Journal of Applied Biological Sciences 2 (2): 73-78, 2008
ISSN: 1307-1130, www.nobel.gen.tr

Genetic Variation Among *Crocus* L. Species from Western Turkey as Revealed by RAPD and ISSR Markers

Levent SIK[1] Feyza CANDAN[1] Seckin SOYA[2] Canan KARAMENDERES[3] Teoman KESERCIOGLU[4] Bahattin TANYOLAC[2*]

[1] Celal Bayar University, Department of Biology 45030, Muradiye, Manisa, Turkey

[2] Ege University, Department of Bioengineering, 35100, Bornova, Izmir, Turkey

[3] Ege University, Department of Pharmacy, Pharmaceutical Botany Section 35100, Bornova, Izmir, Turkey

[*]Corresponding Author
e-mail: bahattin.tanyolac@ege.edu.tr

Received: December 10, 2007
Accepted: March 05, 2008

Abstract

Crocus L. is of interest to the horticultural, culinary and pharmacological fields. Our aims herein are to obtain information based on genetic diversity and to investigate existing levels of genetic variation within and among the *Crocus* species sampled. Since the classification of *this species* has not been clarified to date, this research may shed some more light on methods for classification of *Crocus* L. RAPD and ISSR markers systems were used to estimate genetic diversity of 56 individuals representing 19 *Crocus* taxa from Western Turkey. RAPD and ISSR banding matrix were calculated to construct a dendrogram (Ward) by a cluster analysis performed by JMP software.

High level of genetic variation detected among sampled C*rocus* species. Genetic distance (GD) values ranged between 0.06 and 0.52 GD. DNA markers based clustering of *Crocus* L. did not coincide with morphological classifications.
This taxon should be reconsidered in terms of classification at the DNA-based level using nuclear, mitochondrial, and chloroplast DNA, as well as more types of robust DNA markers such as AFLP and SSR. The existing high level of genetic variation shows that Turkey could be one of the centers of origin for *Crocus* L.

Key Words: Turkey, *Crocus* species, Genetic variation, RAPD, ISSR

INTRODUCTION

The genus *Crocus* L. consists of some 80 species, distributed from South-Western Europe, throughout central Europe to Turkey and Southwestern parts of Asia, and as Far-East as Western China [1]. The genus is well characterized and morphologically distinct but karyologically very heterogeneous [2]. The genus is of interest to the ecological, horticultural, culinary and pharmacological fields. The combination of a wide geographical range and the presence of dispersed populations make it possible for a plant species to harbor a vast amount of genetic variability; this genetic richness can be present either in the form of allelic variability or allelic uniqueness of some populations [3].

Crocus has a relatively large genome size, with 11.000 Mbp in *Crocus vernus* (2n=8) [4]. It also has large cytological variation. The very wide range of chromosome numbers in *Crocus*, 2n=6, 8, 10, 12, 14, 16, 18, 19, 20, 22, 23, 24, 26, 28, 30, 32, 34, 44, 48 and 64 [5], is accompanied by an equally vast amount of variation in morphology. Morphological data in plants can be affected by environment conditions, therefore a single species grown in different environmental conditions may be morphologically different and thus the use of morphological characters for classification of *Crocus* may result in some mistakes. Frello et al (2004) [4] found that DNA marker analysis in the genus showed poor agreement with the taxonomic structure of the *Crocus* genus. Molecular markers such as RAPD [6], ISSR [7], and AFLP [8] have been used extensively for detection of genetic variation at the DNA level. Among these marker types, RAPD and ISSR do not require preliminary sequence information and are less dependent on laboratory conditions. The use of biochemical and molecular markers represents a useful tool for the study of genetic diversity and phylogenetic relationships at different levels of taxa classification [8]. The first classification of *Crocus sativus* L accessions was published by Caiola *et al.* (2004) [6] who studied some *Crocus* species using RAPD markers. In our research, we used 56 samples (representing 19 taxa) collected from different locations of Western Turkey to investigate genetic diversity.

In this research, our aim was to obtain information based on genetic diversity and to investigate existing levels of genetic variation within and among the *Crocus* species sampled. Since the classification of *Crocus* has not been made clear to date, this research may shed some more light on methods for classification of this *genus*. Although RAPD markers have been performed on *Crocus sativus* [6], to our knowledge, this is the most comprehensive study of genetic variation in *Crocus* species using RAPD and ISSR markers to date.

MATERIALS AND METHODS

Plant material and DNA extraction

The *Crocus* L. species sampled from different locations of Western Turkey are presented in Table 1 and sampling locations are shown in Fig 1. Numbers for the collected samples of the taxa is presented in Table 2. Sample locations

Table 1. Locations, altitudes and co-ordinates of collected samples.

