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Genetic Diversity of Cherry Laurel (*Laurocerasus officinalis* Roemer) BY SSR Markers

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ABSTRACT

Cherry laurel (*Laurocerasus officinalis*) belongs to the Rosacea family. The main distribution area for edible cherry laurels is the Blacksea shores in Turkey. In the study, it was aimed to reveal the differences among the various cherry laurel genotypes by using the SSR molecular marker technique. Cherry laurel genotypes were selected from the Black Sea Region of Turkey. A total of 15 SSR primer pairs were developed and used for *Prunus* species, and the phylogenetic relationship and polymorphism rates were also demonstrated. As a result, 13 SSR primers resulted in scorable DNA band profiles. UDAp-401 SSR primer was detected with a minimum of 3 alleles and BBCT001 primer with a maximum of 17 alleles. The average number of alleles was observed at 9 per locus. Whereas, the average number of polymorphic bands per SSR marker was calculated as 8.38. Additionally, 109 polymorphic DNA profiles were obtained from a total of 117, and the polymorphism rate was calculated as 93.5%. The band patterns resulting from SSR analysis showed multiple alleles, suggesting polyploidy in cherry laurel. In conclusion, we determined that the SSR molecular markers could be used to identify the different cherry laurel genotypes. Furthermore, these results depicted that among the different genotypes sampled there is significant genetic variability that can be useful for future research and breeding programs.

Keywords: *Prunus laurocerasus* L, DNA, Polymorphism, primer

1. Introduction

The Rosaceae family's cherry laurel (*Laurocerasus officinalis* L.) is a small tree or also known as an evergreen shrub. Different cherry laurel species or genotypes are grown naturally and used as a fruit and ornamental plants. It grows as a single tree in many places in the Blacksea Region. Cherry laurel cultivated tree's height can range from 5 to 10 m. These plants may differ from each other in terms of leaf size and shape, fruit colour, size and taste (İslam & Vardal 2006).

Cherry laurel might differ in terms of leaves, flower, fruit color and taste. The leaves of the cultivated types are larger and some types are narrow and long. White flowers collect in an upright cluster on an axis of 5 - 15 cm. Each flower has 5 sepals, 5 petals, 1 female and 15-20 male organs (İslam et al. 2020; Turna & Güney 2006). The people consumed it as table fruit, because of its taste & healthy properties of this unique fruit (İslam 2002; Halilova & Ercisli 2010; İslam et al. 2010; Tarakci et al. 2013; Temiz et al. 2014; Eser et al. 2014). The fruits are consumed as fresh after ripening. In addition, its fruits are made into jam, molasses and pickles. It is also used in the pharmaceutical industry (Guven & Geçgil 1961; Islam 2008; Eser et al. 2014). Since, it is an evergreen tree, it isused as an ornamental plant in parks and gardens in floriculture, and as a windbreaker for orchards and houses (İslam & Deligöz 2012).

In recent years, some morphological, biochemical, and molecular studies have been carried on cherry laurel. Different types of molecular markers like RAPD (Aka Kaçar 2001; Sandallı 2002; Aksu et al. 2012; Yılmaz et al. 2012; Pınar et al. 2018), and SSR (Cipriani et al. 1999; Downey & Lezzoni 2000; Aka Kaçar et al. 2005; Kaçar et al. 2006; Wünsch 2009; Türkoğlu at al. 2010; Ercişli et al. 2011; Stanys et al. 2012; Hajyzadeh et al. 2013; Köse 2013) have been used to characterize the genetic diversity in cherry laurel.

Microsatellite markers, known as Simple Sequence Repeats (SSRs), are co-dominant and stable markers, demonstrates high polymorphism, repeatable and suitable for automation. SSRs have a cross-species feature and are an informative marker system.

(Weber & May 1989; Yamamoto et al. 2001; Wünsch & Hormaza 2002). The present study aimed to investigate the genetic diversity of cherry laurel genotypes growing naturally in the regions of the Black Sea using SSR markers.

2. Material and Methods

2.1. Material

A total of 43 cherry laurel genotypes were used as plant materials (Table 1). The plant materials were selected from the Blacksea Region in Turkey considering the morphological, phenological and fruit characteristics with the support of the TUBITAK project (No:107O252) in 2007-2010 and planted at Ordu University, Faculty of Agriculture, Research Field (Figure 1).

