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İLKNUR SOLMAZ

YILDIZ KAÇAR

NEBAHAT SARI

ÖZHAN ŞİMŞEK

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Genetic diversity within Turkish watermelon [*Citrullus lanatus* (Thunb.) Matsumura & Nakai] accessions revealed by SSR and SRAP markers

İlknur SOLMAZ*, Yıldız AKA KAÇAR, Nebahat SARI, Özhan ŞİMŞEK
Department of Horticulture, Faculty of Agriculture, University of Çukurova, Sarıçam, Adana, Turkey

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Abstract: The genetic diversity of Turkish watermelon genotypes selected from the watermelon genetic resource collection was evaluated by simple sequence repeat (SSR) and sequence-related amplified polymorphism (SRAP) markers. Most of the accessions were collected from various geographical regions of Turkey. Different *Citrullus* species, wild relatives, foreign landraces, open pollinated (OP), and commercial hybrid cultivars were also assessed for genetic relatedness. Fourteen SSR primers and 31 SRAP primer combinations were used in the experiment. Both SSR markers (100%) and SRAP markers (97.3%) generated high polymorphisms. Based on the SSR and SRAP data, the genetic similarity coefficients were calculated, and dendrograms were constructed using the unweighted pair-group method with arithmetic average (UPGMA). Cluster and principle coordinate analyses indicated that *Citrullus lanatus* var. *lanatus* subspecies genotypes collected from the different regions of Turkey were closely genetically related. Overall, our results displayed low genetic variability within the Turkish watermelon germplasm in contrast with their high morphological diversity.

Key words: *Citrullus lanatus*, genetic resources, microsatellite, molecular markers, SRAP

1. Introduction

Horticulture is concerned with plants that are used by people for food as edible products, culinary ingredients, or for medicinal or ornamental and aesthetic purposes. They are genetically a very diverse group and play a major role in modern society and the economy. Fruits and vegetables are an important component of traditional food but are also central to healthy diets in modern urban populations (Bajpai et al., 2014; Feng et al., 2014; Ruttanapraset et al., 2014; Mlcek et al., 2015). Watermelon is an economically important vegetable crop of the family *Cucurbitaceae* and belongs to the genus *Citrullus*. Watermelon is a popular vegetable crop grown in almost all regions of Turkey and many areas of the world. To improve modern varieties, genetic diversity studies are needed to identify genes involved in stress resistance, fruit quality, and yield (Heslop-Harrison, 2002; Rao, 2004).

Turkey is an important center of genetic diversity for watermelon. Valuable watermelon genetic resources with distinct morphological differences exist in the Southeastern Anatolia, Aegean, Marmara-Thrace, Middle Anatolia, and Mediterranean regions of Turkey (Sari et al., 2007; Solmaz and Sari, 2009). Characterization of these genetic resources is required to assist in their utilization for breeding studies.

Characterization of genetic resources is usually based on morphological classifications, which are easy to conduct, reliable, and have low operating costs. However, morphological descriptors are limited and influenced by environmental conditions. Another important limiting factor is the use of live plants for assessment (Ferreira, 2005; Zhang et al., 2012). Information on genetic diversity and relationships among and within landraces is beneficial for identification, conservation, and utilization of genetic resources for future breeding and food security.

Several molecular markers have been effectively used to assess the genetic diversity of watermelon. Isozymes (Navot and Zamir, 1987), RAPD (Levi et al., 2001a, 2001b; Solmaz et al., 2010), AFLP (Che et al., 2003; Levi et al., 2004; Nimmakayala et al., 2010), ISSR (Levi et al., 2004), SSR (Jarret et al., 1997; Kwon et al., 2007; Levi et al., 2007; Sheng et al., 2012; Zhang et al., 2012; Nantoume et al., 2013), PCR–RFLP (Dane and Liu, 2007), SRAP (Levi et al., 2007; Uluturk et al., 2011), EST–PCR (Levi et al., 2008), and HFO–TAG markers (Levi et al., 2013) have been used to estimate the genetic relationship among cultivated watermelons and different *Citrullus* species. These studies revealed low levels of DNA polymorphism among cultivated watermelons but high genetic diversity among the *Citrullus* subspecies (Zhang et al., 2012; Levi et al., 2013).

* Correspondence: isolmaz@cu.edu.tr

A few studies (Solmaz et al., 2010; Uluturk et al., 2011; Ocal et al., 2014) were performed on the genetic diversity of Turkish watermelon genetic resources that indicated low levels of DNA polymorphism, in contrast to the broad morphological diversity reported by Solmaz and Sari (2009).

The objective of this study was to determine the genetic relationships among cultivated Turkish watermelon genetic resources and compare them to different species in genus *Citrullus* wild relatives, foreign landraces, open pollinated (OP), and hybrid cultivars by using SSR and SRAP markers.

2. Materials and methods

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

2.1. Plant material

Of the 93 accessions, 88 were selected from the watermelon genetic resources collection of Çukurova University, and 5 commercial cultivars were used in this study. The accessions were collected from various regions of Turkey. Seven accessions of *Citrullus lanatus* var. *lanatus* were obtained from the National Gene Bank of Turkey (Aegean Agricultural Research Institute, Menemen-İzmir). Four accessions were brought from the Turkish Republic of North Cyprus, two from Egypt, one from France, and one from Uzbekistan. The seeds of two *Citrullus lanatus* var. *citroides* (PI 270563, PI 482293), two *Citrullus colocynthis* (PI 220778, PI 432337), one *Citrullus rehmii* (PI 632755), and two *Praecitrullus fistulosus* (PI 174812, PI 212522) accessions were obtained from the USDA, ARS, Plant Genetic Resources Conservation Unit (Griffin, GA, USA). One *Citrullus lanatus* var. *citroides* accession (Kar 26) was provided by INRA (Institut Nationale de la Recherche Agronomique, Avignon, France). The seeds of the PI accession 296341 (*Citrullus lanatus* var. *citroides*) Calhoun Gray, Charleston Gray, and Congo were obtained from Seminis Seeds (USA). OP cultivar Crimson Sweet and hybrid cultivars Crimson Tide, Celebration, and Bolkan were supplied from Çukurova Seed, Syngenta Seed, and Multi Seed. The names, origins, and species of watermelon accessions are presented in Table 1.

2.2. DNA isolation

Young leaves were collected from each watermelon genotype and immediately frozen in liquid nitrogen and stored at –80 °C. High molecular weight genomic DNA was extracted from the leaf samples following the CTAB miniprep protocol (Edwards et al., 1991). DNA concentration was measured with 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.