Sample No	Taxon Name	Locations, altitudes and co-ordinates of collected samples	Sample No	Taxon Name	Locations, altitudes and co-ordinates of collected samples
01	***C. chrysanthus*** **Herb.**	Kütahya, 1113 m, 39°19'N 30°03' E	21	***C. chrysanthus*** **Herb.**	Manisa- Spil 1240 m, 38°32' N 27°25' E
02	***C. pallasii*** **Goldb. subsp.*pallasii***	İzmir-Bergama, 477 m., 39°15,'N27°05' E	22	***C. chrysanthus*** **Herb.**	Manisa-Spil 1240 m, 38°32' N 27°25' E
03	***C. flavus*** **Haw. subsp. *dissectus*** **Baytop & B.Mathew**	Kütahya-Emet, 1123 m, 39°22' N 29°20' E	23	***C. chrysanthus*** **Herb.**	İzmir-Kemalpaşa, 785 m, 38°24' N 27°23' E
04	***C. chrysanthus*** **Herb.**	Kütahya-Gediz 1185 m, 39°05' N 29°28' E	24	***C. chrysanthus*** **Herb.**	İzmir-Yamanlar 700 m, 38°32' N 27°09' E
05	***C. candidus*** **Clarke**	Çanakkale-Çan, 120 m, 39°58'N26°53' E	25	***C. flavus*** **Haw. subsp. *flavus***	Çanakkale-Truva, 72 m, 39°57' N 26°16' E
06	***C. flavus*** **Haw. subsp. *dissectus*** **Baytop & B.Mathew**	Denizli-Cankurtaran 1040 m, 37°39' N 29°13' E	26	***C. flavus*** **Haw. subsp. *flavus***	Çanakkale-Ayvacık 270 m, 39°37' N 26°24' E
07	***C. danfordiae*** **Maw**	Kütahya-Aslanapa, 1063 m, 39°14' N 29°49' E	27	***C. antalyensis*** **B.Mathew**	Kütahya-Domaniç, 760 m, 39°55'N29°39' E
08	***C. olivieri*** **J.Gay subsp. *balansae*** **(Gay ex Bak.) B.Mathew**	Manisa-Spil 998 m, 38°34'N27°23' E	28	***C. flavus*** **Haw. subsp. *dissectus*** **Baytop & B.Mathew**	Kütahya-Domaniç, 811 m, 39°39'N29°29' E
09	***C. pulchellus*** **Herb.**	Çanakkale-Yenice, 296 m, 39°55'N27°14' E	29	***C. antalyensis*** **B.Mathew**	Muğla-Fethiye, 1350 m, 36°48'N29°40' E
10	***C. chrysanthus*** **Herb.**	Manisa-Spil, 998 m, 38°34' N 27°23' E	30	***C. biflorus*** **Mill. subsp. *biflorus***	Çanakkale-Ayvacık, 352 m, 39°32' N 26°19' E
11	***C. cancellatus*** **Herb. subsp. *lycius*** **B.Mathew**	Muğla-Fethiye, 340 m., 36°44'N29°00' E	31	***C. gargaricus*** **Herb. subsp. gargaricus**	Balıkesir-Edremit, 1310 m, 39°41' N, 26°54' E
12	***C. pulchellus*** **Herb.**	Manisa-Avdal 126 m, 38°43'N27°16' E	32	***C. biflorus*** **Mill. subsp. *nubigena*** **(Herbert) B.Mathew**	İzmir-Bergama, 459 m, 39°15' N 27°00' E
13	***C. pulchellus*** **Herb.**	Manisa-Avdal , 126 m, 38°43' K, 27°16' E	33	***C. olivieri*** **J.Gay subsp. *olivieri***	Çanakkale-Ayvacık, 249 m, 39°31'N26°19' E
14	***C. pulchellus*** **Herb.**	İzmir-Bergama, 472 m, , 39°13'N27°08' E	34	***C. candidus*** **Clarke**	Çanakkale-Çan 247 m, 40°02'N26°38' E
15	***C. pallasii*** **Goldb. subsp.*pallasii***	Manisa-Kırkağaç, 240 m, 39°08'N27°39' E	35	***C. flavus*** **Haw. subsp. *flavus***	Çanakkale-Ayvacık, 160 m, 39°57' N 26°16' E
16	***C. fleischeri*** **J.Gay**	Manisa-Maldan 352 m, 38°50' N 27°16' E	36	***C. chrysanthus*** **Herb.**	Manisa,-Avdal 126 m, 38°43' N 27°16' E
17	***C. flavus*** **Haw. subsp. *dissectus*** **Baytop & B.Mathew**	Manisa-Kırkağaç, 253 m, 39°08' N 27°39' E	37	***C. pulchellus*** **Herb.**	Çanakkale-Çan, 247 m, 40°02'N26°38' E
18	***C. fleischeri*** **J. Gay**	Denizli-Honaz 502 m, 37°45' N 29°15' E	38	***C. baytopiorum*** **B.Mathew**	Denizli-Honaz 2156 m, 37°41' N 29°16' E
19	***C. biflorus*** **Mill. subsp. *nubigena*** **(Herbert) B.Mathew**	İzmir-Bergama 226 m, 39°08' N 27°10' E	39	***C. pulchellus*** **Herb.**	Çanakkale-Çan, 120 m, 39°58'N26°53' E
20	***C. biflorus*** **Mill. subsp. *nubigena*** **(Herbert) B.Mathew**	İzmir-Bergama, 442 m, 39°15' N 27°01' E	40	***C. pulchellus*** **Herb.**	Manisa-Maldan 352 m, , 38°50'N27°16' E
41	***C. fleischeri*** **J. Gay**	Denizli-Pamukkale, 363 m, 37°56' N 29°07' E	49	***C. cancellatus*** **Herb. subsp. *mazziaricus*** **(Herbert) B.Mathew**	Aydın-Karacasu, 393 m, 37°45'N28°37' E
42	***C. flavus*** **Haw. subsp. *dissectus*** **Baytop & B.Mathew**	Manisa-Akhisar, 150 m, 38°25' N 27°02' E	50	***C. danfordiae*** **Maw**	Kütahya-Yenice, 1193 m, 39°16'N30°03' E
43	***C. chrysanthus*** **Herb.**	Manisa-Spil 1250 m, 38°33' N 27°26' E	51	***C. olivieri*** **J.Gay subsp. *olivieri***	Kütahya-Yenice, 1193 m, 39°16'N30°03' E
44	***C. olivieri*** **J.Gay subsp. *olivieri***	Kütahya-Tavşanlı 850 m, 39°32'N29°33' E	52	***C. fleischeri*** **J.Gay**	Manisa-Kırkağaç, 240 m, 39°08' N 27°39' E
45	***C. chrysanthus*** **Herb.**	Manisa-Maldan 290 m, 38°49' N 27°16' E	53	***C. chrysanthus*** **Herb.**	Manisa-Maldan 352 m, 38°50' N 27°16' E
46	***C. chrysanthus*** **Herb.**	Muğla-Göktepe, 1315 m, 37°12' N 28°27' E	54	***C. pulchellus*** **Herb.**	İzmir-Bergama, 500 m, 39°14'N27°01' E
47	***C. biflorus*** **Mill. subsp. *biflorus***	Çanakkale, Çan, 327 m, 40°01' K 26°49' D	55	***C. antalyensis*** **B.Mathew**	Muğla-Göktepe, 1315 m, 37°21'N28°27' E
48	***C. biflorus*** **Mill. subsp. *crewei*** **(Hooker f.) B.Mathew**	Denizli,-Cankurtaran 1040 m,37°39' N29°13' E	56	***C. biflorus*** **Mill. subsp. *isauricus*** **(Siehe ex Bowles) Mathew**	Muğla-Fethiye, 1250 m., 36°32' N 29°11' E

