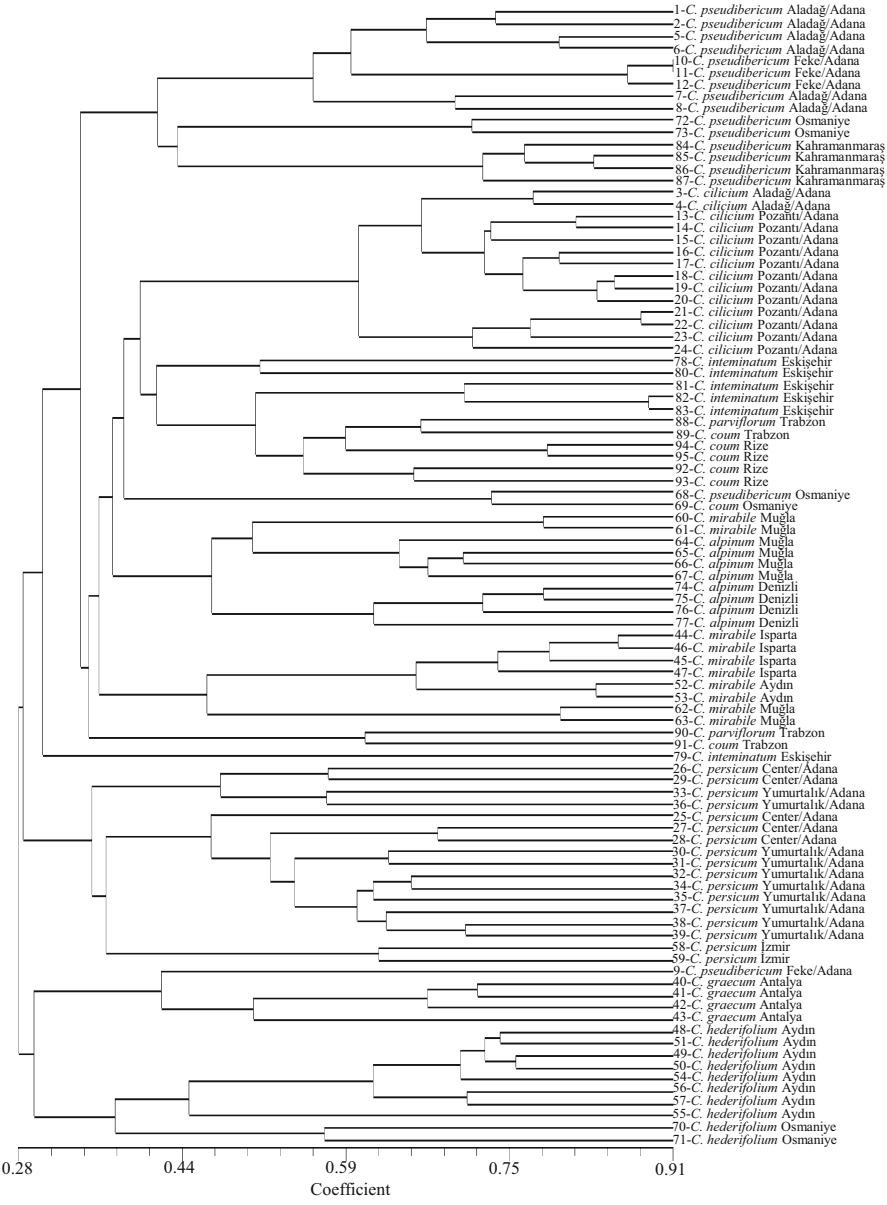

Fig. 4 SRAP dendrogram differentiating 95 Turkish *Cyclamen* genotypes

intaminatum has a confusing past. It was first discovered by E.K. Balls and collected as number EKB669a in June 1934. However, he simply described it as *C. cilicium* var. and although it was briefly erroneously referred to as *C. alpinum*, it was not until 1978 that it received a formal name as *C. cilicium* var. intaminatum Meikle. Undoubtedly, the plant is closely allied to *C. cilicium* and shared the chromosome

