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LEAF AND FRUIT CHARACTERISTICS AND GENETIC DIVERSITY OF WILD FRUIT *Cerasus prostrata* GENOTYPES COLLECTED FROM THE CENTRAL ANATOLIA, TURKEY

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ABSTRACT

Cerasus prostrata (Lab.) Ser. is quite widespread in some regions of Turkey. It is a wild and deciduous fruit species. The species is commonly encountered in Central Anatolia over the foothills of Erciyes Mountain. In this study, some fruit and leaf characteristics of 30 *C. prostrata* genotypes collected from the foothills of Erciyes Mountain were determined and genetic diversity among them was presented. Fruit weights of the genotypes varied between 0.66–0.23 g and fruit flesh ratios varied between 84.59–63.11%. Leaf width, leaf length and petiole lengths of the genotypes respectively varied between 1.61–0.68 cm; 4.02–1.82 cm and 0.60–0.28 cm. In genetic analyses, 17 ISSR primers were used and 115 bands were obtained. Of these bands, 98 were polymorphic. All genotypes were distinguished from each other. Relatively high variation was identified between the genotypes and similarity levels varied between 0.70–0.95. Current findings revealed significant information for the preservation and appraisal of *C. prostrata*. Further studies are recommended for breeding and protection of this species.

Key words: characterization, genetic analysis, mountain cherry, *Prunus prostrata*

INTRODUCTION

Turkey is among the most significant countries with regard to plant diversity and genetic resources. There are more than 85 fruit types, mostly composed of deciduous species, sub-tropic species and some tropical species, grown in Turkey. While deciduous species are widespread nationwide, sub-tropic and tropical species are mostly grown in hotter southern regions of the country [Ercisli 2004, Pınar et al. 2019]. Turkey hosts about 11 000 plant species and about 3 000 of them are endemic species. Among them, there are several naturally grown trees, small trees, shrubs and pasture plants. Naturally grown species can adapt to humid or arid areas, sunny or shadow environments, acidic or alkaline sites and they provide significant contributions to natural habitat and landscape and play various roles in preservation of biodiversity [Irmak 2013].

Cerasus prostrata (Lab.) Ser. is a shrub-type wild fruit species with about 1 m height and naturally growing over rocky sites at 940–2400 m altitudes in Turkey. The species is especially widespread in West, South and Central Anatolia. The species took the name from its creeping growth form [Ercisli 2004]. It has white-pink flowers, green small fruits and red round ripened fruits (Fig. 1). In some literatures, *Prunus prostrata* (Lab.) Ser. is used as the synonym of *Cerasus prostrata* [Gonulsen 1996]. Hanelt [1997] reported the spread of *P. prostrata* as southern European countries from Spain to and Crete and Eastern

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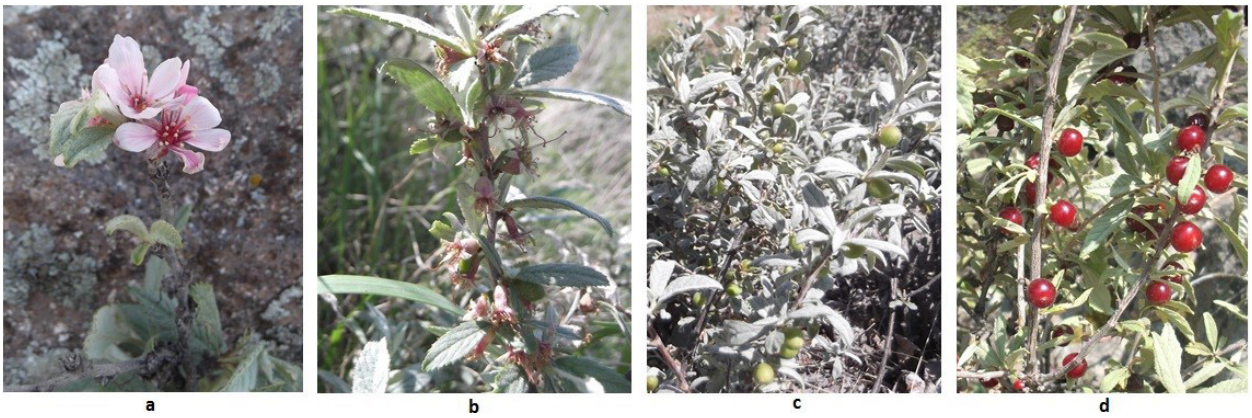