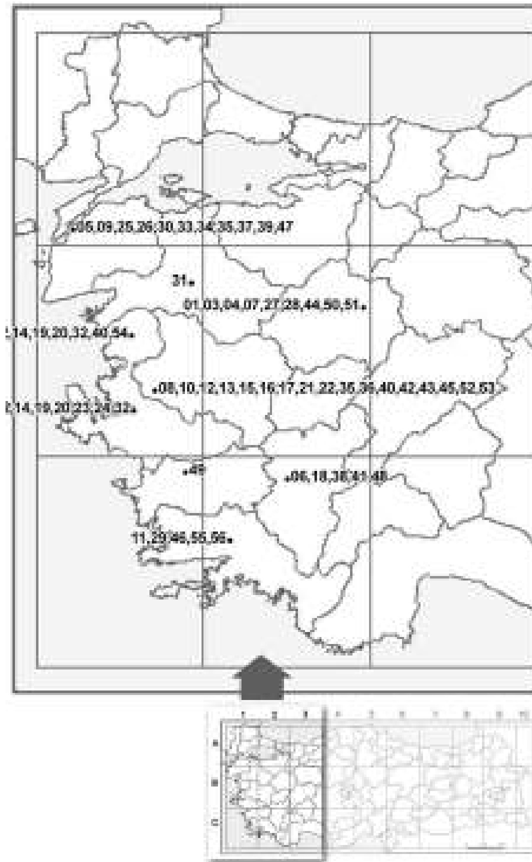

Figure 1. Map locations of the samples. The numbers indicate sample number.

Table 2. Number of samples collected for the taxa

Taxon	Number of samples
C. baytopiorum **B. Mathew**	1
C. fleischeri **J.Gay**	4
C. gargaricus **Herb. subsp. gargaricus**	1
C. chrysanthus **Herb.**	12
C. danfordiae **Maw**	2
C. biflorus **Mill. subsp.** ***biflorus***	2
C. biflorus **Mill. subsp.** ***crewei*** **(Hooker f.) B. Mathew**	1
C. biflorus **Mill. subsp.** ***nubigena*** **(Herbert) B. Mathew**	3
C. biflorus **Mill. subsp.** ***isauricus*** **(Siehe ex Bowles) Mathew**	1
C. flavus **Haw. subsp.** ***flavus***	3
C. flavus **Haw. subsp.** ***dissectus*** **Baytop & B. Mathew**	5
C. antalyensis **B. Mathew**	3
C. olivieri **J.Gay subsp.** ***olivieri***	3
C. olivieri **J.Gay subsp.** ***balansae*** **(Gay ex Bak.) B. Mathew**	1
C. candidus **Clarke**	2
C. pallasii **Goldb. subsp.*****pallasii***	2
C. cancellatus **Herb. subsp.** ***mazziaricus*** **(Herbert) B. Mathew**	1
C. cancellatus **Herb. subsp.** ***lycius*** **B. Mathew**	1
C. pulchellus **Herb.**	8
Total	**56**

are shown in Fig 1. Numbers for the collected samples of the taxa is presented in Table 2. Sample coordinates were identified by GPS (GARMIN, Global Positioning System V model, Taiwan). Plant materials were morphologically identified by Dr Levent SIK, from the Department of Botany at Celal Bayar University, as described in the Flora of Turkey [9, 10] from 2004-2005. Voucher specimens were deposited in the Herbarium of Ege University, Department of Pharmacy, Izmir, Turkey.

Total genomic DNA was extracted according to Doyle (1991) [11] with minor modifications. Isolated DNA was diluted in 100 µl of sterile, distilled water and kept at -80^0 C.