Figure 1- Cherry laurel orchard in Ordu University Faculty of Agriculture, Research Field

Table 1- The accession list of cherry laurel used in the present study

Code Numbers	*Genotypes*	*Sampling site*	*Code Numbers*	*Genotypes*	*Sampling site*
1	R126	Rize	23	R137	Rize
2	R135	Rize	24	R19	Rize
3	T214	Trabzon	25	G40	Giresun
4	T203	Trabzon	26	R25	Rize
5	R27	Rize	27	R149	Rize
6	A19	Artvin	28	S24	Samsun
7	R20	Rize	29	S37	Samsun
8	O44	Ordu	30	T193	Trabzon
9	R24	Rize	31	T219	Trabzon
10	A4	Artvin	32	T217	Trabzon
11	T87	Trabzon	33	S16	Samsun
12	A14	Artvin	34	T94	Trabzon
13	O20	Ordu	35	R142	Rize
14	R27x	Rize	36	S51	Samsun
15	R5	Rize	37	R24X	Rize
16	O26	Ordu	38	T159	Trabzon
17	O29	Ordu	39	O27	Ordu
18	T303	Trabzon	40	T216	Trabzon
19	Keller	Trabzon	41	S3	Samsun
20	Sarı	Trabzon	42	A23	Artvin
21	S25	Samsun	43	R14	Rize
22	S21	Samsun			

2.2. Method

The research was carried out in Plant Biotechnology Laboratory, Department of Horticulture, Çukurova University, Adana, Turkey

2.2.1. DNA isolation

Young leaves were collected from each accession, immediately frozen in liquid nitrogen (-196 ºC), and stored at –80 °C. Genomic DNA was extracted from the leaf samples following the protocol for minipreps using CTAB (Simsek et al., 2008). The DNA quality and quantity were measured using a NanoDrop ND 100 spectrophotometer (NanoDrop Technologies) and agarose gel electrophoresis.

2.2.2. PCR amplification and gel electrophoresis

A total of 15 SSR primers (PMS49, PceG25, PMS40, PMS67, PceGA34, UDP98-21, PMS2, PMS3, PceGA59, UDP96-005, UCD-CH17, UDAp-401, BBCT001, BBCT002 and BBCT005) were tested. Information about SSR primers were shown in Table 2. Amplification was conducted at a total volume of 20 µl (25 ng of genomic DNA, 1X PCR buffer, 0.02 mM of each dNTP, 2.5 µmol primer (forward + reverse) and 0.8 units of DNA Taq polymerase, 5 µl dd H_2O). The amplifications were as follows: 94 ºC /5 min, 35 cycles/94 ºC /1 min, 55 ºC /30 sec, 72 ºC /1 min, and a final extension at 72 ºC / 5 min. Blue stop solution (95% formamide, 25 mM EDTA, and 2% bromophenol blue) was added to each PCR reaction as well. The PCR products were denatured at 95 ºC for 3 min and resolved in denaturing gel containing 6.5% polyacrylamide (40:2 acrylamide/bisacrylamide), 8.4 gr urea, 10 mL ddH_2O, and 2 mL 10X TBE buffer. PCR products were run in a 4.300 DNA Analyzer (LI-COR). Parameters for each run were 1.5 h, 1.500 V, 40 W, 40 mA, and 45 ºC respectively.