Fig. 1. *Cerasus prostrata* images at flowering (a), flower shedding (b), small fruit (c) and ripened fruit (d) stages (phot. A. Uzun)

Source: wikipedia.org

Fig. 2. Map showing the location of Kayseri province where the study was conducted

Mediterranean countries. On the other hand, Dudley and Stolton [2003] indicated that among the European countries, *C. prostrata* was encountered most in Turkey. It was reported in previous studies for Turkey that *C. prostrata* naturally grow at 1500–1600 m altitudes of Balıkesir in the west [Dirmenci et al. 2006], at 2000 m altitude of Hatay in the south [Aytac and Semenderoglu, 2011], at 1150–1200 m altitudes of Aksaray in Central Anatolia [Baskose and Dural 2011] and at high mountains of Erzurum in the east [Irmak 2013, Öztürk et al. 2015].

P. prostrata was assessed as diploid and self-incompatible wild species [Hanelt 1997]. Fruits are mostly consumed in nature by birds and mammals [Valido et al. 2011]. Fruits were also reported to be used in folk medicine for gastrointestinal disorders. In a study, eight flavonols and pro-anthocyanidins (of which one was a new A-type pro-anthocyanidins and

two were new monomeric flavonols) were isolated from this species [Bilia et al. 1996].

Kayseri province is located in Central Anatolia and the city was established right at the foothills of Erciyes Mountain (3917 m), the highest mountain of the region. Erciyes and surrounding mountainous sites are quite rich in plant diversity and there identified 1170 genotypes and sub-taxa belonging to 89 families and 433 species [Vural and Aytaç 2005]. *C. prostrata* is also widespread over the mountainous sites at foothills of Erciyes Mountain. The encountered sites are generally mountainous and rocky and it is not encountered much at low altitude plateaus and smooth terrains. There isn't any report in Turkey about the fruit characteristics, genetic diversity, culture and use of this species. Therefore, in this study, fruit and leaf characteristics of 30 *C. prostrata* genotypes collected from the foothills of Erciyes Mountain were determined and genetic diversity was presented for this species.

MATERIALS AND METHODS

Plant materials. In this study, 30 *C. prostrata* populations collected from the mountainous sections of Ali Dagi (38°39'10"N, 35°28'36"E) and Hacilar (38°38'27"N, 35°24'03"E) regions located at foothills of Erciyes Mountain of Central Anatolia, Turkey were used as the plant material (Tab. 1). In the region, the climate is continental, where winters are cold and snow is dominant, and summers are hot and dry. The soil characteristic of the region where the material is collected is rocky soil in slope land, which is poor in organic matter and has poor water holding capacity.

Fruit and leaf characterization. For fruit and leaf characterization, ripened fruits and normal-size leaves were sampled from each genotype (30 samples from each genotype) at the beginning of August 2014. For the purpose of characterization, leaves that have completed their development in annual shoots were used. Fruit samples were subjected to fruit weight (g) and fruit flesh ratio (%) analyses and leaf samples were subjected to leaf blade length (cm), leaf blade width (cm) and petiole length (cm) measurements. All fruit samples of each genotype were weighed with precision scale (±0.001 g) and the average fruit weight was estimated. The width and length values of each leaf were measured with a digital caliper (±0.01 mm).

Results of each character for genotypes were transformed to standardize units. The principal component analysis was held using PAST3 software.

Molecular analysis. Young leaves of *C. prostrata* genotypes collected from directly on sites were used for genomic DNA extraction through CTAB method as described by Doyle and Doyle [1990]. DNA concentrations were determined with a spectrophotometer (BioTek Instruments, Inc., Winooski, VT, United States) and 10 ng/µL DNA solutions were prepared. For PCR processes, 17 ISSR primers were used. PCR components and cycles were arranged in accordance with the method specified by Uzun et al. [2009] and Pınar et al. [2017]. PCR products were run in 2% agarose gel at 110 volts for 2–3 hours. A 100 bp DNA ladder was used to determine band sizes. Bands were then imaged under UV-light.