2.3. SSR analysis

Fourteen SSR primer pairs (CMCT44, CMACC146, CMTC168, Cgb4765, CLG7992, Cgb476, ASUW2, ASUW13, ASUW19, Cgb5009, C.1. 1-06, C.1. 1-20, C.1. 2-23, and C.1. 2-140) previously tested for watermelon and melon (Jarret et al., 1997; Danin-Poleg et al., 2001; Levi et al., 2006; Aka Kacar et al., 2012) were used. Amplification reactions were performed in 10 µL volumes containing 2X PCR Mastermix (Fermentas K0171), 1 unit of Taq DNA polymerase (Fermentas EP0402), 25 mM $MgCl_2$, 1 µM each of the forward and the reverse primers, and 25 ng of watermelon DNA. The mixtures were assembled at 0–4 °C and then transferred to the thermal cycler. The amplification was performed in a Master Gradient thermal cycler (Eppendorf) using a program consisting of an initial denaturation step of 2 min at 94 °C followed by 35 cycles of 2 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C; the program ended with a 10 min elongation step at 72 °C. PCR products were stored at 4 °C prior to analysis. After amplification, 1–25 µL of loading buffer containing 95% formamide, 10 mM EDTA (pH 8.0), 0.025% xylene cyanol, and 0.025% bromophenol blue was added to each reaction tube. The samples were heat denatured for 5 min at 95 °C and quickly transferred to ice. After loading 1.0 µL of each sample, PCR products were separated in a 25-cm, 6% denaturing polyacrylamide gel (Long Ranger, FMC Biozym, Hessisch Oldendorf, Germany) that had been preheated for 25 min. Electrophoresis was conducted at 1500 V, 50 W, 35 mA, and 48 °C using a Li-Cor DNA Analyzer 4300 (Licor Biosciences, Bad Homburg, Germany). A 50–350 bp DNA ladder (MWG Biotech AG, Ebersberg, Germany) was used to determine DNA sizes.

2.4. SRAP analysis

PCR amplifications were carried out using 31 SRAP primer combinations (me1em3, me1em4, me1em6, me1em11, me2em3, me2em4, me2em5, me2em6, me3em1, me3em2, me3em3, me3em4, me3em5, me3em6, me4em1, me4em2, me4em3, me4em4, me4em6, me5em1, me5em2, me5em3, me5em4, me5em5, me5em6, me5em10, me5em12, me6em6, me7em8, me13em4, me9em11) using 9 forward and 10 reverse SRAP primers (Li and Quiros, 2001). Amplification reactions were performed in a 25 µL volume containing 25 ng of watermelon genomic DNA, 2X PCR Master Mix (Fermentas K0171), 1 unit of Taq DNA polymerase (Fermentas EP0402), 25 mM $MgCl_2$, 20 µM each of the forward and the reverse primers. The PCR amplifications were performed using a Master Gradient thermal cycler (Eppendorf) using a program as follows: 2 min initial denaturation at 94 °C and 5 cycles of 3 steps. First, a 1 min denaturation at 94 °C, 1 min annealing at 37 °C, 2 min extension at 72 °C; followed by 35 cycles with 1 min denaturation at 94 °C, 1 min annealing at 50 °C, 2 min extension at 72 °C, and 5 min final extension at 72 °C. The

Table 1. The 93 watermelon accessions evaluated by 14 SSR and 31 SRAP primers.

No.	Genotype	Local name	Origin	Variety
1	Kar 23	Tat karpuzu	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
2	Kar 24	Unknown	Kafr el Sheikh, Egypt	*C. lanatus* var. *lanatus*
3	Kar 25	Unknown	Kafr el Sheikh, Egypt	*C. lanatus* var. *lanatus*
4	Kar 26	Pastèque à chair vert	INRA, France	*C. lanatus* var. *citroides*
5	Kar 28	Beyaz kışlık karpuz	Diyarbakır, Turkey	*C. lanatus* var. *lanatus*
6	Kar 29	Beyaz karpuz	Diyarbakır, Turkey	*C. lanatus* var. *lanatus*
7	Kar 35	Sugar baby	Adana, Turkey	*C. lanatus* var. *lanatus*
8	Kar 36	Sugar baby DH	Adana, Turkey	*C. lanatus* var. *lanatus*
9	Kar 37	Halep karası	Adana, Turkey	*C. lanatus* var. *lanatus*
10	Kar 38	Halep karası DH	Adana, Turkey	*C. lanatus* var. *lanatus*
11	Kar 58	TR 48528	AARI, Turkey	*C. lanatus* var. *lanatus*
12	Kar 59	TR 48544	AARI, Turkey	*C. lanatus* var. *lanatus*
13	Kar 70	TR 43889	AARI, Turkey	*C. lanatus* var. *lanatus*
14	Kar 77	TR 40374	AARI, Turkey	*C. lanatus* var. *lanatus*
15	Kar 78	TR 43066	AARI, Turkey	*C. lanatus* var. *lanatus*
16	Kar 84	TR 64153	AARI, Turkey	*C. lanatus* var. *lanatus*
17	Kar 86	TR 66064	AARI, Turkey	*C. lanatus* var. *lanatus*
18	Kar 92	Yerli karpuz	North Cyprus Turk. Rep.	*C. lanatus* var. *lanatus*
19	Kar 93	Beyaz karpuz	North Cyprus Turk. Rep.	*C. lanatus* var. *lanatus*
20	Kar 97	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
21	Kar 98	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
22	Kar 100	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
23	Kar 102	Unknown	Nusaybin-Cizre, Turkey	*C. lanatus* var. *lanatus*
24	Kar 104	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
25	Kar 105	Unknown	Mardin, Turkey	*C. lanatus* var. *lanatus*
26	Kar 109	Unknown	Mardin, Turkey	*C. lanatus* var. *lanatus*
27	Kar 114	Unknown	Nusaybin-Cizre, Turkey	*C. lanatus* var. *lanatus*
28	Kar 116	Unknown	Nusaybin-Cizre, Turkey	*C. lanatus* var. *lanatus*
29	Kar 117	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
30	Kar 121	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
31	Kar 129	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
32	Kar 139	Unknown	Nusaybin-Cizre, Turkey	*C. lanatus* var. *lanatus*
33	Kar 142	Çakal karpuzu	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
34	Kar 146	Çakal karpuzu	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
35	Kar 147	Medine karpuzu	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
36	Kar 149	Beyaz kışlık karpuz	Diyarbakır, Turkey	*C. lanatus* var. *lanatus*
37	Kar 150	Amerikan karpuzu	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
38	Kar 151	Yaylak karpuzu	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
39	Kar 152	Yerli yuvarlak	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
40	Kar 153	Sürme	Diyarbakır, Turkey	*C. lanatus* var. *lanatus*
41	Kar 154	Unknown	Batman, Turkey	*C. lanatus* var. *lanatus*
42	Kar 160	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*

Table 1. (Continued).

