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Genetic Characterization of Bay Laurel (*Laurus nobilis* L.) Populations Using Microsatellite Markers and Flow Cytometry

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Abstract

Turkey is one of the few countries that exports the highest quality bay laurel leaf and covers about 90% of the world bay laurel leaf trade. In this study, 95 bay laurel genotypes selected from flora of Hatay province for their superior characteristics were used. Selected genotypes were *genetically* characterized by 6 SSR markers and the DNA contents were determined by Flow Cytometry. No polyploidy was determined as a result of flow cytometry analysis and 2C DNA values were observed between 5.91 and 6.36 pg. As a result of the SSR analysis, a total of 82 alleles were obtained with a mean of 16.4 of 5 polymorphic loci, while LnD106 loci were observed monomorphic. The highest number of alleles (24 bp) was observed in the LnA2 locus. Generally, a low similarity is determined among the genotypes. The highest genetic similarity was seen in E6 and O6 genotypes with 80%.This situation revealed the importance of genetic diversity in Hatay bay laurel populations. The results are important as regard to reveal and protect the genetic diversity of bay laurel existence in Hatay.

Key words: Genetic diversity, *L. nobilis*, DNA content, SSR

Defne (*Laurus nobilis* L.) Populasyonlarının Mikrosatellit Markörler ve Flow Sitometri ile Genetik Karakterizasyonu

Özet

Türkiye, yüksek kaliteli defne yaprağı ihraç eden birkaç ülkeden biridir ve dünya defne yaprağı ticaretinin yaklaşık % 90'ını Türkiye yapmaktadır. Bu çalışmada üstün özellikleri nedeniyle Hatay florasından seçilen 95 adet defne genotipi kullanılmıştır. Seçilen genotipler, genetik olarak 6 SSR markörü ile karakterize edilmiş ve DNA içerikleri Flow Cytometry ile belirlenmiştir. Flow sitometri analizi sonucunda poliploidi saptanmamış ve 2C DNA değerleri 5.91 ile 6.36 pg arasında gözlenmiştir. SSR analizi sonucunda, 5 polimorfik lokusta ortalama 16.4 ile toplam 82 allel elde edilirken, LnD106 lokusu monomorfik olarak gözlenmiştir. En yüksek allel sayısı (24 bp) LnA2 loküsünde gözlenmiştir. Genel olarak, genotipler arasında benzerlik düşük olmuştur. En yüksek genetik benzerlik oranı % 80 ile E6 ve O6 genotiplerinde görülmüştür Bu durum Hatay defne popülasyonlarındaki genetik çeşitliliğin önemini ortaya çıkarmıştır. Sonuçlar, Hatay'da defne varlığının genetik çeşitliliğini ortaya koymak ve korumak açısından önemlidir.

Anahtar Kelimeler: Genetik çeşitlilik, *L. nobilis*, DNA içeriği, SSR

Introduction

Bay laurel (*Laurus nobilis* L.) is an evergreen, dioecious plant in the form of a pyramidal-shaped tree or large bush of the *Laurus* genus of the Lauraceae family. Besides bay laurel, there are about 2500 species in Lauraceae family including plant species such as cinnamon and avocado (Heywood, 1978; Christenhusz and Byng, 2016). *L. nobilis* L., also known as Mediterranean bay laurel, is widely grown in Turkey, Greece, Italy, Spain, Portugal, France, Yugoslavia, Syria, Morocco, Algeria, Mediterranean Islands and California (Baytop,

1999; Ross, 2001; Kumar et al., 2003; Rodriguez-Sanchez et al., 2009).