Data analysis. Data on fruit and leaf characteristics were subjected to statistical analyses through JMP 5.0 (SAS Institute Inc., Cary, NC, USA) software and means were grouped through Tukey test ($P < 0.05$). Molecular analyses were performed as follows: Net bands obtained from ISSR primers were scored. Cluster analysis was performed in accordance with unweighted pair group method with arithmetic averages (UPGMA) and a dendrogram was created with NTSYS pc 2.11 software [Rohlf 2000]. The genetic similarity matrix and ultrametric distance matrix produced from UPGMA-based dendrogram with COPH module nested in the same software was compared using Mantel's matrix correspondence test [Mantel 1967]. The result of this test is a cophenetic correlation coefficient, *r*, that indicates how well dendrogram represents similarity data. 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: $fi\ 2$ – frequency of the i^{th} allele.

PIC provides an estimate of the discriminatory power of a locus by taking into account not only the number of alleles that are expressed but also the relative frequencies of those alleles [Smith et al. 1997]. The resolving powers (RP) of the primers were deter-

Table 1. Genotype numbers (GN), sampling altitudes, fruit weights (FW), flesh ratios (FR), leaf length (LL), leaf width (LW) and petiole length (PL) of *C. prostrata* genotypes (the genotypes 1–12 were collected from Ali Dagi and 13–30 from Hacilar region)

GN	Altitude (m)	FW (g)	FR (%)	LL (cm)	LW (cm)	PL (cm)
1	1332	0.34 h-m	73.01 c-f	0.94 f-k	2.02 j-k	0.34 h-j
2	1357	0.56 ab	73.76 c-f	1.57 a-b	3.68 a-b	0.49 a-g
3	1329	0.41 c-k	84.59 a	1.26 b-f	2.76 d-ı	0.35 g-j
4	1319	0.50 be	75.69 b-f	1.04 e-j	2.33 h-k	0.38 e-j
5	1330	0.38 f-l	72.73 d-f	1.49 a-c	2.94 c-h	0.50 a-f
6	1328	0.35 g-l	76.62 a-e	1.61 a	3.19 b-e	0.51 a-e
7	1318	0.29 l-m	72.62 d-f	1.41 a-d	2.92 c-h	0.41 b-j
8	1315	0.44 c-ı	79.57 a-d	0.84 ı-k	2.37 h-k	0.35 g-j
9	1312	0.45 b-h	80.40 a-d	1.05 e-j	3.09 b-f	0.39 d-j
10	1314	0.33 ı-m	80.29 a-d	0.68 k	1.82 k	0.34 h-j
11	1313	0.46 b-g	80.26 a-d	1.31 a-e	3.20 b-e	0.40 c-j
12	1330	0.52 b-c	83.71 ab	1.49 a-c	3.64 a-b	0.60 a
13	1352	0.49 b-f	77.36 a-e	1.22 c-g	3.47 a-c	0.44 b-ı
14	1386	0.23 m	77.58 a-e	1.57 a-b	3.35 b-d	0.44 b-ı
15	1343	0.30 k-m	70.60 e-g	1.07 e-j	2.26 ı-k	0.34 h-j
16	1340	0.32 j-m	67.75 fg	1.10 d-j	2.87 c-ı	0.28 j
17	1265	0.66 a	75.40 b-f	1.42 a-d	4.02 a	0.45 b-h
18	1414	0.38 e-l	74.10 c-f	1.17 c-h	2.78 d-ı	0.53 a-d
19	1436	0.47 b-g	63.11 g	0.98 e-k	2.77 d-ı	0.55 a-b
20	1438	0.43 c-j	71.90 d-g	1.12 d-j	2.61 e-j	0.33 h-j
21	1427	0.40 d-l	73.67 c-f	0.81 j-k	2.42 h-k	0.30 ı-j
22	1454	0.44 c-ı	77.34 a-e	1.06 e-j	2.62 e-j	0.36 f-j
23	1440	0.41 c-k	74.39 c-f	0.90 g-k	2.78 d-ı	0.41 c-j
24	1441	0.44 c-ı	77.76 a-e	1.27 b-e	3.21 b-e	0.53 a-c
25	1455	0.44 c-ı	80.63 a-d	0.88 h-k	2.65 e-ı	0.43 b-ı
26	1453	0.50 b-d	77.91 a-e	1.26 b-f	3.06 b-g	0.34 h-j
27	1455	0.51 b-c	83.30 ab	1.17 c-ı	2.57 f-j	0.37 e-j
28	1450	0.45 b-h	77.92 a-e	1.11 d-j	2.26 ı-k	0.39 d-j
29	1427	0.43 c-j	81.71 a-c	1.04 e-j	2.38 h-k	0.34 h-j
30	1429	0.44 c-ı	75.55 b-f	1.05 e-j	2.45 g-j	0.33 h-j