43	Kar 162	Unknown	Siirt, Turkey	*C. lanatus* var. *lanatus*
44	Kar 163	Gelin karpuzu	Siirt, Turkey	*C. lanatus* var. *lanatus*
45	Kar164	Unknown	Siirt, Turkey	*C. lanatus* var. *lanatus*
46	Kar 171	Unknown	Manisa, Turkey	*C. lanatus* var. *lanatus*
47	Kar 173	Unknown	Manisa, Turkey	*C. lanatus* var. *lanatus*
48	Kar 174	Unknown	Manisa, Turkey	*C. lanatus* var. *lanatus*
49	Kar 175	Unknown	Manisa, Turkey	*C. lanatus* var. *lanatus*
50	Kar 176	Unknown	Tekirdağ, Turkey	*C. lanatus* var. *lanatus*
51	Kar 177	Unknown	Tekirdağ, Turkey	*C. lanatus* var. *lanatus*
52	Kar 178	Komando karpuzu	Manisa, Turkey	*C. lanatus* var. *lanatus*
53	Kar 181	Unknown	Manisa, Turkey	*C. lanatus* var. *lanatus*
54	Kar 192	Unknown	Tekirdağ, Turkey	*C. lanatus* var. *lanatus*
55	Kar 197	Unknown	İstanbul, Turkey	*C. lanatus* var. *lanatus*
56	Kar 200	Unknown	Uşak, Turkey	*C. lanatus* var. *lanatus*
57	Kar 203	Unknown	Gediz-Uşak, Turkey	*C. lanatus* var. *lanatus*
58	Kar 205	Unknown	Ankara, Turkey	*C. lanatus* var. *lanatus*
59	Kar 208	Unknown	Konya, Turkey	*C. lanatus* var. *lanatus*
60	Kar 212	Unknown	Konya, Turkey	*C. lanatus* var. *lanatus*
61	Kar 215	Ak karpuz	Çanakkale, Turkey	*C. lanatus* var. *lanatus*
62	Kar 216	Kore karpuzu	Çanakkale, Turkey	*C. lanatus* var. *lanatus*
63	Kar 217	Söbü karpuz	Çanakkale, Turkey	*C. lanatus* var. *lanatus*
64	Kar 218	Kara karpuz	Çanakkale, Turkey	*C. lanatus* var. *lanatus*
65	Kar 222	Unknown	Şanlıurfa, Turkey	*C. lanatus* var. *lanatus*
66	Kar 224	Unknown	Mardin, Turkey	*C. lanatus* var. *lanatus*
67	Kar 230	31-04	Hatay, Turkey	*C. lanatus* var. *lanatus*
68	Kar 232	Congo (PI 385964)	Seminis, USA	*C. lanatus* var. *lanatus*
69	Kar 233	Calhoun Gray	Seminis, USA	*C. lanatus* var. *lanatus*
70	Kar 234	PI 296341	Seminis, USA	*C. lanatus* var. *citroides*
71	Kar 235	Charleston Gray	Seminis, USA	*C. lanatus* var. *lanatus*
72	Kar 237	All Sweet	-	*C. lanatus* var. *lanatus*
73	Kar 238	Dixilee	-	*C. lanatus* var. *lanatus*
74	Kar 241	Unknown	Uzbekistan	*C. lanatus* var. *lanatus*
75	Kar 242	Unknown	Hatay, Turkey	*C. lanatus* var. *lanatus*
76	Kar 243	Zerzuri	Hatay, Turkey	*C. lanatus* var. *lanatus*
77	Kar 254	Adıbudu	Niğde, Turkey	*C. lanatus* var. *lanatus*
78	Kar 268	Unknown	Niğde, Turkey	*C. lanatus* var. *lanatus*
79	Kar 277	Unknown	Nevşehir, Turkey	*C. lanatus* var. *lanatus*
80	Kar 285	Unknown	Adıyaman, Turkey	*C. lanatus* var. *lanatus*
71	Kar 298	Unknown	Osmaniye, Turkey	*C. lanatus* var. *lanatus*
82	Kar 310	Unknown	North Cyprus Turk. Rep.	*C. lanatus* var. *lanatus*
83	Kar 318	PI 220778	Afghanistan, USDA, USA	*C. colocynthis*
84	Kar 319	PI 432337	Cyprus, USDA, USA	*C. colocynthis*
85	Kar 324	PI 270563	South Africa, USDA, USA	*C. lanatus* var. *citroides*

Table 1. (Continued).

86	Kar 327	PI 482293	Zimbabwe, USDA, USA	*C. lanatus* var. *citroides*
87	Kar 330	PI 632755	France, USDA, USA	*C. rehmii*
88	Kar 331	PI 174812	India, USDA, USA	*Praecitrullus fistulosus*
89	Kar 333	PI 217522	Pakistan, USDA, USA	*Praecitrulluus fistulosus*
90	Crimson Tide		Syngenta Seed, Turkey	*C. lanatus* var. *lanatus*
91	Celebration		Syngenta Seed, Turkey	*C. lanatus* var. *lanatus*
92	Bolkan		Multi Seed, Turkey	*C. lanatus* var. *lanatus*
93	Crimson Sweet		Çukurova Seed, Turkey	*C. lanatus* var. *lanatus*

amplification products were separated by electrophoresis in 2.5% agarose gels containing 1X TAE buffer (40 mM Tris–Acetate, 1 mM EDTA, pH 8.0) for 3.5 h at 110 V. Gels were visualized under UV light and photographed. A 100 bp DNA ladder marker (GeneRuler, Fermentas) was used as a standard marker for assessing the molecular size of the PCR products.