Bay laurel grows naturally starting from the province of Hatay along the Mediterranean, Aegean and Black Sea coasts, up to 1200 m altitudes in the inner parts of these coastal areas (Kayacık, 1977; Davis, 1982; Anonymous, 2016). In Turkey, 5500 tons of bay laurel seeds and 21634 tons of dry bay laurel leaves are produced, 12741 tons of the dry bay laurel leaves are exported every year. Comparing the export values of medicinal and aromatic plants in recent years, the export of bay laurel dry leaf is one of the frontrunners in terms of the amount and the economic value in Turkey (Anonymous, 2014; Anonymous, 2016; Şafak and Okan, 2004; Kurt et al. 2016). Turkey holds approximately 90% of the world bay laurel leaf trade.

The main constituents of bay laurel essential oil are 1,8-cineole, trans-sabinene hydrate, α-terpinyl acetate, methyl eugenol, sabinene, eugenol and α-Pinene (Kekelidze et al., 987; Ceylan and Özay 1990; Kılıç et al., 2004; Verdian-Rizi, 2008; Ayanoğlu et al., 2013). Leaves of bay laurel with aromatic odor are used in cooking to give fragrance and flavor to soups, stews, seafood, and etc in many cuisines. Bay laurel oil is commonly used as a moisturizer and fragrance ingredient in soap and other cosmetic skin moisturizing products in the industry. As a healing herb; it is known that the essential oil of bay laurel leaves are used for treatment of rheumatism, skin rashes, and ear pain. It is specified that bay laurel leaves have the benefits as antioxidant (Simic et al., 2003), analgesic (pain reliever), anti-inflammatory (Sayyah et al., 2003) and antifungal (Rodilla et al., 2008).

The evaluation of morphological, biochemical characteristics and the DNA markers both in research and in practice, has gained importance in terms of properly orienting the genetic potentials of plants and the opportunity of benefiting these markers in plant breeding is increasing day by day. SSR markers have been identified as the advantageous technique for genomic studies in terms of high polymorphism and repeatability (Powell et al., 1996).

Flow cytometry, which is widely used today in cytogenetic definitions; is an efficient, reliable, rapid method that is particularly effective in determination of the amount of DNA in plant cells, in the detection of cell cycle analyzes, and in the investigation of variations in ploidy status (Suda et al., 2003, Galbraith 2004, Bennett and Leitch, 2011).

The aim of the present study is to assess population structure of bay laurel in the region, the level of genetic variability as well as the relationship among the selected genotypes to aid in the selection of promising genotypes and to enhance the efficiency of bay laurel breeding program. In the study; among the 203 bay laurel genotypes collected from different locations of Hatay province (Ayanoğlu et al., 2013), a total of 95 bay laurel genotypes showing superior characteristics in terms of various characters were genetically characterized by scanning with SSR markers. In addition, polyploidy levels were compared by determining the nuclear DNA content of the genotypes by flow cytometry.

Materials and Methods

Plant material

In the previous selection studies conducted in Hatay province, 203 genotypes were examined and 95 genotypes were selected for their superior characteristics (Ayanoğlu et al., 2013). These characteristics are fruit weight (A2, B23, H3, SY3, SY9), kernel weight (B30, E10, YY1, B1), kernel ratio (B5, B6, B33, ER3, K2), ovality coefficient (ER20, O9, ER4), berry oil content (ER1, ER6, ER16, ER17, ER29, ER41), berry flesh oil content (B26, ER12, ER13), kernel oil content (E6, E9, ER14, ER17, ER22, ER24), lauric acid ratio (HB7, K9, BA9, ER42, ER8, K1), oleic acid ratio (S4, S7, H1, O12), palmitic acid ratio (BA13), chlorophyll SPAD value (H7, H11, HB11, SY7, O17), dry leaf ratio (AY4, ŞK3, YY2, YY3), leaf area (B11, B21, H5, HB10), essential oil contents (B29, B34, HB8A, HB8B, K4, SY10, YY7, YY8, E1, ER7, ER35, O6, O8), 1,8 cineol content (AY3, AY5, B10, ER11, ER26, O4, O13), essential oil components (B4, B25, ER3, ER15, ER18, H2, HU2, HU3, K10, K12, S6, SY2, SY5, ŞK4, YY5, BA3, E5, S3, D2).