RAPD and ISSR analysis

Twenty nanograms of DNA were used as a template in both RAPD and ISSR analysis. Primers for RAPD analysis were purchased from OPERON tech (Alameda Ca, US). Primers for ISSR (800-899 series) analysis were purchased from University of British Columbia (UBC). PCR amplifications for RAPD were performed in a volume of 15 µl containing 20 ng of genomic DNA, 1.5 µl of 10 X buffer (50 mM of KCl, 10 mM of Tris-Cl at pH 8.3, 0.001 % gelatin), 1.5 mM $MgCl_2$, 0.2 mM each dNTPs, 10 pM primer and 1 unit of *Taq* DNA polymerase enzyme (MBI Fermentas). A control PCR tube containing all components except genomic DNA was run with each primer to check for contamination. DNA amplification was carried out in MJ Research Tetrad Thermal Cycler (Nevada US) with following conditions: 94°C (1 min) initial denaturation, 35 cycles at 94° C (30 s), 36° C (45 s) and 72° C (1 min), 72° C (5 min) final extension, and the last cycle was 4° C for unlimited time. PCR amplifications for ISSR were performed in a volume of 15 µl containing 20 ng of genomic DNA, 10X buffer (50 mM of KCl, 10 mM of Tris-Cl at pH: 8.3, 0.001 % gelatin), 400 mM of each d NTPs, 2.5 mM of $MgCl_2$, 1 µM of primer and 1 unit of *Taq* DNA polymerase enzyme (MBI Fermentas). Reactions were carried out in MJ Research Tetrad Thermal Cycler (Nevada US) with following conditions: 94° C (1 min) initial denaturation, 45 cycles of 94° C (30 s), annealing temperature of each primer was calculated according to nucleotide contents of primers (varied 48-55° C) for 45 s and 72° C for 2 min final extension. Last cycle was at 4°C for unlimited time. All amplification reactions were repeated three times.

RAPD and ISSR amplification products were resolved in a 2% agarose gel (in 1XTAE) and visualized by staining with EtBr. The lambda DNA (digested EcoR I/Hind III) size marker was used to estimate amplification products. KODAK 2D gel imaging system was used to transfer the gel image into computer.

Data Analysis

DNA fragment sizes on agarose gel were estimated by comparisons with DNA size markers (lambda DNA EcoR I/Hind III) run on the same gel. The bands were scored ‘1’ for presence and ‘0’ for absence in DNA sample amplified to

create a binary data matrix. Data from both methods (RAPD and ISSR) were combined into a single matrix. Pair wise comparisons of the PCR phenotypes were used to calculate genetic distance (GD) according to Jaccard (1908) [12]. GD values were used to construct a dendrogram (Ward) by a cluster analysis performed by JMP software (version 3.1, SAS Institute, 1995)

RESULTS AND DISCUSSION

A total of 96 primers were used to detect genotypic variation. Fifteen out of 96 primers (16%) did not produce any amplification product; 39 out of 96 primers (40%) did not yield scorable bands and the rest of the primers (a total of 42, 44%) produced scorable bands. Among these scorable primers, 23 (52%) produced polymorphic bands and 19 (48%) were monomorphic. The polymorphic primers amplified 253 bands. The average number of bands per primer was 11. Caiola et al (2004) [6] found that amplification of seven *Crocus* species' DNAs with 21 primers provided 217 repeatable and interpretable fragments. The primer that produced the most bands was M11 (16 bands) and the least bands were amplified by primer I04 (6 bands). Molecular weight of the amplification products varied between 0.5-2.0 kb. Among all the individuals subjected in this research, no species specific band was detected. An example of the RAPD profile obtained from some of the sampled locations is presented in Figure 2.

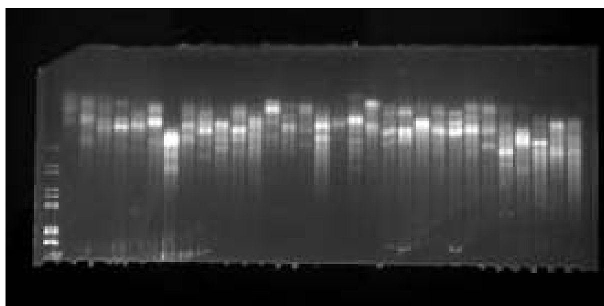

Figure 2. An RAPD profile obtained from some of the sampled locations.

The presence and absence of the amplification products were used to calculate GD among the samples. GD values are presented in Table 3. GD values varied between 0.06 and 0.52. According to GD values, the most closely related two species (GD 0.06) were *C. pulchellus* (9) and *C. chrysanthus* (10), and the most genetically distant two species (GD 0.52) were *C. antalyensis* (27) and *C. chrysanthus* (52). Caiola et al (2004) [6] found the GD values between 0.47 and 0.77 in *C. sativus* (collected from different countries) and five closely related species.

A dendrogram was drawn using RAPD and ISSR data matrices (Figure 3). Fifty-six samples representing 19 taxa formed eight groups as revealed by DNA markers. The taxa subject to the analysis neither were grouped in to geographical origins and nor were clustered according to taxonomic structures. Similarly, Caiola et al (2004) [6] reported no location specific differences could find in their study. Some of the taxa distributed over all clustered groups such as *C. chrysanthus, C. pulchellus, C. flavus, C. olivieri, and C. biflorus*. This shows high level of variation within the genus. Frello et al (2004) [4] found that individuals karyotypes were characterized by high polymorphism in the distribution of repetitive DNA in *C. vernus*.

This study examined a substantially larger number of individuals of *Crocus* and loci than examined in previous studies and allows a thorough overview of RAPD and ISSR diversity across a wide range of *Crocus*. Our results indicate that RAPD and ISSR markers are particularly valuable in the study of the genus *Crocus*, where extensive genetic characterization of the nuclear genome is lacking. Results reported in this study confirm those on DNA content and base composition obtained by cytofluorimetric analysis [13]. RAPD, ISSR data and cytofluorimetric data are in

with karyotypic distance for *Crocus* L. of different geographical origins as reported by Brighton (1977) [2]. Presents results (GD 0.06-0.52) based on DNA markers demonstrated that *Crocus* taxa collected from western Turkey possessed high genetic diversity. These results confirm those on The *Crocus*, a revision of the genus in Flora of Turkey [9, 10].