Table 2- Information about SSR primers used in the present study

No	*SSR Marker*	*Primers Sequence*	*Tm (°C)*	*Type name*	*Bp*	*References*
1	PMS49	F: TCA CGA GCA AAA GTG TCT CTG	50	Cherry laurel		Hajyzadeh et al. 2013
		R: CAC TAA CAT CTC TCC CCT CCC		Cherry	79-185	Cantini et al. 2001
2	PceGA25	F: GCA ATT CGA GCT GTA TTT CAG ATG	49	Cherry laurel		Hajyzadeh et al. 2013
		R: CAG TTG GCG GCT ATC ATG TCT TAC		Cherry	141-198	Cantini et al. 2001
3	PMS40	F: TCA CTT TCG TCC ATT TTC CC	50	Cherry laurel		Hajyzadeh et al., 2013
		R: TCA TTT TGG TCT TTG AGC TCG		Cherry	181-226	Cantini et al. 2001
4	PMS67	F: AGT CTC TCA CAG TCA GTT TCT	48	Cherry laurel		Hajyzadeh et al. 2013
		R: TTA ACT TAA CCC CTC TCC CTC C		Cherry	144-191	Cantini et al. 2001
					149-161	Struss et al. 2003
5	PceGA34	F: GAA CAT GTG GTG TGC TGG TT	45	Cherry laurel		Hajyzadeh et al. 2013
		R: TCC ACT AGG AGG TGC AAA TG		Sour cherry	140-174	Downey & Lezzoni 2000
6	UDP98-021	F: AAG CAG CAA TTG GCA GAA TC	54	Cherry laurel		Hajyzadeh et al. 2013
		R: GAA TAT GAG ACG GTC CAG AAG C		Peach	145	Testolin et al. 2000
7	PMS2	F: CAC TGT CTC CCA GGT TAA ACT	-	Cherry laurel		Hajyzadeh et al. 2013
		R: CCT GAG CTT TTG ACA CAT GC		Cherry	132-152	Cantini et al. 2001
					127-151	Struss et al. 2003
8	PMS3	F: TGG ACT TCA CTC ATT TCA GAG A	-	Cherry laurel		Hajyzadeh et al. 2013
		R: ACT GCA GAG AAT TTC ACA ACC A		Cherry	152-200	Cantini et al. 2001
					153-203	Struss et al. 2003
9	PceGA59	F: AGA ACC AAA AGA ACG CTA AAT C	-	Cherry laurel		Hajyzadeh et al., 2013
		R: CCT AAA ATG AAC CCC TCT ACA AAT		Cherry	181-256	Cantini et al. 2001
10	UDP96-005	F: GTA ACG CTC GCT ACC ACA AA	55	Cherry laurel	93-101	Türkoğlu et al. 2010
		R: CCT GCA TAT CAC CAC CCA G		Cherry	115-135	
				Sour cherry	99-113	
				Mahaleb	117-119	
				Silverberry	156-186	
				Peach	155	Cipriani et al. 1999 Testoline et al. 2000
11	UCD-CH17	F:TGG ACT TCA CTC ATT TCA GAG A	58	Cherry	180-200	Türkoğlu et al. 2010
		R: ACT GCA GAG AAT TTC CAC AAC CA		Sour cherry	178-202	
				Mahaleb	164	
				Silverberry	14-160	
				Cherry	186-190	
12	UDAp-401	F:AAA CCC TAG CCG CCA TAA CT	60	Cherry laurel	106-116	
		R: GCT AAA GGC CTT CCG ATA CC		Cherry	260-270	
				Sour cherry	262-272	
				Mahaleb	138-146	
				Silverberry	146-162	
				Apricot	201	Messine et al. 2004

2.2.3. Data analysis

The SSR primer pairs which produced clear PCR fragments were scored and indicated as present (1) or absent (0). Genetic similarity was calculated using Jaccard's coefficient to obtain a pairwise similarity matrix (Jaccard 1908). The Principle Coordinate (PCoA) and cluster analysis was constructed using the unweighted pair-group method with arithmetic mean (UPGMA) using the PAST program (Hammer et al., 2001). The bootstrap values for the clusters were calculated by 1000 replicates using the PAST program. The representativeness of the dendrogram was evaluated by estimating cophenetic correlation for the dendrogram and comparing it with the similarity matrix, using Mantel's matrix correspondence test (Mantel, 1967). PIC values for each locus were measured by using the following equation: $PIC=1-\Sigma Pi^2$, where Pi is the frequency of the ith allele) (Perrier & Jacquemond-Collet 2006).

3. Results and Discussion

Allele sizes of 15 SSR markers are presented in Table 3.

Table 3- Allele sizes (bp) of the SSR markers used in the study

SSR Marker	*Amplicon size found in this study*	*Amplicon size reported in the literature*		*Reference*
	Cherry laurel	**Cherry laurel**	**Cherry**	
PMS67	154-180	-	144-191 149-161	Cantini et al. 2001 Struss et al. 2003
PMS49	180-186	-	79-185	Cantini et al. 2001
UDAp-401	150-154	106-116	260-270	Messiani et al. 2010
PMS2	146-162		132-152 127-151	Cantini et al. 2001 Struss et al. 2003
UDP98-021	108-124	-	-	Hajzadeh et al. 2013 Testolin et al. 2000
UDP96-005	112-146	93-101	115-135	Türkoğlu et al. 2010
PceGA59	220-236	-	181-256	Cantini et al. 2001
PMS3	135-160	-	152-200 153-203	Hajzadeh et al. 2013 Struss et al. 2003
UCD-CH17	150-162	-	180-200	Türkoğlu et al. 2010
PceGA34	142-155	-	-	Hajzadeh et al. 2013 Downey & Lezoni 2000
BBCT001	315-350	-	-	-
BBCT002	194-222	-	-	-
BBCT005	220-246	-	-	-
PceGA25	-	-	141-198	Cantini et al. 2001
PMS40	-	-	181-226	Cantini et al. 2001