2.5. Data collection and analyses

SSR and SRAP fragments were scored based on presence (1) or absence (0) of bands. Then the data were used to generate a pair-wise similarity matrix using Jaccard coefficients (Jaccard, 1908). The unweighted pair-group method (UPGMA) was employed to create clustering dendrograms using the NTSYS-PC program (Rohlf, 1998). Mantel's matrix correspondence test (Mantel, 1967) was used to evaluate the representativeness of the dendrogram by estimating the cophenetic correlation for the dendrogram compared with the similarity matrix. The results of this test show a cophenetic correlation coefficient (r), indicating how well the dendrogram represents similarity data. The principle coordinate (PCoA) analyses were performed based on the same similarity matrix using the Statistical Analysis Software (SAS, 2006) program. Polymorphism information content (PIC) values were calculated according to Smith et al. (1997) using the algorithm for all primer combinations as follows:

PIC = $1 - \Sigma fi^2$, where fi^2 is the frequency of the ith allele.

3. Results

3.1. SSR analysis

Primer sequence information and the range of amplified product sizes among the watermelon accessions are presented in Table 2. The 14 SSR primer pairs amplified 63 alleles in 93 different watermelon accessions. The number of alleles detected by each primer set ranged from 2 to 7, with an average of 4.5. The polymorphism rate was 100%. In terms of total number of alleles, Cgb4767 and Cgb4765 loci produced the highest number of alleles (7), while CLG7992 and ASUW2 loci produced only two alleles. PIC values ranged between 0.40 (Cgb4765) and 0.83 (CMCT44).

Genetic similarities among all accessions ranged from 0.00 to 1.00. The highest similarity rate (1.00) was between *Citrullus lanatus* var. *lanatus* accessions Kar 78 - Kar 200, Kar 86 - Kar 162, Kar 154 - Kar 162, and Kar 162 - Kar 171. The lowest similarity rate was 0.00, and it was obtained between *Praecitrullus fistulosus* genotypes (PI 174812 and PI 217522) and accessions belong to a different *Citrullus* species. The similarity rate between *Citrullus lanatus* var. *citroides* accessions PI 296341, PI 270563, and PI 482293 (except Kar 26) ranged from 0.60 to 0.86. *Citrullus rehmii* was represented by one genotype (PI 632755), and this accession was closer to *Citrullus colocynthis* accession PI 432337 with a 0.45 similarity rate. The similarity index value 0.59 was obtained between *Citrullus colocynthis* accessions PI 220778 and PI 432337.

By utilizing the similarity index, a clustering dendrogram (Figure 1) was constructed using UPGMA. The dendrogram separated all accessions except Kar 78 - Kar 200 and Kar 86 - Kar 162. The dendrogram grouped all accessions into two main clusters. The first main cluster (1) was also divided into two subgroups (1.1 and 1.2). While subgroup 1.1 included two accessions of *Praecitrullus fistulosus* species (PI 217522 and PI 174812), subgroup 1.2 contained only Kar 26, a *Citrullus lanatus* var. *citroides* accession.

The second main cluster (2) included 90 accessions and consisted of 2.1 and 2.2 subgroups. Subgroup 2.1 was also divided into two subgroups (2.1.A and 2.1.B). The subgroup 2.1.A comprised *Citrullus lanatus* var. *citroides* accessions PI 482293, PI 270563, and PI 296341. The subgroup 2.1.B was separated into two groups (2.1.B1 and 2.1.B2). While 2.1.B1 subgroup included the only representative accession of *Citrullus rehmii* (PI 632755), subgroup 2.1.B2 comprised PI 220778 and PI 432337, which were *Citrullus colocynthis* accessions. Subgroup 2.2 included 2.2.A subgroup, which contained only one accession (Kar 109) of *Citrullus lanatus* var. *lanatus*, while 2.2.B subgroup involved all *Citrullus lanatus* var. *lanatus* accessions, hybrid, and open pollinated varieties. This subgroup was the largest and divided into two more subgroups, 2.2.B1

Table 2. SSR markers used to assess the genetic diversity of 93 watermelon accessions.

Primer	Total allele number	Polymorphic allele number	Allele size (bp)	Polymorphism (%)	PIC
CMCT44	4	4	102, 120, 125, 135	100	0.83
CMACC146	3	3	140, 168, 170	100	0.50
CMTC168	5	5	160, 163, 175, 200, 204	100	0.81
Cgb4765	7	7	155, 160, 161, 175, 185, 200, 204	100	0.40
CLG7992	2	2	145, 180	100	0.72
Cgb4767	7	7	185, 188, 195, 198, 204, 206, 215	100	0.71
ASUW2	2	2	185, 188	100	0.79
ASUW13	4	4	122, 140, 142, 150	100	0.73
ASUW19	4	4	140, 143, 160, 165	100	0.79
Cgb5009	6	6	185, 187, 220, 225, 230, 232	100	0.79
C.1. 1-06	4	4	118, 155, 157, 165	100	0.66
C.1. 1-20	6	6	165, 170, 173, 177, 180, 185	100	0.73
C.1. 2-23	6	6	198, 200, 205, 208, 225, 235	100	0.53
C.1. 2-140	3	3	200, 208, 211	100	0.78
Total	63	63		-	
Mean	4.5	4.5		100	

and 2.2.B2. Subgroup 2.2.B1 contained more accessions than 2.2.B2 and included 5 subgroups (I, II, III, IV, and V), while 2.2.B2 subgroup comprised 4 subgroups (VI, VII, VIII, and IX). The cophenetic correlation coefficient was 0.90 between the similarity index and dendrogram.

Principle coordinate analysis (PCoA) was also performed using the similarity matrix, and the two-dimensional dendrogram corroborated UPGMA analyses (Figure 2). While the first axis (D1) explained 13.70% of the total molecular variance, the second axis (D2) explained 10.10%. The accessions of *Citrullus lanatus* var. *lanatus* species collected from Turkey and commercial varieties were separated from *Praecitrullus fistulosus* (PI 217522 and PI 174812), *Citrullus colocynthis* (PI 220778 and PI 432 337), *Citrullus rehmii* (PI 632755), and *Citrullus lanatus* var. *citroides* (PI 482293, PI 296341, PI 270563, Kar 26) species. The Turkish accessions and open pollinated and hybrid cultivars were very closely related and are shown as groups A and B in the dendrogram.