In conclusion, our results did not support morphological classification. This taxon should be reconsidered in terms of classification at the DNA-based level using nuclear, mitochondrial, chloroplast DNA and types of robust DNA markers such as AFLP and SSR. The existing high level of genetic variation shows that Turkey could be one of the centers of origin of *Crocus*. Kerndorf and Pasche (2006) [14] concluded in their survey of genetic variation of *Crocus* in Western Turkey that the complex flora of *Crocus* is a marvelous species in a wonderful country and, hopefully, will open up new areas for further research.

ACKNOWLEDGEMENTS

This research was supported by Turkish Scientific and the Technical Research Council (TUBITAK), project number: TBAG 2342. We would like to thank Dr Anne Frary from Izmir High Tech Institute in Turkey and Dr Bethany Case from Mississippi University, US for critical reading of the manuscript.

Table 3. GD values among the taxa used in the study calculated by using RAPD and ISSR markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
01	0.00	0.14	0.14	0.24	0.21	0.19	0.14	0.14	0.11	0.13	0.15	0.35	0.28	0.24	0.25	0.26	0.36	0.18	0.21	0.22	0.35	0.17	0.24	0.41	0.38	0.28	0.35	0.31	0.13	0.20	0.16	0.39	0.45	0.39	0.27	0.29	0.21	0.24	0.18	0.16	0.35	0.24	0.27	0.35	0.33	0.31	0.27	0.25	0.37	0.29	0.39	0.45	0.46	0.35	0.18	0.18
02		0.00	0.10	0.20	0.16	0.18	0.17	0.13	0.12	0.14	0.16	0.35	0.27	0.28	0.20	0.25	0.35	0.20	0.22	0.24	0.35	0.19	0.27	0.40	0.36	0.27	0.32	0.29	0.14	0.20	0.18	0.38	0.43	0.35	0.30	0.33	0.22	0.19	0.18	0.17	0.29	0.22	0.26	0.27	0.31	0.31	0.26	0.26	0.18	0.27	0.39	0.43	0.45	0.32	0.18	0.20
03			0.00	0.15	0.13	0.12	0.14	0.12	0.13	0.15	0.18	0.31	0.27	0.26	0.19	0.26	0.31	0.21	0.24	0.24	0.33	0.17	0.26	0.40	0.31	0.22	0.29	0.24	0.17	0.21	0.16	0.35	0.42	0.33	0.25	0.30	0.24	0.23	0.18	0.16	0.29	0.22	0.28	0.27	0.28	0.27	0.24	0.26	0.19	0.25	0.34	0.40	0.40	0.30	0.18	0.17
04				0.00	0.14	0.13	0.22	0.15	0.24	0.21	0.23	0.35	0.27	0.27	0.22	0.30	0.32	0.28	0.28	0.26	0.29	0.21	0.21	0.32	0.30	0.25	0.26	0.25	0.22	0.21	0.24	0.32	0.40	0.33	0.25	0.27	0.23	0.21	0.26	0.26	0.30	0.27	0.26	0.27	0.28	0.25	0.28	0.29	0.28	0.29	0.36	0.40	0.41	0.31	0.26	0.23
05					0.00	0.09	0.18	0.12	0.16	0.18	0.18	0.31	0.26	0.25	0.15	0.27	0.32	0.23	0.24	0.27	0.35	0.21	0.23	0.33	0.27	0.25	0.29	0.27	0.18	0.18	0.19	0.34	0.40	0.30	0.25	0.29	0.25	0.19	0.20	0.21	0.27	0.24	0.27	0.26	0.30	0.28	0.22	0.26	0.22	0.27	0.31	0.39	0.40	0.30	0.18	0.16
06						0.00	0.14	0.12	0.16	0.16	0.17	0.27	0.26	0.26	0.20	0.29	0.28	0.25	0.25	0.24	0.31	0.20	0.20	0.33	0.26	0.21	0.26	0.23	0.17	0.22	0.20	0.33	0.36	0.34	0.23	0.29	0.21	0.25	0.16	0.20	0.29	0.25	0.30	0.29	0.27	0.26	0.23	0.23	0.26	0.29	0.36	0.41	0.42	0.30	0.21	0.19
07							0.00	0.09	0.11	0.11	0.13	0.32	0.28	0.23	0.25	0.26	0.32	0.17	0.24	0.24	0.33	0.22	0.28	0.43	0.36	0.28	0.36	0.31	0.16	0.19	0.14	0.43	0.44	0.38	0.26	0.27	0.20	0.22	0.19	0.14	0.28	0.23	0.28	0.30	0.33	0.34	0.26	0.28	0.19	0.30	0.34	0.42	0.43	0.34	0.25	0.21
08								0.00	0.12	0.13	0.14	0.28	0.25	0.24	0.20	0.26	0.33	0.17	0.19	0.23	0.28	0.16	0.21	0.33	0.32	0.22	0.30	0.25	0.16	0.20	0.16	0.35	0.38	0.33	0.25	0.28	0.21	0.21	0.20	0.17	0.30	0.22	0.25	0.25	0.30	0.27	0.25	0.24	0.19	0.26	0.31	0.38	0.40	0.30	0.18	0.17
09									0.00	0.06	0.11	0.35	0.22	0.20	0.18	0.25	0.30	0.15	0.18	0.22	0.33	0.15	0.24	0.41	0.35	0.29	0.36	0.34	0.12	0.17	0.11	0.38	0.45	0.35	0.23	0.25	0.17	0.19	0.13	0.10	0.29	0.21	0.24	0.31	0.30	0.30	0.22	0.22	0.15	0.25	0.31	0.40	0.39	0.30	0.15	0.16