Note: Means indicated with different letters denote significant differences between the cultivars (Tukey test, $p < 0.05$)

mined by the following formula [Prevost, Wilkinson 1999]. According to the formula, it is expressed as the ratio of p = I band in the total number of genotypes.

$$RP = \sum Ib;\ \ Ib = 1 - (2 \times |0.5 - p|)$$

RESULTS

Fruit and leaf characteristics. Significant differences were observed in fruit weight (g) and flesh ratio (%) of the genotypes. A high-level variation was observed among the genotypes with regard to fruit weight. The prominent genotypes in terms of this parameter were the genotypes 17 (0.66 g) and genotype 2 (0.56 g). Genotypes 14 had the least fruit weight (0.23 g). Flesh ratio also exhibited a significant variation among the genotypes. The values varied between 84.59% (genotype 3) and 63.11% (genotype 19) (Tab. 1).

Significant differences were also observed in leaf traits (leaf width, leaf length, petiole length) of the genotypes. The greatest leaf widths were observed in genotypes 6 (1.61 cm) and genotype 2 (1.58 cm) and the lowest leaf width was observed in genotype 10 (0.68 cm). The higher leaf lengths were observed in genotypes 17 (4.02 cm) and 2 (3.68 cm) and the lowest leaf length was observed in genotype 10 (1.82 cm). With regard to leaf petiole lengths, the higher values were observed in genotypes 12 (0.60 cm) and 19 (0.55 cm) and the lowest value was seen in genotype 16 (0.28 cm).

According to results of principal component analysis (PCA), two principal components (PC1 and PC2) with Eigenvalues >1 accounted with individual variance values and contributed 71.88 % of the total variation of genotypes (Tab. 2). The proportion of total variation explained by these principal components used for next step. The percentages of cumulative variation accounted for by each of the five PCs are 48.79% and 23.09% respectively. The coefficients defining the five principal components of these data are given in Table 3.

The first principal component shows for highest variability in the data with respect to succeeding components. The value of all character in the first principal component has been above |0.3| except for FR (%). These total four traits possessive high component value conceive more genetic diversity. The first component (PC1) has high value for LL (|0.594|). In the second principal component FW and FR have been determined as prominent characters. Principal component analysis reduce the complex data by transforming the number of correlated variables into a smaller number of variables. The results of our study indicate that assignment of genetic variability of

Table 2. Eigen values and percentage of variation for each principal components

PC Number	Eigen value	Variance (%)	Cumulative variance (%)
1	2.43	48.79	48.79
2	1.15	23.09	71.88
3	0.78	15.64	87.52
4	0.47	9.48	97.01
5	0.14	2.99	100

Table 3. The coefficients defining the five principal components

FW (g)	0.3124	0.56498	-0.6926	-0.0908	0.3085
FR (%)	0.0821	0.76778	0.60668	0.17280	-0.07631
LW (cm)	0.5382	-0.2183	0.38629	-0.3713	0.6128
LL (cm)	0.5944	-0.0307	-0.0333	-0.3485	-0.7232
PL (cm)	0.5025	-0.2065	-0.0428	0.8381	0.0199