3.2. SRAP analysis

A total of 31 SRAP primer combinations were used to determine the genetic diversity of Turkish watermelon germplasm. Total allele number, polymorphic allele number, polymorphism rate, and allele sizes are presented in Table 3. Thirty-one primer combinations produced a total of 472 bands ranging in size from 100 to 2200 bp. The number of polymorphic markers was 461, providing 97.3% polymorphism. A range of 10–25 alleles was amplified by each SRAP primer set with an average of 15.2 alleles. While me5em12 primer set produced the highest (25), me4em1 primer set produced the lowest number (8) of polymorphic fragments. The PIC values ranged from 0.48 (me3em5) to 0.84 (me3em2 and me9em11). The similarity index value among 93 watermelon accessions varied between 0.18 and 0.97. The closest accessions were Kar 142 - Kar 146 and Kar 146 - Kar 152 (0.97 similarity rate). All these accessions had similar morphological characters in terms of fruit shape and stripes. Genetically, the most distant accessions were *Praecitrullus fistulosus* accessions PI 174812 and *Citrullus colocynthis* accessions PI 220778 and PI 432337 (0.18 similarity index value). While the similarity index value between *Citrullus colocynthis* accessions PI 220778 and PI 432 337 was 0.66, it ranged from 0.72 to 0.97 between *Citrullus lanatus* var. *citroides* accessions. The only *Citrullus rehmii* accession (PI 632755) was genetically closer (0.51 similarity index) to *Citrullus colocynthis* genotype PI 432337 than all other accessions in germplasm. The majority of accessions belong to *Citrullus lanatus* var. *lanatus* accessions, and their genetic similarity index value varied between 0.61 and 0.97.

Results of UPGMA analysis are presented in Figure 3. Similarity levels ranged from 0.22 to 0.97 for all 93 accessions, and two main groups (1 and 2) were observed. The first main cluster (1) contained only two *Praecitrullus fistulosus* accessions (PI 217522 and PI 174812). The second main cluster (2) included 91 *Citrullus* accessions

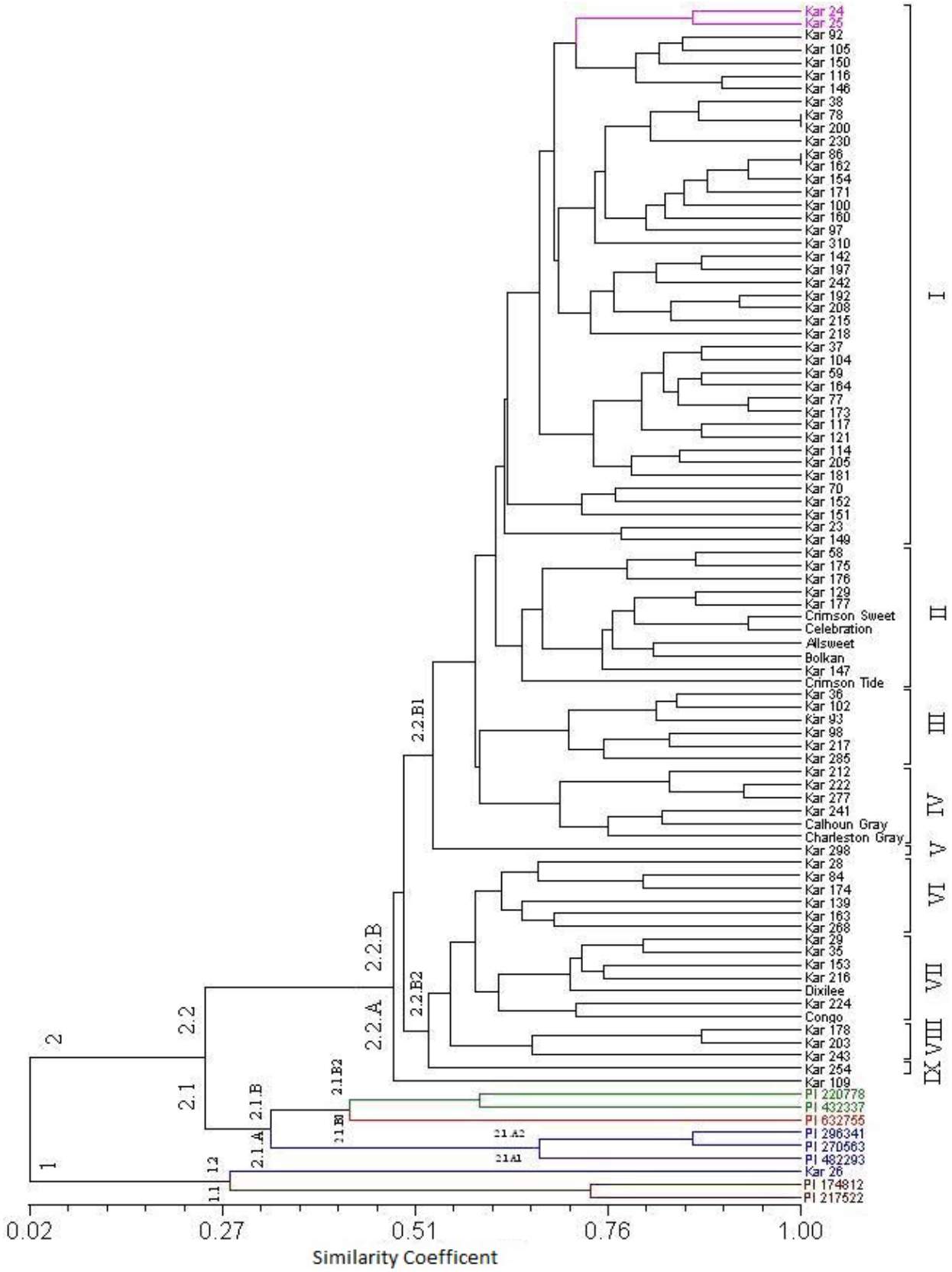

Figure 1. Dendrogram of 93 watermelon accessions generated by the data from 14 SSR primers.