15 SSR primer pairs were used to determine the genetic relationship among the cherry laurel genomes. Among these 15 primers, 13 SSR primer pairs produced a scorable amplicon. The number of scorable bands from 13 SSR primer pairs ranged from 3 (UDAp-401) to 17 (BBCT001), as presented in Table 4. The total number of bands obtained was 117. The average number of bands per primer was 9. The average number of polymorphic bands per SSR marker was 8.38. The highest polymorphic band number was 17 and was obtained from BBCT001 primer. 109 polymorphic fragments were obtained from a total of 117 bands, and the polymorphism rate was calculated as 93.5%.

Table 4- Information obtained with SSR markers

SSR Primer	*Total number of bands*	*Number of polymorphic bands*	*Polymorphism rate*	*PIC*
PMS67	11	9	81.81	0.93
PMS49	4	4	100	0.92
UDAp-401	3	3	100	0.85
PMS2	9	9	100	0.76
UDP98-021	9	8	88.88	0.68
UDP96-005	14	13	91.85	0.92
PceGA59	7	7	100	0.78
PMS3	10	8	80	0.86
UCD-CH17	6	5	83.33	0.74
PceGA34	7	7	100	0.82
BBCT001	17	17	100	0.85
BBCT002	10	10	100	0.81
BBCT005	10	9	90	0.83
Average	**9**	**8.38**	**93.52**	
TOTAL	**117**	**109**		

3.1. Dendrogram and evaluation of SSR analysis

The similarity index was calculated according to the Jaccard dissimilarities (Figure 3). Dendrograms were constructed based on the UPGMA method using Past software (Figure 4).