10										0.00	0.09	0.34	0.13	0.17	0.21	0.20	0.30	0.13	0.18	0.20	0.29	0.14	0.26	0.39	0.30	0.29	0.36	0.31	0.12	0.16	0.13	0.36	0.42	0.35	0.25	0.24	0.15	0.20	0.14	0.10	0.25	0.19	0.24	0.31	0.27	0.30	0.22	0.25	0.15	0.27	0.30	0.35	0.35	0.28	0.16	0.15
11											0.00	0.36	0.25	0.23	0.24	0.26	0.32	0.15	0.20	0.22	0.33	0.18	0.27	0.39	0.34	0.30	0.36	0.32	0.17	0.20	0.17	0.43	0.43	0.36	0.27	0.30	0.21	0.24	0.19	0.16	0.31	0.23	0.28	0.32	0.31	0.34	0.26	0.25	0.19	0.31	0.32	0.40	0.44	0.32	0.20	0.20
12												0.00	0.30	0.30	0.35	0.34	0.33	0.38	0.36	0.34	0.37	0.33	0.33	0.34	0.32	0.31	0.37	0.32	0.31	0.34	0.32	0.38	0.40	0.44	0.38	0.43	0.39	0.40	0.30	0.32	0.39	0.40	0.38	0.43	0.38	0.36	0.40	0.36	0.39	0.41	0.43	0.49	0.44	0.40	0.37	0.35
13													0.00	0.13	0.28	0.25	0.34	0.25	0.29	0.26	0.33	0.26	0.31	0.38	0.35	0.36	0.38	0.36	0.25	0.26	0.25	0.36	0.45	0.47	0.34	0.32	0.27	0.35	0.21	0.22	0.38	0.35	0.31	0.38	0.35	0.35	0.31	0.31	0.27	0.35	0.39	0.45	0.45	0.30	0.25	0.21
14														0.00	0.24	0.25	0.36	0.22	0.27	0.24	0.33	0.25	0.32	0.42	0.39	0.36	0.39	0.33	0.21	0.22	0.22	0.39	0.49	0.49	0.35	0.32	0.25	0.32	0.21	0.21	0.36	0.31	0.29	0.42	0.34	0.34	0.29	0.30	0.27	0.35	0.40	0.42	0.38	0.32	0.25	0.25
15															0.00	0.24	0.28	0.21	0.22	0.23	0.33	0.20	0.26	0.39	0.33	0.30	0.31	0.28	0.21	0.22	0.19	0.32	0.36	0.39	0.27	0.27	0.23	0.26	0.20	0.20	0.27	0.27	0.33	0.34	0.33	0.32	0.26	0.26	0.24	0.27	0.37	0.34	0.36	0.34	0.20	0.18
16																0.00	0.31	0.15	0.29	0.25	0.28	0.27	0.32	0.33	0.38	0.32	0.35	0.32	0.25	0.27	0.25	0.39	0.45	0.43	0.30	0.33	0.30	0.26	0.27	0.23	0.30	0.32	0.30	0.34	0.34	0.37	0.32	0.32	0.28	0.42	0.43	0.40	0.42	0.38	0.26	0.28
17																	0.00	0.34	0.32	0.32	0.31	0.34	0.36	0.37	0.31	0.30	0.29	0.34	0.35	0.33	0.34	0.43	0.39	0.44	0.34	0.37	0.35	0.35	0.31	0.32	0.38	0.34	0.38	0.36	0.39	0.36	0.36	0.36	0.37	0.41	0.40	0.45	0.45	0.38	0.35	0.34
18																		0.00	0.21	0.21	0.33	0.17	0.26	0.43	0.40	0.30	0.36	0.31	0.18	0.20	0.19	0.38	0.42	0.38	0.29	0.28	0.22	0.22	0.20	0.15	0.24	0.25	0.28	0.32	0.31	0.34	0.25	0.27	0.21	0.29	0.35	0.34	0.39	0.30	0.21	0.20
19																			0.00	0.15	0.30	0.17	0.25	0.40	0.39	0.32	0.36	0.35	0.21	0.22	0.23	0.35	0.40	0.36	0.32	0.30	0.24	0.27	0.19	0.22	0.30	0.23	0.29	0.31	0.30	0.30	0.28	0.24	0.23	0.29	0.34	0.33	0.35	0.31	0.22	0.22
20																				0.00	0.32	0.21	0.28	0.41	0.42	0.30	0.36	0.31	0.24	0.21	0.26	0.33	0.42	0.38	0.32	0.33	0.26	0.27	0.23	0.24	0.32	0.25	0.31	0.36	0.29	0.27	0.28	0.29	0.26	0.36	0.47	0.42	0.42	0.36	0.25	0.27
21																					0.00	0.22	0.21	0.21	0.42	0.37	0.41	0.35	0.36	0.36	0.38	0.45	0.48	0.52	0.45	0.39	0.37	0.36	0.33	0.38	0.42	0.43	0.30	0.38	0.34	0.35	0.35	0.36	0.38	0.38	0.42	0.39	0.40	0.43	0.34	0.34
22																						0.00	0.13	0.29	0.34	0.26	0.34	0.28	0.19	0.21	0.20	0.31	0.38	0.32	0.27	0.26	0.22	0.27	0.18	0.19	0.27	0.25	0.28	0.36	0.29	0.32	0.22	0.25	0.22	0.24	0.35	0.34	0.37	0.35	0.18	0.19
23																							0.00	0.22	0.40	0.35	0.40	0.36	0.24	0.28	0.27	0.36	0.46	0.36	0.35	0.28	0.30	0.29	0.26	0.28	0.31	0.36	0.25	0.38	0.34	0.32	0.28	0.29	0.30	0.29	0.41	0.40	0.40	0.39	0.27	0.25
24																								0.00	0.38	0.40	0.42	0.36	0.39	0.43	0.45	0.40	0.48	0.43	0.43	0.38	0.43	0.42	0.40	0.46	0.31	0.49	0.28	0.43	0.35	0.36	0.39	0.44	0.46	0.44	0.48	0.41	0.43	0.50	0.35	0.36
25																									0.00	0.20	0.20	0.24	0.29	0.33	0.34	0.38	0.31	0.36	0.30	0.38	0.32	0.35	0.34	0.35	0.38	0.33	0.44	0.29	0.36	0.37	0.35	0.36	0.39	0.36	0.40	0.46	0.39	0.38	0.39	0.35
26																										0.00	0.13	0.16	0.28	0.28	0.29	0.31	0.34	0.35	0.27	0.34	0.32	0.30	0.29	0.30	0.38	0.29	0.41	0.33	0.34	0.33	0.31	0.32	0.33	0.33	0.39	0.45	0.47	0.39	0.32	0.31