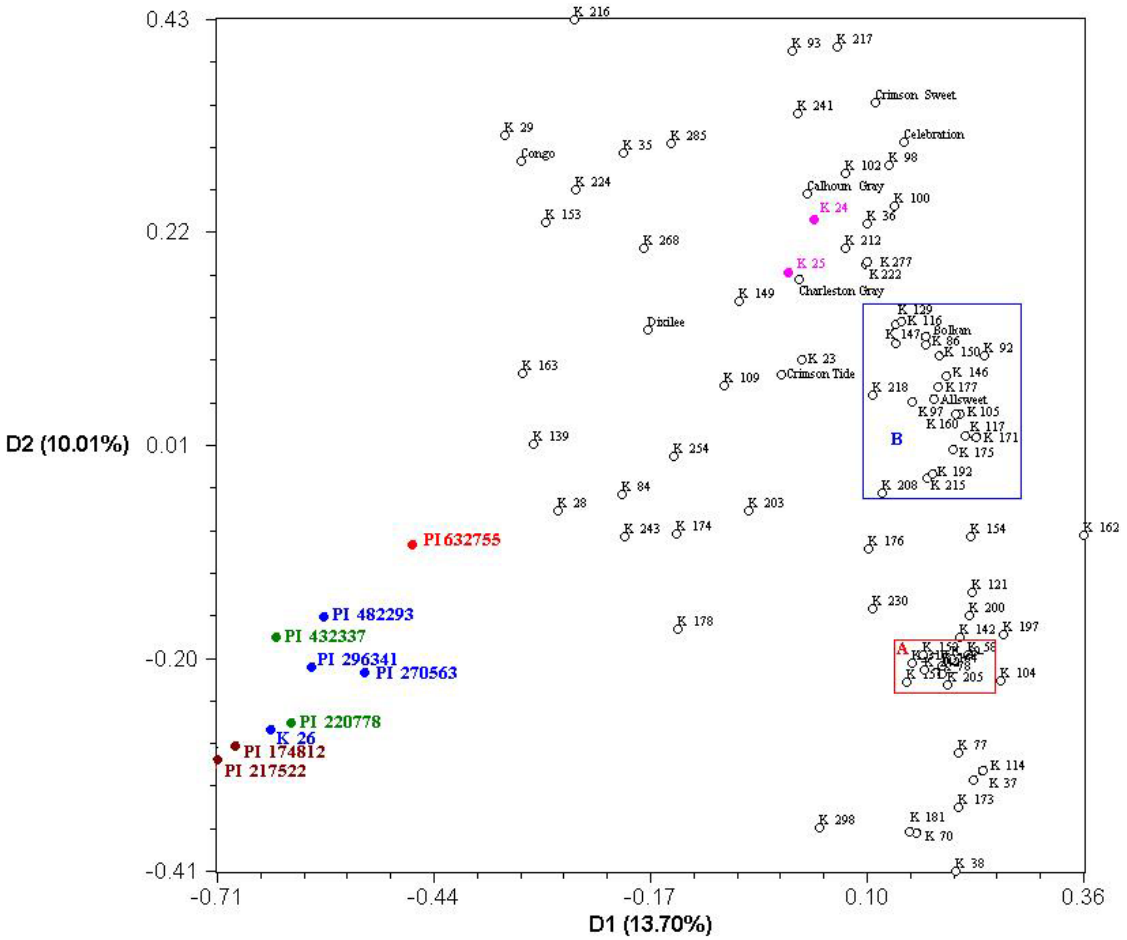

Figure 2. Biplot (the first two principle coordinate analysis) of 93 watermelon accessions generated by the data from 14 SSR primers.

and was divided into two subgroups (2.1 and 2.2), with a 0.51 similarity level. Subgroup 2.1 was also separated into two new subgroups: 2.1.A with *Citrullus rehmii* accession PI 632755 and 2.1.B, with *Citrullus colocynthis* accessions PI 432337 and PI 220778. Subgroup 2.2 was divided into two new subgroups (2.2.A and 2.2.B), with a 0.55 similarity level. Subgroup 2.2.A involved *Citrullus lanatus* var. *lanatus* accessions, hybrids, and open pollinated varieties and formed two subgroups (2.2.A1 and 2.2.A2). While subgroup 2.2.A.1. consisted of accessions Kar 24 and Kar 25, which originated from Egypt and were genetically very close (91%) to each other, subgroup 2.2.A2 was a large group including highly related *Citrullus lanatus* var. *lanatus* accessions. Four *Citrullus lanatus* var. *citroides* accessions (PI 296341, PI 270563, PI 482293, and Kar 26) were grouped in 2.2.B subgroup. The cophenetic correlation coefficient was 0.99, between similarity index and dendrogram.

According to the principle coordinate analysis (PCoA) (Figure 4), the first axis of the plot explained 20% and the second axis explained 7% of the total molecular variation. The two main groups from the dendrogram (A and B) were observed. Group A was the largest group and had 72 accessions of *Citrullus lanatus* var. *lanatus* species. The second group (B) also included *Citrullus lanatus* var. *lanatus* accessions (Kar 243, Kar 277, and Kar 285); open pollinated Crimson Sweet; and F_1 commercial cultivars Crimson Tide, Celebration, and Bolkan. *Citrullus lanatus* var. *citroides* accessions (Kar 26, PI 296341, PI 270563, and PI 482293), *Citrullus colocynthis* accessions (PI 432337 and PI 220778), and *Citrullus rehmii* accession (PI 632755) were located separately from the two groups but closely within the same species. Two *Praecitrullus fistulosus* accessions (PI 174812 and PI 217522) were located quite far from all *Citrullus* species but close to each other.

4. Discussion

We examined the genetic diversity among Turkish watermelon accessions in comparison with different *Citrullus* species, wild relatives, foreign landraces, OP,

Table 3. SRAP markers used to assess the genetic diversity of 93 watermelon accessions.

Primer combination	Total allele number	Polymorphic allele number	Allele sizes (bp)	Polymorphism (%)	PIC
me1em3	12	11	175–900	91.7	0.65
me1em4	15	15	100–950	100	0.75
me1em6	11	10	175–1000	90.9	0.42
me1em11	18	18	100–900	100	0.63
me2em3	14	14	150–900	100	0.56
me2em4	16	16	150–900	100	0.77
me2em5	11	11	150–900	100	0.59
me2em6	16	16	150–1000	100	0.74
me3em1	11	11	100–750	100	0.82
me3em2	17	17	125–1000	100	0.84
me3em3	15	15	100–1000	100	0.67
me3em4	15	15	100–950	100	0.66
me3em5	15	15	125–900	100	0.48
me3em6	17	17	125–1000	100	0.63
me4em1	11	8	125–900	72.7	0.67
me4em2	17	17	100–950	100	0.73
me4em3	14	14	100–900	100	0.52
me4em4	14	14	150–900	100	0.75
me4em6	16	16	175–1000	100	0.56
me5em1	10	9	100–900	90	0.78
me5em2	16	16	150–1000	100	0.65
me5em3	13	13	175–900	100	0.67
me5em4	23	22	100–925	95.7	0.67
me5em5	12	12	175–925	100	0.61
me5em6	15	15	150–1000	100	0.70
me5em10	16	15	150–900	93.8	0.56
me5em12	25	25	125–1000	100	0.74
me6em6	18	18	125–900	100	0.61
me7em8	15	13	100–900	86.7	0.83
me9em11	18	18	125–800	100	0.84
me13em4	16	15	125–925	93.8	0.68
Total	472	461	-	-	
Mean	15.2	14.9	-	97.3	