	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
2	0.46																																									
3	0.50	0.51																																								
4	0.50	0.52	0.06																																							
5	0.61	0.44	0.47	0.48																																						
6	0.62	0.59	0.42	0.43	0.49																																					
7	0.53	0.47	0.45	0.40	0.48	0.55																																				
8	0.62	0.59	0.61	0.62	0.63	0.64	0.60																																			
9	0.60	0.60	0.47	0.48	0.54	0.62	0.49	0.60																																		
10	0.53	0.39	0.46	0.44	0.40	0.57	0.41	0.55	0.48																																	
11	0.59	0.63	0.39	0.37	0.56	0.57	0.56	0.53	0.54	0.50																																
12	0.50	0.55	0.08	0.13	0.46	0.42	0.49	0.62	0.46	0.44	0.37																															
13	0.59	0.61	0.47	0.48	0.56	0.53	0.62	0.61	0.61	0.57	0.45	0.45																														
14	0.68	0.71	0.58	0.59	0.64	0.61	0.66	0.64	0.67	0.62	0.57	0.57	0.65																													
15	0.68	0.71	0.45	0.49	0.58	0.5	0.66	0.60	0.66	0.66	0.52	0.43	0.60	0.57																												
16	0.65	0.56	0.37	0.38	0.55	0.48	0.43	0.66	0.51	0.54	0.39	0.35	0.50	0.64	0.53																											
17	0.59	0.61	0.44	0.47	0.53	0.53	0.65	0.59	0.65	0.56	0.46	0.42	0.19	0.60	0.52	0.52																										
18	0.56	0.56	0.17	0.18	0.53	0.48	0.52	0.65	0.49	0.47	0.38	0.17	0.51	0.60	0.52	0.33	0.50																									
19	0.56	0.60	0.32	0.37	0.53	0.56	0.53	0.55	0.54	0.54	0.27	0.33	0.47	0.61	0.55	0.41	0.42	0.41																								
20	0.58	0.51	0.22	0.24	0.40	0.42	0.51	0.64	0.55	0.51	0.48	0.23	0.50	0.62	0.46	0.41	0.42	0.30	0.42																							
21	0.54	0.43	0.49	0.52	0.43	0.54	0.44	0.67	0.60	0.33	0.62	0.50	0.63	0.62	0.64	0.55	0.55	0.50	0.59	0.50																						
22	0.62	0.58	0.37	0.38	0.44	0.52	0.54	0.70	0.59	0.55	0.46	0.40	0.64	0.63	0.52	0.50	0.59	0.41	0.47	0.34	0.50																					
23	0.65	0.50	0.48	0.49	0.28	0.61	0.53	0.66	0.61	0.48	0.55	0.49	0.60	0.59	0.57	0.54	0.55	0.52	0.55	0.49	0.46	0.48																				
24	0.51	0.55	0.43	0.48	0.53	0.54	0.59	0.59	0.58	0.50	0.51	0.42	0.46	0.54	0.47	0.60	0.42	0.54	0.48	0.46	0.55	0.57	0.45																			
25	0.57	0.61	0.15	0.20	0.52	0.46	0.55	0.67	0.50	0.48	0.42	0.12	0.52	0.58	0.45	0.39	0.46	0.20	0.42	0.25	0.51	0.37	0.53	0.46																		
26	0.53	0.62	0.16	0.18	0.50	0.43	0.51	0.68	0.51	0.51	0.43	0.13	0.48	0.61	0.46	0.40	0.47	0.25	0.40	0.27	0.57	0.41	0.56	0.50	0.09																	
27	0.50	0.57	0.33	0.35	0.52	0.49	0.50	0.68	0.56	0.43	0.50	0.34	0.50	0.61	0.56	0.50	0.49	0.38	0.48	0.37	0.49	0.55	0.60	0.53	0.39	0.32																
28	0.57	0.53	0.39	0.40	0.51	0.57	0.50	0.57	0.42	0.50	0.46	0.40	0.54	0.65	0.60	0.52	0.56	0.46	0.53	0.45	0.55	0.50	0.52	0.55	0.44	0.45	0.47															