27																											0.00	0.15	0.36	0.33	0.38	0.41	0.37	0.42	0.32	0.38	0.37	0.36	0.35	0.36	0.40	0.32	0.43	0.38	0.36	0.42	0.40	0.38	0.42	0.40	0.44	0.52	0.50	0.39	0.39	0.35
28																												0.00	0.30	0.36	0.30	0.30	0.32	0.40	0.32	0.34	0.33	0.34	0.32	0.32	0.33	0.30	0.42	0.39	0.33	0.34	0.36	0.38	0.39	0.36	0.38	0.38	0.40	0.40	0.34	0.30
29																													0.00	0.13	0.13	0.32	0.40	0.31	0.25	0.23	0.38	0.21	0.14	0.13	0.29	0.21	0.24	0.28	0.27	0.27	0.19	0.23	0.16	0.16	0.34	0.36	0.33	0.31	0.19	0.14
30																														0.00	0.20	0.31	0.42	0.32	0.24	0.22	0.39	0.18	0.18	0.18	0.30	0.22	0.26	0.32	0.27	0.34	0.20	0.23	0.18	0.27	0.36	0.39	0.32	0.28	0.22	0.17
31																															0.00	0.33	0.38	0.35	0.25	0.26	0.18	0.22	0.16	0.11	0.27	0.23	0.28	0.31	0.30	0.28	0.23	0.22	0.16	0.27	0.38	0.35	0.38	0.34	0.20	0.17
32																																0.00	0.34	0.33	0.36	0.28	0.36	0.34	0.30	0.35	0.37	0.35	0.42	0.44	0.36	0.30	0.31	0.37	0.34	0.30	0.38	0.35	0.35	0.38	0.39	0.31
33																																	0.00	0.29	0.32	0.40	0.39	0.45	0.40	0.38	0.40	0.39	0.49	0.42	0.45	0.41	0.39	0.36	0.44	0.38	0.40	0.38	0.44	0.39	0.47	0.39
34																																		0.00	0.27	0.32	0.32	0.27	0.32	0.32	0.34	0.27	0.35	0.29	0.38	0.40	0.32	0.36	0.38	0.34	0.32	0.39	0.35	0.35	0.38	0.32
35																																			0.00	0.25	0.28	0.29	0.25	0.25	0.27	0.24	0.31	0.30	0.33	0.34	0.27	0.30	0.30	0.31	0.35	0.40	0.39	0.31	0.31	0.27
36																																				0.00	0.21	0.25	0.23	0.23	0.27	0.28	0.27	0.38	0.25	0.33	0.24	0.31	0.30	0.29	0.37	0.35	0.29	0.32	0.32	0.23
37																																					0.00	0.17	0.18	0.15	0.29	0.24	0.29	0.33	0.31	0.35	0.27	0.25	0.20	0.28	0.38	0.36	0.39	0.27	0.24	0.21
38																																						0.00	0.25	0.19	0.29	0.22	0.28	0.26	0.32	0.32	0.25	0.29	0.22	0.31	0.35	0.43	0.40	0.32	0.24	0.23
39																																							0.00	0.09	0.28	0.23	0.24	0.33	0.28	0.31	0.24	0.24	0.20	0.24	0.35	0.36	0.32	0.28	0.18	0.14
40																																								0.00	0.25	0.21	0.22	0.31	0.26	0.29	0.21	0.21	0.14	0.26	0.32	0.33	0.35	0.26	0.19	0.12
41																																									0.00	0.26	0.28	0.33	0.32	0.30	0.25	0.33	0.31	0.36	0.45	0.32	0.34	0.39	0.34	0.26
42																																										0.00	0.25	0.25	0.27	0.29	0.19	0.24	0.22	0.28	0.34	0.38	0.39	0.34	0.27	0.23
43																																											0.00	0.28	0.22	0.29	0.22	0.27	0.24	0.30	0.38	0.36	0.32	0.34	0.31	0.24
44																																												0.00	0.27	0.29	0.27	0.29	0.29	0.35	0.36	0.40	0.38	0.34	0.35	0.29
45																																													0.00	0.21	0.22	0.28	0.29	0.32	0.35	0.29	0.27	0.30	0.35	0.27
46																																														0.00	0.19	0.28	0.28	0.36	0.40	0.42	0.36	0.34	0.36	0.28
47																																															0.00	0.21	0.21	0.28	0.35	0.36	0.30	0.30	0.27	0.19
48																																																0.00	0.19	0.29	0.39	0.37	0.33	0.33	0.24	0.18
49																																																	0.00	0.27	0.35	0.35	0.35	0.31	0.22	0.19
50																																																		0.00	0.30	0.32	0.29	0.28	0.32	0.25
51																																																			0.00	0.32	0.31	0.32	0.36	0.30
52																																																				0.00	0.23	0.35	0.41	0.32
53																																																					0.00	0.29	0.41	0.32
54																																																						0.00	0.36	0.30
55																																																							0.00	0.11
56																																																								0.00