and commercial hybrid cultivars by using SSR and SRAP markers. While 14 SSRs produced 100% polymorphism, 31 SRAP primer combinations provided 97.3% polymorphism; these results were higher than the results of previous genetic diversity studies (RAPD 60.2%: Solmaz et al., 2010; ISSR 80.2%: Levi et al., 2004; 40.0%: Levi et al., 2007; AFLP 81%: Levi et al., 2007; SRAP 80.5%: Levi et al., 2007) conducted by different molecular marker systems. In SSR analysis we detected the number of alleles by each primer set ranging from 2 to 7, with an average of 4.5. These results are consistent with the findings of Jarret et al. (1997). Researchers used 8 SSR primers to detect the genetic diversity of 33 *Citrullus* accessions belonging to *C. lanatus* var. *lanatus*, *C. lanatus* var. *citroides*, and *C. colocynthis* species and obtained 3–7 alleles with an average of 4.7. Guerra-Sanz (2002) reported 1–8 alleles

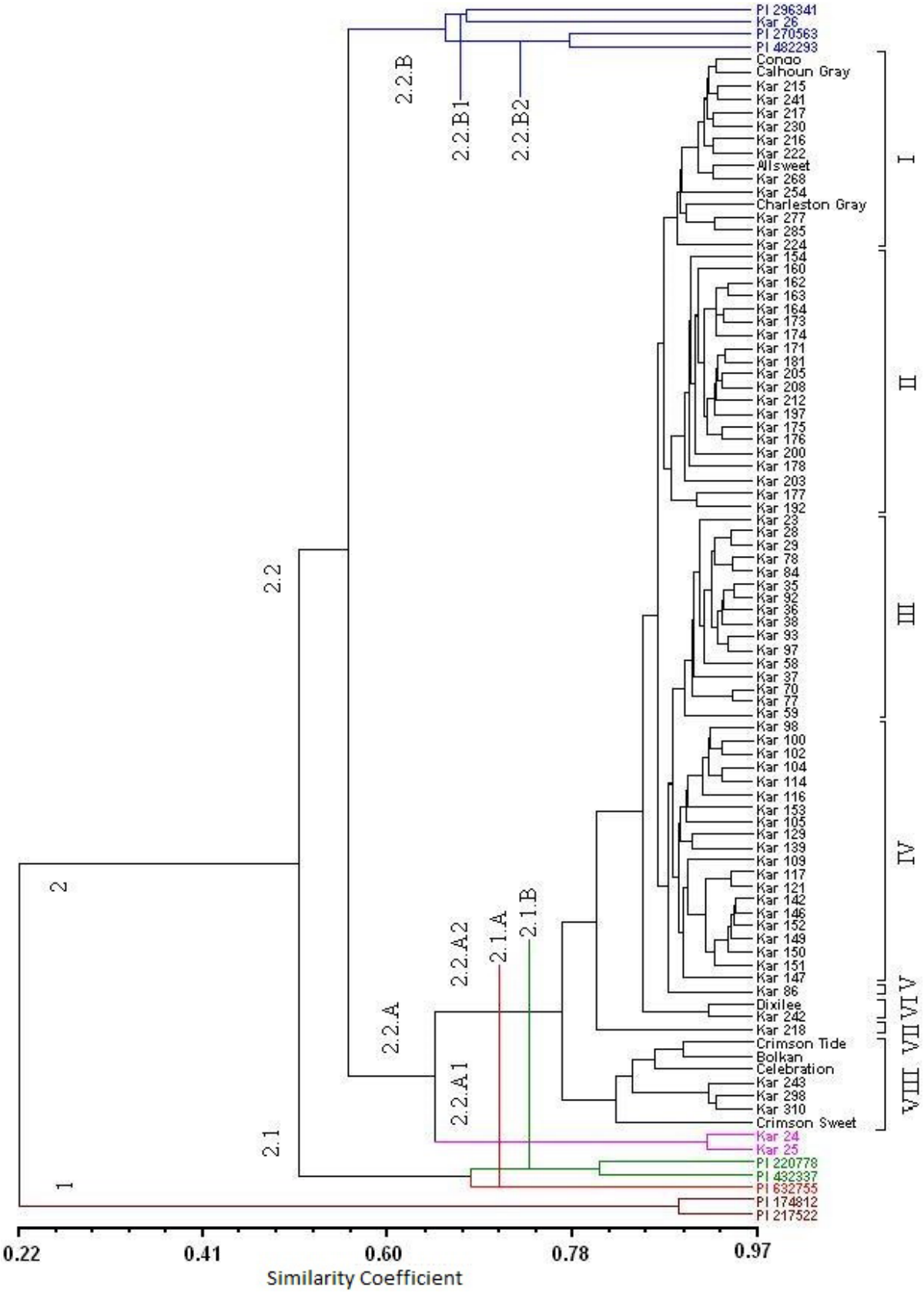

Figure 3. Dendrogram of 93 watermelon accessions generated by the data from 31 SRAP primer combinations.