29	0.55	0.57	0.21	0.22	0.5	0.44	0.58	0.68	0.58	0.53	0.42	0.18	0.52	0.58	0.42	0.43	0.46	0.26	0.45	0.14	0.54	0.36	0.53	0.43	0.14	0.19	0.39	0.44														
30	0.53	0.60	0.21	0.23	0.53	0.47	0.57	0.67	0.56	0.56	0.42	0.18	0.50	0.60	0.43	0.44	0.44	0.30	0.43	0.18	0.60	0.41	0.54	0.40	0.18	0.196	0.37	0.45	0.08													
31	0.50	0.57	0.16	0.18	0.48	0.43	0.52	0.64	0.51	0.49	0.40	0.13	0.50	0.61	0.43	0.38	0.47	0.25	0.40	0.24	0.57	0.39	0.51	0.44	0.16	0.15	0.37	0.45	0.15	0.12												
32	0.44	0.48	0.37	0.41	0.55	0.52	0.47	0.62	0.54	0.37	0.57	0.39	0.54	0.63	0.62	0.52	0.56	0.45	0.55	0.46	0.41	0.54	0.59	0.53	0.46	0.44	0.33	0.50	0.49	0.50	0.44											
33	0.50	0.53	0.48	0.51	0.52	0.56	0.61	0.66	0.53	0.53	0.63	0.46	0.59	0.68	0.61	0.61	0.61	0.54	0.57	0.46	0.59	0.58	0.63	0.49	0.50	0.51	0.53	0.55	0.48	0.51	0.51	0.48										
34	0.53	0.61	0.33	0.37	0.55	0.59	0.58	0.64	0.54	0.56	0.53	0.30	0.58	0.63	0.52	0.55	0.52	0.41	0.50	0.34	0.60	0.40	0.54	0.43	0.30	0.34	0.48	0.53	0.32	0.26	0.31	0.50	0.51									
35	0.55	0.46	0.39	0.40	0.49	0.50	0.58	0.63	0.62	0.42	0.57	0.40	0.53	0.61	0.55	0.55	0.50	0.41	0.55	0.35	0.47	0.54	0.51	0.47	0.40	0.40	0.37	0.47	0.34	0.34	0.40	0.40	0.45	0.46								
36	0.57	0.60	0.41	0.42	0.50	0.57	0.54	0.57	0.38	0.48	0.43	0.39	0.48	0.64	0.55	0.49	0.54	0.45	0.53	0.46	0.57	0.49	0.53	0.54	0.39	0.40	0.50	0.26	0.43	0.44	0.42	0.50	0.51	0.51	0.50							
37	0.58	0.55	0.49	0.50	0.55	0.56	0.59	0.64	0.32	0.60	0.58	0.50	0.55	0.74	0.66	0.55	0.59	0.55	0.60	0.42	0.64	0.58	0.67	0.58	0.51	0.47	0.53	0.44	0.46	0.50	0.50	0.54	0.50	0.59	0.57	0.38						
38	0.56	0.58	0.29	0.31	0.58	0.59	0.60	0.68	0.55	0.53	0.52	0.28	0.57	0.64	0.54	0.51	0.54	0.29	0.55	0.35	0.60	0.46	0.56	0.50	0.21	0.27	0.46	0.50	0.26	0.25	0.29	0.47	0.51	0.25	0.34	0.44	0.56					
39	0.56	0.57	0.32	0.33	0.47	0.47	0.53	0.63	0.45	0.52	0.50	0.30	0.47	0.66	0.48	0.37	0.46	0.42	0.51	0.33	0.53	0.47	0.55	0.52	0.30	0.31	0.45	0.42	0.32	0.32	0.31	0.44	0.48	0.48	0.45	0.37	0.34	0.40				
40	0.50	0.56	0.41	0.40	0.52	0.52	0.51	0.66	0.51	0.47	0.52	0.40	0.52	0.68	0.66	0.50	0.54	0.47	0.57	0.44	0.56	0.59	0.60	0.56	0.41	0.37	0.48	0.43	0.43	0.44	0.40	0.45	0.47	0.51	0.50	0.40	0.41	0.45	0.40			
41	0.53	0.58	0.25	0.23	0.53	0.52	0.55	0.70	0.51	0.50	0.44	0.22	0.55	0.62	0.55	0.40	0.53	0.26	0.48	0.34	0.57	0.44	0.52	0.51	0.21	0.23	0.41	0.46	0.27	0.27	0.23	0.48	0.52	0.44	0.47	0.43	0.52	0.23	0.34	0.43		
42	0.54	0.53	0.44	0.42	0.44	0.51	0.49	0.67	0.50	0.38	0.52	0.44	0.50	0.69	0.63	0.56	0.54	0.47	0.57	0.44	0.52	0.55	0.53	0.53	0.46	0.42	0.41	0.46	0.48	0.49	0.47	0.42	0.53	0.55	0.48	0.36	0.45	0.50	0.42	0.39	0.47	
43	0.52	0.56	0.20	0.21	0.47	0.44	0.50	0.67	0.49	0.48	0.42	0.16	0.52	0.60	0.50	0.4	0.49	0.25	0.43	0.26	0.51	0.40	0.50	0.50	0.16	0.18	0.37	0.42	0.22	0.22	0.18	0.44	0.50	0.40	0.42	0.39	0.49	0.28	0.27	0.39	0.12	0.42