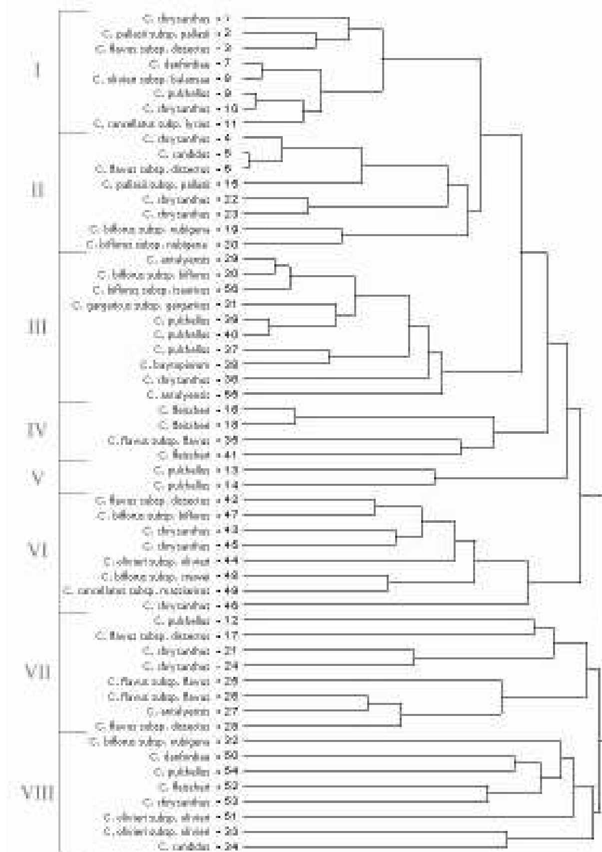

Figure 3. Cluster analysis of 56 samples collected from different regions of Western-Turkey using RAPD and ISSR fingerprinting data.

LITERATURE CITED

[1] Mathew B. 1982. The *Crocus*, a revision of the genus *Crocus* (Iridaceae). B.T. Batsford Ltd London.

[2] Brighton CA, 1977. Cytological problems in the genus *Crocus* (Iridaceae): II. *Crocus cancellatus* aggregate. Kew Bulletin **32(1)**:33-47.

[3] Petit J, El Mousadik A, Pons O. 1998. Identification of populations for conservation on the basis of genetic markers. *Conservation Biology* **12**: 844-855.

[4] Frello S, Ørgaard M, Jacobsen N, Heslop-Harrison JS. 2004. The genomic organization and evolutionary distribution of a tandemly repeated DNA sequence family in the genus *Crocus* (Iridaceae). *Hereditas* **141**: 81 88.

[5] Brighton CA, Mathew B, Marchant CJ. 1973. Chromosome counts in the genus *Crocus* (Iridaceae). *Kew Bulletin* **28**: 450-464.

[6] Caiola MG, Caputo P, Zanier R. 2004. RAPD analysis in

Crocus sativus L. accessions and related *Crocus* species. *Biologia Plantarum* **48**: 375-380.

[7] Tanyolac B. 2003. Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (*Hordeum vulgare* spp. *spontaneum*) populations from west Turkey. *Genetic Resources and Crop Evolution* **50**: 611-614.

[8] Ozkan H, Kafkas S, Ozer MS, Brondolini A. 2005. Genetic relationships among South-East Turkey wild barley populations and sampling strategies of *Hordeum spontaneum*. *Theo Appl Genet* **112**: 12-20.

[9] Mathew, B. 1984 *Crocus* L. in: Davis, P.H. (ed.), Flora of Turkey and The East Aegean Islands, Vol. 8:413-438.

[10] Mathew, B.F. 2000 *Crocus* L. In: Güner, A., Özhatay, N., Ekim, T., Başer, KHC (eds.) Flora of Turkey and The East Aegean Islands (supplement II), Vol. 11:271-274.

[11] Doyle JJ. 1991. DNA protocols for plants-CTAB total DNA isolation. in: Hewitt GM, Johnston A (eds), molecular techniques in taxonomy. Springer, Berlin, pp. 283-293.

[12] Jaccard P. 1908. Nouvelles reserches sur la distribution florale. *Bull Vaud. Soc. Nat.* **44**: 233-270.

[13] Brandizzi, F., Caiola, M. G. 1998 Flow cytometric analysis of nuclear DNA in *Crocus sativus* and allies (Iridaceae). Pl. Syt. Evol. 211:149-154.

[14] Kerndorf H, Pasche E. 2006. *Crocus biflorus* (Liliiflorae, Iridaceae) in Anatolia (Part three). *Linzer Biol. Beitr*. **38:** 165-187.