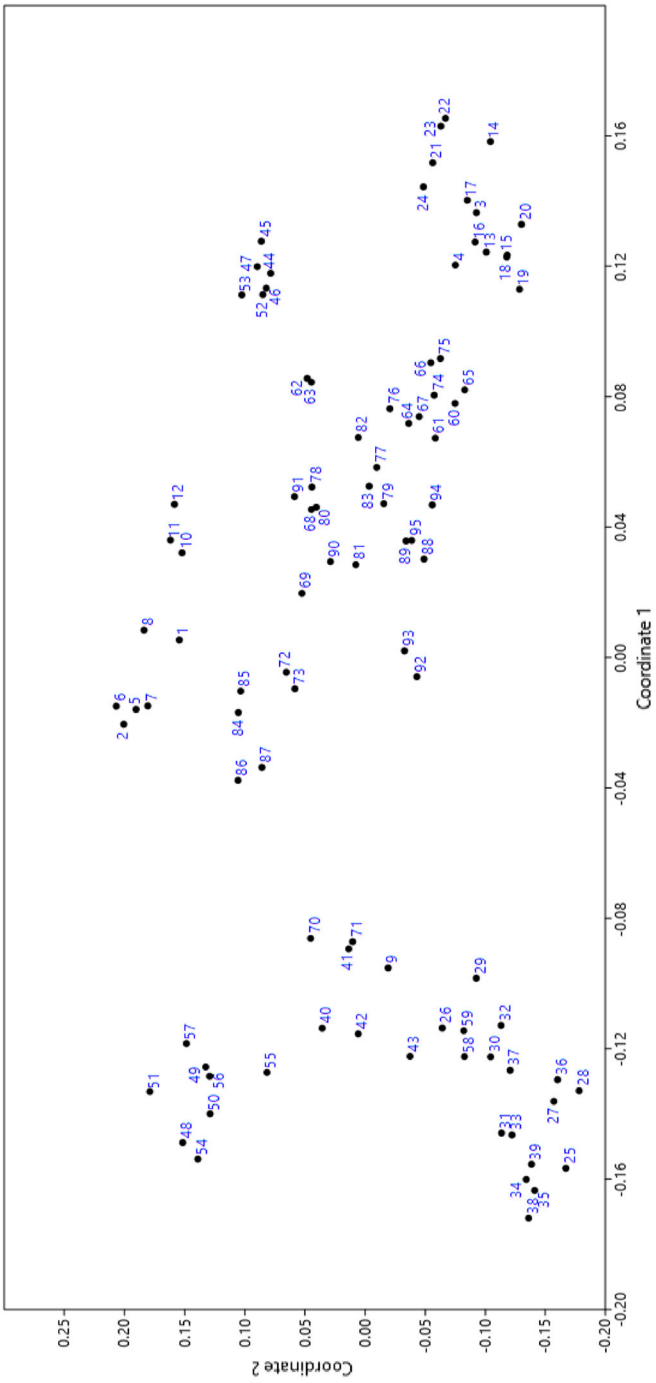

Fig. 5 Biplot (the first two principle coordinates analysis) of 95 cyclamen genotypes generated by the data from SRAP

count $2n = 30$, but in 1988, Grey-Wilson recognized that it was distinct and elevated it to specific status as *C. intaminatum* (Grey-Wilson 1997).

C. graecum genotypes collected from southwest Turkey (Antalya) were placed in the same group in both RAPD- and SRAP-derived dendrograms and showed narrow genetic diversity. *C. persicum* and *C. graecum* genotypes were clustered in the same sub-branches for both marker systems. Based on the nrDNA ITS, cpDNA *trn*L intron sequence data performed by Compton et al. (2004), *C. persicum* and *C. graecum* have been detected to be close to each other.

Curuk et al. (2015) investigated a total of 27 phenotypic characters (13 flower, 11 leaf, 2 plant, and 1 tuber) and 13 quantitative traits (7 flower, 5 leaf, and 1 tuber) in *C. persicum*, *C. cilicium*, *C. coum*, and *C. pseudibericum* species collected from different parts of Turkey. Based on the principal component analysis, the grouping of characters was determined using species-specific clusters, although one or two clusters could not differentiate species, indicating that morphological and cluster analyses alone are not enough for characterizing this complex *Cyclamen* germplasm and that molecular techniques may reveal more intricate and useful relationships.

Molecular markers have also mainly been used for testing genetic purity in cyclamen seeds (Zhang et al. 1997) and somaclonal variation within *C. persicum* callus (Laura et al. 2003; Aka Kacar et al. 2013) as well as in molecular characterization studies. However, there are few studies on the molecular characterization of *Cyclamen* by RAPD. In addition, this study represents the first attempt at the use of SRAP markers for cyclamen. SRAP and RAPD markers were equally powerful tools to separate different cyclamen species and/or genotypes, and in order to determine genetic diversity among the same species, different markers can be used. Especially, SSR markers can be employed for the genetic characterization of *Cyclamen* species, but there are no currently available SSR markers for cyclamens. The development of SSRs is an important and necessary issue for cyclamen molecular studies.

The conservation of plant genetic resources is an important issue for Turkish *Cyclamen*. At first, these genetic resources should be accurately characterized allowing them to be preserved in vitro or in situ (Jalali et al. 2012).

Acknowledgments This research was funded by the Scientific and Technical Research Council of Turkey (TÜBİTAK, project number 110O102).

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