Figure 3- Similarity (Jaccard) index of cherry laurel genotypes

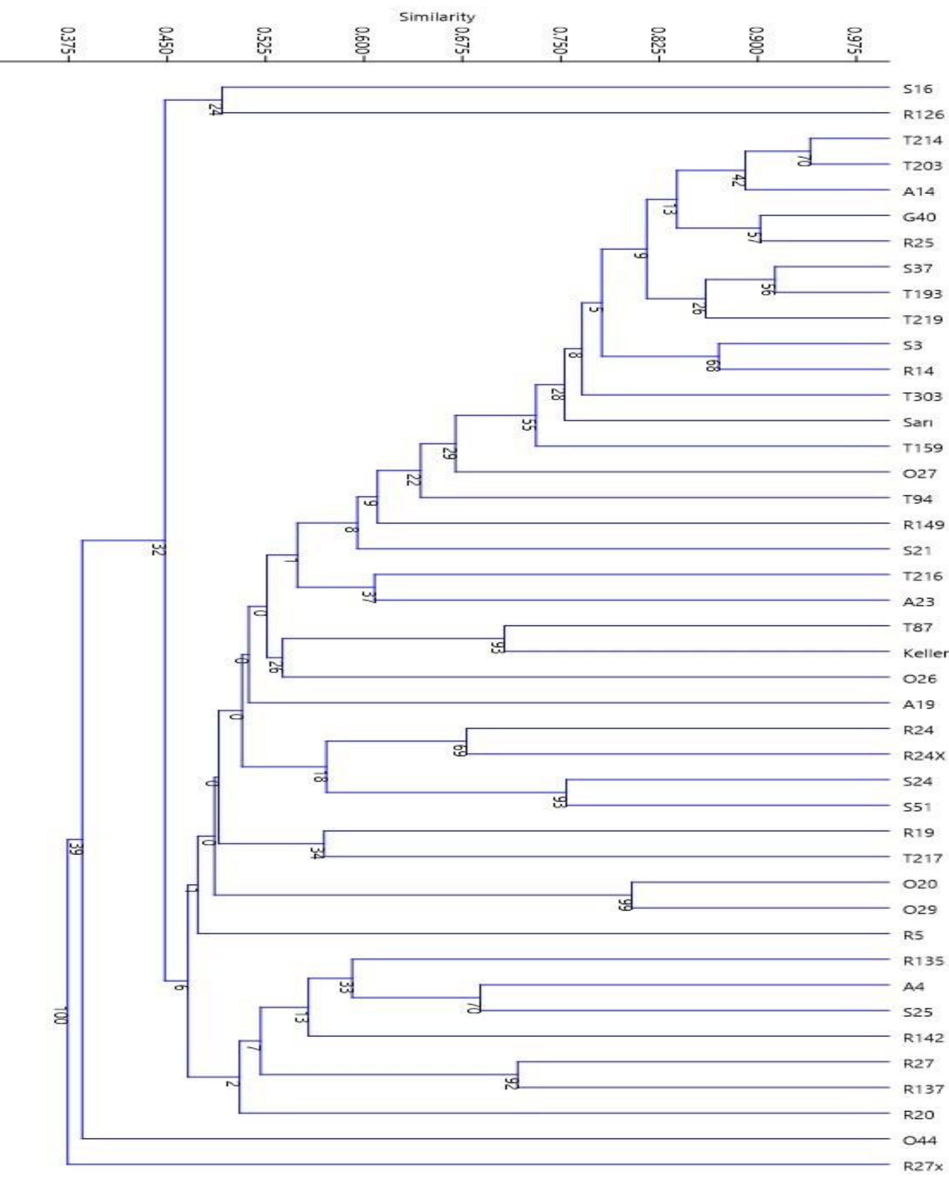

Figure 4- SSR dendrogram of cherry laurel

The dendrogram separated 43 accessions of cherry laurel collected from 6 different sites in the Black Sea Region of Turkey. UPGMA cluster analysis showed that all cultivars are clustered in two major groups. The similarity rate was found to be between 0.94 and 0.26. In the first major group, the R27x genotype selected from Rize was separated from the other 42 genotypes. Its fruits ripen too late. In the second major group, the O44 genotype selected from Ordu separated all other genotypes. This genotype has pointed and dark black fruit and produces large trees. The validity of the dendrogram in reflecting the genetic relationships among the cherry laurel genotypes is indicated by a high cophenetic correlation coefficient (r) of 0.98. Principle coordinate analysis (PCoA) was also performed using the similarity matrix, and the two-dimensional dendrogram corroborated UPGMA analyses (Figure 5).

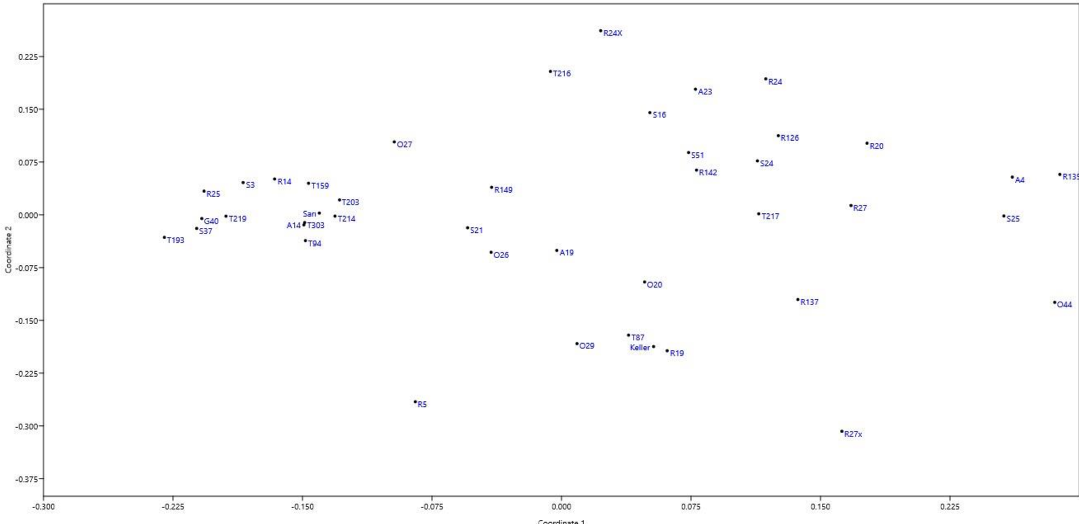

Figure 5- Principle coordinate analysis (PCoA) of 43 cherry laurel genotypes generated by the data from SSR analyses