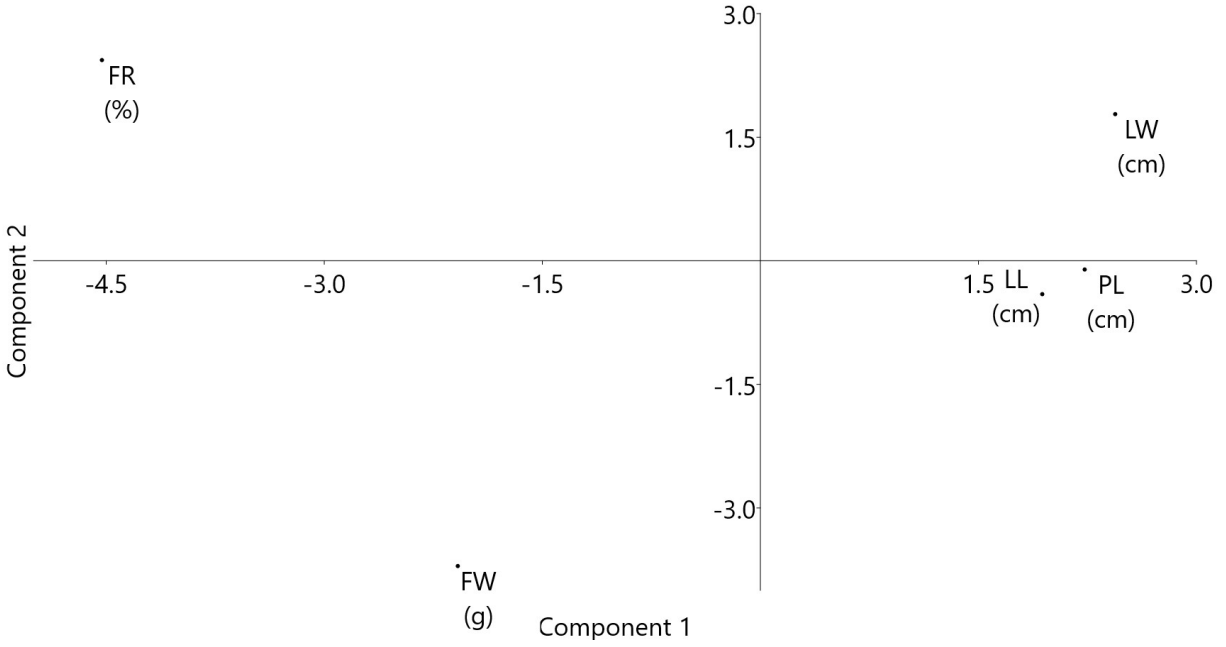

Fig. 3. Distribution of observed characters based on the first and second component