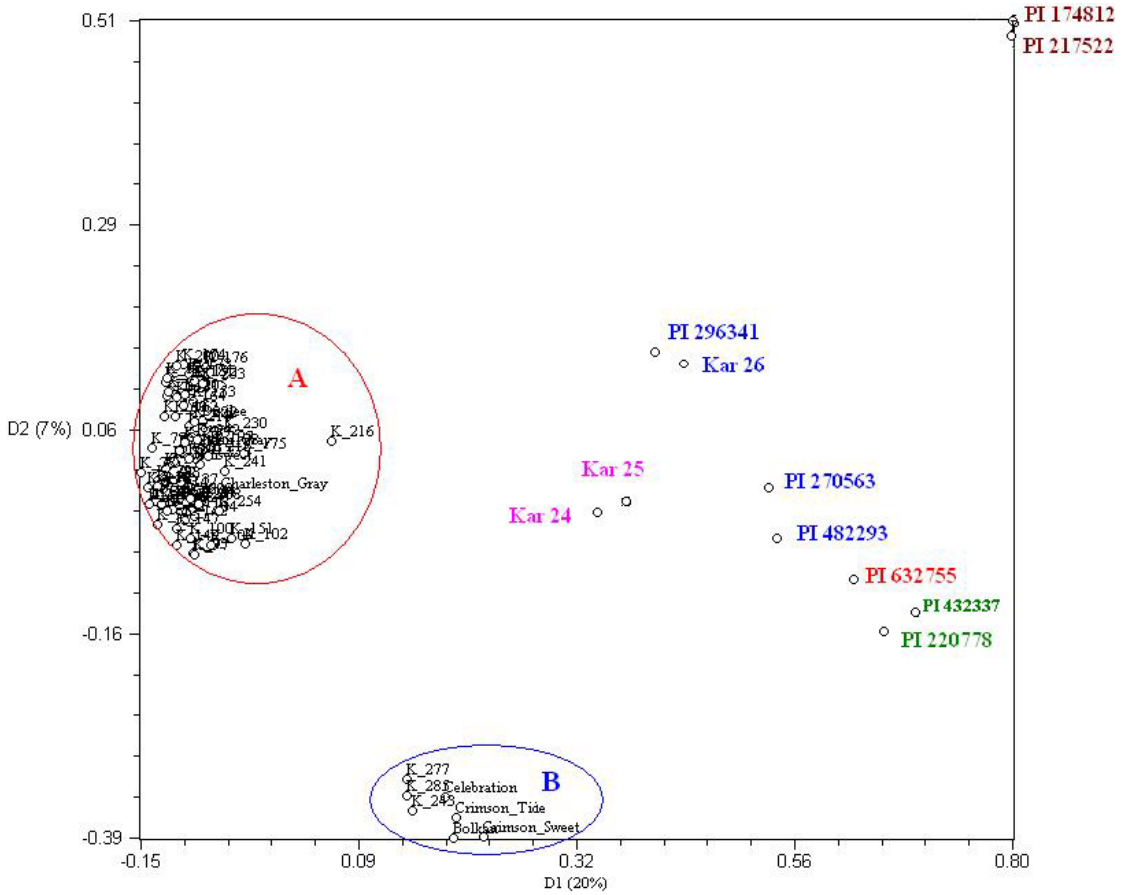

Figure 4. Biplot (the first two principle coordinate analysis) of 93 watermelon accessions generated by the data from 31 SRAP primer combinations.

in *C. lanatus* genotypes and varieties, 0–2 alleles in *C. colocynthis* genotypes, and 0–4 alleles in hybrids by using 18 SSR markers. In another study, Nimmakayala et al. (2010) estimated the phylogenetic relations of 31 accessions of *C. lanatus* var. *lanatus*, *C. lanatus* var. *citroides*, and *C. colocynthis* by SSR and AFLP markers; 30 SSR primer pairs produced a total of 169 alleles ranging between 2 and 12. Genetic diversity of 134 watermelon landrace accessions from Mali were analyzed using 24 microsatellite primer sets, which differentiated 129 alleles across all loci (Nantoume et al., 2013). The number of alleles varied from 1 to 11 with an average of 5.4 alleles detected per SSR marker, and the researchers stated that SSR markers are an efficient means to distinguish different accessions. Mujaju et al. (2010) also used SSR markers and reported that nine SSR primer pairs produced a total of 43 alleles with an average of 4.8 alleles per locus. Genetic diversity within Chinese watermelon ecotypes was determined and compared with germplasm from other countries. The average number of polymorphic bands among 96 accessions was 2.4 (Sheng et al., 2012).

We found a total of 472 fragments, 461 of which were polymorphic, by using 31 SRAP primer combinations and obtained a high polymorphism rate (97.3%). Levi and Thomas (2007) reported that 33 of the 41 SRAP markers were polymorphic with an 80.5% polymorphism rate in 24 watermelon genotypes that have low genetic variability. The researchers stated that SRAP markers were effective, like AFLP markers, and represented different linkage regions of watermelon.

The genetic diversity of hybrid watermelon varieties was assessed by 25 SRAP primer combinations, and 20 of them produced 135 polymorphic alleles with an average of 7.11 for each primer pair (Yan and Zhang, 2005). In our study, we obtained 15.2 alleles and 14.2 polymorphic alleles on average, and these were higher than those of previous reports. This may be due to presence of different *Citrullus* species and related species (*Praecitrullus fistulosus*) in our germplasm. Uluturk et al. (2011) determined the genetic diversity and relatedness of 90 watermelon (*Citrullus lanatus* var. *lanatus* and *Citrullus lanatus* var. *citroides*) accessions by using 30 sequence-related amplified polymorphism (SRAP) marker combinations. According to their results, the SRAP combinations were highly polymorphic (97%), and they reported that the SRAP

marker system is efficient for detection of polymorphism in watermelon, which has low levels of polymorphism.

The highest genetic similarity coefficient was found within the Turkish accessions belonging to *Citrullus lanatus* var. *lanatus*, despite their high level of morphological diversity, as reported by Solmaz and Sari (2009). In a previous study, the genetic diversity of Turkish watermelon accessions was estimated by RAPD markers, and the average genetic similarity coefficient of Turkish accessions was 0.94, which indicated that they are closely related (Solmaz et al., 2010).

Several markers were used to estimate the genetic structure of *C. lanatus* var. *lanatus* cultivars and accessions. However, they all revealed low genetic variability due to their narrow genetic base (Zhang et al., 2012).

We found that higher genetic variation exists within *C. colocynthis* and *C. lanatus* var. *citroides* than *C. lanatus* var. *lanatus* accessions. This result is supported by different studies (Jarret et al., 1997; Levi et al. 2000, 2001a, 2001b; Solmaz et al., 2010). The lowest similarity rate was obtained between *Praecitrullus fistulosus* accessions and all other *Citrullus* accessions. It is reported by Levi et al. (2005) that *Praecitrullus* fistulosus appeared to be distant from all *Cucumis* and *Citrullus* species.

The UPGMA clustering dendrograms constructed separately by using SSR and SRAP data clearly demonstrated that Turkish watermelon accessions collected from different regions, foreign accessions, OP, and hybrid cultivars of *Citrullus lanatus* var. *lanatus* clustered together, indicating that they have a close genetic relationship. There was no correlation between the geographical origin and molecular data consistent with previous report by Solmaz et al. (2010). The PCoA and clustering dendrograms revealed similar groupings. Most of the Turkish accessions (*Citrullus lanatus* var. *lanatus*) formed a compact group, indicating low genetic diversity. This finding is also supported by Ocal et al. (2014). Accessions of different *Citrullus* and *P. fistulosus* species were dispersed on the dendrogram, representing high genetic diversity.

In conclusion, our study revealed that Turkish watermelon genetic resources are molecularly different from other *Citrullus* species and *Praecitrullus fistulosus*. They possessed low levels of genetic variation even though they were morphologically diverse.

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