When all genotypes were evaluated, the most distant were R27x and R24x genotypes with a ratio of 0.26. In our study, when genotypes were evaluated in terms of the province where they were collected, the genotypes selected from Rize had the lowest similarity by 0.49, while the genotypes selected from Trabzon had the highest similarity with a mean ratio of 0.61. The genotypes selected from Rize showed the most distant distribution. When the genotypes selected from all the provinces are evaluated as a whole, it can be said that there is a gene flow in the cherry laurel genotypes among the provinces. The UCDCH-17, PMS67, PMS49, PMS2, PMS3 loci, which were developed from cherry (Struss et al. 2003), the UDAp-401 locus developed from apricot (Messina et al. 2004), the UDP 98-021, UDP 96-005 loci developed from peach (Cipriani et al. 1999), and PceGA34 and PceGA59 loci developed from sour cherry were used in the definition.

DNA markers are frequently used in the genetic identification of *Prunus* species. Of the 15 SSR primers used in the study of Mnejja et al. (2004), 13 SSR primers yielded a scorable band pattern. This study showed that SSR markers can be used among the *Prunus* species (Mnejja et al. 2004; Wünsch 2009). Also, Celikkol (2011), and Hayaski et al. (2009) stated that crossing between species could be possible. The primers used by Hajyzadeh et al. (2013) included PMS 49, PMS 67, Pce GA34, UDP98-021, PMS2, PMS3, PceGA59, and the number of alleles supported our work. The scorable band number of the SSR primers was found to be a total of 117. They ranged from 3 alleles (UDAp-401) to 17 (BBCT001) alleles. The average number of bands was recorded as 9 per primer. The average number of polymorphic bands per primer was 8.38. The highest polymorphic band number, obtained from the BBCT001 primer, was 17. 109 bands were polymorphic, and the polymorphism rate was calculated as 93.52% (Table 4). The polymorphism rates of the SSR markers used in this study were close to those of Testolin et al. (2000) and Cantini et al. (2001).

Cherry, sour cherry and cherry laurel belong to the family Rosaceae. The haploid chromosome number of the cherry progenitor was eight (n= 8) and cultivated cherry varieties were obtained by selection from this progenitor (Acunalp 2012). Peach, apricot and plum species are diploid, with chromosome number 24, 16 and 16 respectively (Çelikkol 2011). However, when the gel images of SSR analysis were examined it was determined that more than 2 DNA band profiles were formed in a single genotype suggesting polyploidy in *Prunus laurocerasus* L. Furthermore, SSR analyzes were carried out on a diploid plant species, up to two DNA bands are obtained in the case that the amplified region is heterozygous. When SSR-PCR results were examined, it was determined that more than 2 DNA band profiles were formed in a single genotype. Meurman (1929) stated that the number of chromosomes increased up to 2n= 22x in cherry laurel species. Likewise, Zahra et al. (2010) stated that the basic chromosome sequence was 8x in *P. laurocerasus*. Additionally, Hajyzadeh et al. (2013) observed that more than 2 DNA band profiles were detected in a single cherry laurel genotype.

Based on the dendrogram, the genotype of O44 sampled in Ordu and the genotype R27x sampled in Rize were found to be quite different from the other genotypes (Figure 4). Except for these two genotypes, all the other genotypes (selected from Trabzon, Artvin, Rize, Giresun, Samsun and Ordu) were clustered close to each other. Moreover, Türkoğlu et al. (2010) exhibit a similarity ratio of 0.95 among five *P. laurocerasus* and 20 cherry rootstock genotypes in the Black Sea and Northeast regions of Turkey. When the similarity index was examined, the highest ratio was recorded between T203 and T214, with a ratio of 0.94. It was followed by a ratio of 0.92 between T193 and S37 genotypes, and a ratio of 0.91 between the R25 and G40 genotypes. These values are the closest of all the genotypes. The most distant individuals are R27x-R24x genotypes with a ratio of 0.26, followed by R27x-R135 and R27x- R5 with a ratio of 0.29. Additionally, Ercişli et al., (2011) determined the genetic variation

of 18 wild cherry genotypes with 10 SSR primers. They found that 9 genotypes had a high similarity ratio, and 2 genotypes differed in terms of tree and fruit characteristics.

4. Conclusions

In conclusion, we determined that by SSR analysis it is possible to differentiate the 43 different cherry laurel genotypes sampled in the Black Sea region. Furthermore, these results depicted that among the different genotypes sampled there is significant genetic variability that can be useful for future research and breeding programs. Since studies related to cherry laurel are limited around the world these results will provide the pathway for future studies.

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