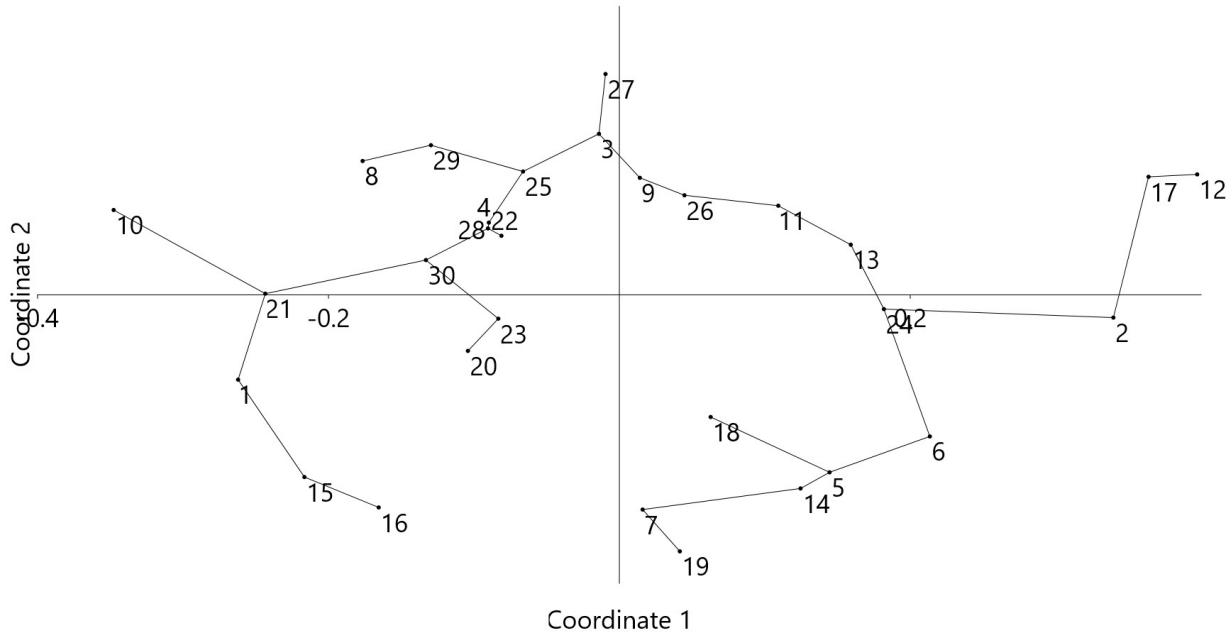

Fig. 4. Distribution of *C. prostrata* genotypes based on the first and second component

Table 4. Primers, number of polymorphic bands (NPB), total number of bands (TNB), polymorphism ratio (PR), polymorphism information content (PIC) and resolving power (RP) values

Primers	NPB	TBN	PR (%)	PIC	RP
$(AGC)_6G$	9	9	100	0.46	12.85
$VHV(GTG)_7$	7	9	77.7	0.48	11.84
$(AG)_8T$	4	8	50	0.26	13.41
$(CA)_8R$	10	10	100	0.71	10.07
$(CT)_8TG$	8	8	100	0.51	10.12
$(CAA)_6$	6	6	100	0.33	9.45
$(CT)_8YA$	5	6	83.3	0.43	8.76
$HVH(TCC)_7$	3	5	60	0.28	8.17
$HVH(CA)_7T$	6	7	85.7	0.46	10.05
$(GT)_6GG$	4	7	57.1	0.45	8.99
$(AG)_7YC$	6	7	85.7	0.58	8.02
$(CAC)_3GC$	8	9	77.7	0.55	9.57
$BDB(CA)_7C$	3	3	100	0.85	2.06
$DBDA(CA)_7$	3	3	100	0.43	4.45
$(CA)_8Y_6$	7	8	87.5	0.75	6.61
$(CA)_6AC$	3	4	75	0.21	7.05
$(TCC)_5RY$	6	6	100	0.54	7.64
Mean	5.8	6.8	84.7	0.49	8.77
Total	98	115			

C. prostrata based on these genotypes can likely be obtained by selecting these characters. The bi-plot from the PCA analysis is presented with the five characters in Figure 3. In the PCA Plots graph, the characters that LW, LL, and PL are clustered together (Fig. 3).

The projections of the all *C. prostrata* genotypes in a 2- dimensional graph have been shown in Figure 4. The first (PC1) and second (PC2) coordinates of the PCA implemented using morphological data accounted for 71.88 % of the diversity monitored. Based on the Principal Coordinate analysis, any prominent groups did not composed (Fig. 4).

Molecular characterization. For molecular analyses, 17 ISSR primers were used. A total of 115 net readable bands were obtained from these primers and 98 of them were polymorphic (84.7% polymorphism). Number of bands per primer varied between 3 ($BDB(CA)_7C$ and $DBDA(CA)_7$) and 10 (CA_8R) with an average value of 6.8. Average number of polymorphic bands was 5.8. In 7 primers, entire bands were polymorphic. PIC values varied between 0.21 (CA_6AC) and 0.85 ($BDB(CA)_7C$) with an average value of 0.49. RP values of the primers ranged between 2.06 ($BDB(CA)_7C$) and 13.41 (AG_8T) (Tab. 4).

Similarity index was calculated by using ISSR data in accordance with Dice's coefficient (Dice 1945). Cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be relatively high ($r = 0.75$, $P < 0.01$), suggesting that the cluster analysis represented the similarity matrix. According to resultant dendrogram, similarity levels among 30 *C. prostrata* genotypes varied between 0.70–0.95 (Fig. 5). All genotypes were genetically distinguished from each other. Relatively high variation was identified among the genotypes. Genotype 7 with a similarity level of 0.70 was the distinct one to others. Again the genotype 30 with a similarity level of 0.72 was also separated from the others. The genotypes 2 and 20 as pair were also separated from the others. The genotype 18 was also placed alone in the dendrogram.

Remaining 25 genotypes were separated into two groups. The lower one (group A) had four sub-groups.

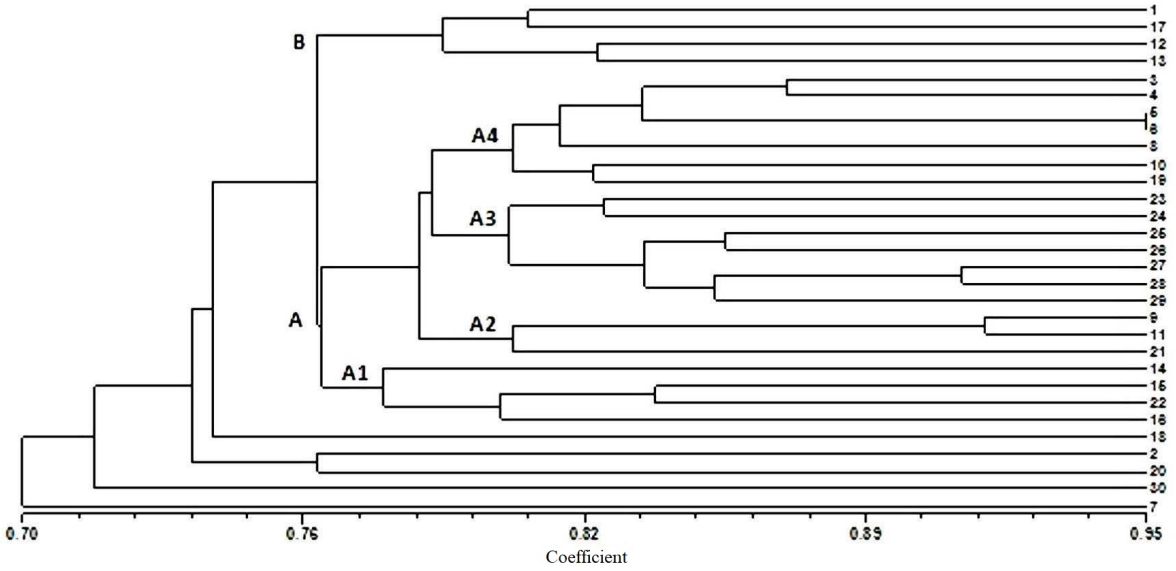

Fig. 5. UPGMA dendrogram of the 30 *C. prostrata* genotypes based on ISSR data

The first sub-group (A1) included the genotypes 16, 22, 15 and 14. These genotypes were sampled from Hacilar region. The second sub-group (A2) included the genotypes 21, 11 and 9. The third sub-group (A3) included the genotypes between 23–29 (7 genotypes) and all these genotypes were from Hacilar region. The fourth sub-group (A4) included 7 genotypes of which 6 from Ali Dagi region (genotypes 3, 4, 5, 6, 8, 10) and one from Hacilar region (genotype 19). The upper group (group B) was composed of 4 genotypes of which two from Hacilar region (genotypes 13, 17) and two from Ali Dagi (genotypes 1, 12) region.

DISCUSSION

In sistematics, *C. prostrata* (synonym = *P. prostrata*) is classified in *Microcerasus* section of *Cerasus* sub-species of *Prunus* species. The *P. besseyi* L.H. Bailey, *P. bifrons* Fritsch, *P. glandulosa* Thunb., *P. jacquemontii* Hook. f., *P. microcarpa* (Boiss.) C.A. Mey, and *P. tomentosa* Thunb. are also classified under this section [Bortiri et al. 2006, Bouhadida et al. 2007]. Fruit and leaf traits of *C. prostrata* haven't been studied before. Therefore, the present study can be considered as the first report on this subject matter. Thun, the present findings were not able to be discussed with the similar studies. However, there are some previous studies carried out on *P. tomentosa* classified under the *Microcerasus* section. Fruit weights of this species were reported as between 0.41–2.85 g, leaf lengths between 3.24–6.70 cm and leaf widths between 1.82–4.27 cm [Zhang et al. 2008]. On the other hand, Kawecki et al. [2002] reported fruit weights of *P. tomentosa* as between 1.01–1.29 g. These findings were generally higher than the current findings on *C. prostrata*. Similar to current study, significant differences were also observed in fruit and leaf traits of *P. tomentosa*.

Some kind of relationships was observed among the investigated genotypes with regard to morphological characteristics. The genotype 2 was prominent for both leaf width and leaf length. Such a case was found to be significant for this genotype to produce larger leaf areas. Similarly, the genotype 17 had also the highest leaf length and quite high leaf width. These two genotypes had also the first two ranks in fruit weight. Then, a positive correlation was identified between leaf area and fruit weight within the same species. Thusly, it was reported in previous studies that high leaf areas resulted in greater growth power in plants and small leaves had low photosynthetic capacity because of lower light and CO_2 diffusion [Hunt and Cornelissen 1997, Gulias et al. 2003]. A correlation was also reported between leaf photosynthesis rates and leaf areas [Meziane and Shipley 2001].

Genetic analysis revealed that investigated *C. prostrata* genotypes were all different from each other. This species is open pollinated in nature and pollens are transported to different locations through natural means (birds, mammals, winds and etc.). Seed-propagated genotypes may exhibit significant variations in genetic characteristics. Current dendrogram revealed in general that the genotypes collected from Hacilar and Ali Dagi regions grouped together within the subgroups. However, such a separation didn't reflect over the entire dendrogram. Some results were obtained in this study where molecular and morphological data did not overlap. In molecular studies, genotypes 6 and 8 have been identified as the closest individuals with a similarity rate of 0.95. Although these two individuals showed closeness in molecular analysis, some differences emerged in morphological analysis. Molecular markers are not influenced by ecological conditions, but morphological attributes are largely influenced by environmental conditions. Present primers might have scanned the regions that cannot designate the exact morphological characters.

There aren't any studies in literature indicating genetic diversity in *P. prostrata*. Thus the present study is the first one in this respect. However, there are only one phylogenetic study carried on *Prunus* species and only one *P. prostrata* accession was used in that study. Bortiri et al. [2006] assessed the molecular and morphological data and found *P. prostrata* as closely related to other members of *Microcerasus* section (*P. tomentosa*, *P. jacquemontii*, *P. bifrons* and *P. microcarpa*). On the other hand, Bouhadida et al. [2007] carried out a study on *Prunus* species and identified 33 chloroplast DNA haplotypes among 84 materials composed of species and inter-species accessions. The researchers identified 2 haplotypes (H18 and 21) in *P. prostrata* and indicated that H18 haplotype also existed in *P. cerasifera*. A dendrogram was also created in that study with these haplotypes and it was pointed out that these two haplotypes were placed in the same group. It was concluded that *P. prostrata* was closely related to *P. pumila*, *P. besseyi*, plums (*P. salicina* and *P. cerasifera*) and apricot (*P. armeniaca*).

Besides significant contributions provided to environment and wildlife, wild fruit species are also used in human nutrition in several countries. Some wild species may contain higher nutritional attributes than the culture fruits. Such valuable species should be selected, hybridized with other species, developed and cultured as new species [Mahapatra et al. 2011].

CONCLUSIONS

Present findings demonstrated significant morphological and molecular variations in *C. prostrata*. Further studies are recommended to investigate the resistance of this species against biotic and abiotic stress conditions. Possible hybridizations between *Prunus* species may set a light for hybridization of *C. prostrata* with the other species and to obtain new genotypes for different purposes. In this way, new rootstock and cultivar candidates may be find out.

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