Table 1. Locations, coordinates and altitudes (m) of bay laurel genotypes

Genotype	Location	m	Coordinate	Genotype	Location	m	Coordinate	Genotype	Location	m	Coordinate
A2	Altınözü	311	N 36 11 615 E 36 11 573	ER6	Eriklikuyu	268	N 36 09 022 E 36 00 044	K1	Kapısuyu	130	N 36 07 204 E 35 56 319
AY3	Karşıyaka	28	N 36 04 438 E 36 02 649	ER7	Eriklikuyu	270	N 36 09 017 E 36 00 033	K2	Kapısuyu	128	N 36 07 207 E 35 56 329
AY4	Karşıyaka	29	N 36 04 442 E 36 02 658	ER8	Eriklikuyu	271	N 36 09 017 E 36 00 030	K4	Kapısuyu	126	N 36 07 223 E 35 56 337
AY5	Karşıyaka	29	N 36 04 450 E 36 02 664	ER11	Eriklikuyu	276	N 36 09 038 E 36 00 035	K9	Kapısuyu	319	N 36 07 789 E 35 57 407
B1	Batıayaz	462	N 36 09 974 E 35 59 511	ER12	Eriklikuyu	275	N 36 09 039 E 36 00 045	K10	Kapısuyu	192	N 36 07 642 E 35 58 293
B4	Batıayaz	460	N 36 09 851 E 35 59 468	ER13	Eriklikuyu	275	N 36 09 040 E 36 00 051	K12	Kapısuyu	251	N 36 08 099 E 35 58 532
B5	Batıayaz	460	N 36 09 851 E 35 59 469	ER14	Eriklikuyu	275	N 36 09 040 E 36 00 052	O4	Olgunlar	680	N 35 58 936 E 36 03 166
B6	Batıayaz	460	N 36 09 851 E 35 59 470	ER15	Eriklikuyu	276	N 36 09 041 E 36 00 057	O6	Olgunlar	676	N 35 58 964 E 36 03 183
B10	Batıayaz	445	N 36 09 851 E 35 59 442	ER16	Eriklikuyu	275	N 36 09 041 E 36 00 060	O8	Olgunlar	634	N 35 59 190 E 36 03 126
B11	Batıayaz	444	N 36 09 846 E 35 59 432	ER17	Eriklikuyu	279	N 36 09 042 E 36 00 068	O9	Olgunlar	633	N 35 59 193 E 36 03 125
B21	Batıayaz	429	N 36 09 819 E 35 59 390	ER18	Eriklikuyu	280	N 36 09 042 E 36 00 066	O12	Olgunlar	631	N 35 59 221 E 36 03 130
B23	Batıayaz	440	N 36 09 799 E 35 59 426	ER20	Eriklikuyu	280	N 36 09 045 E 36 00 066	O13	Olgunlar	629	N 35 59 219 E 36 03 141
B25	Batıayaz	442	N 36 09 803 E 35 59 426	ER22	Eriklikuyu	279	N 36 09 046 E 36 00 051	O17	Olgunlar	628	N 35 59 223 E 36 03 160
B26	Batıayaz	454	N 36 09 823 E 35 59 433	ER24	Eriklikuyu	283	N 36 09 050 E 36 00 046	S3	Sinanlı	93	N 36 05 320 E 36 04 628
B29	Batıayaz	464	N 36 09 878 E 35 59 479	ER26	Eriklikuyu	286	N 36 09 053 E 36 00 054	S4	Sinanlı	70	N 36 05 336 E 36 04 607
B30	Batıayaz	463	N 36 09 899 E 35 59 479	ER29	Eriklikuyu	289	N 36 09 061 E 36 00 067	S6	Sinanlı	63	N 36 05 356 E 36 04 596
B33	Batıayaz	459	N 36 09 894 E 35 59 502	ER35	Eriklikuyu	301	N 36 09 105 E 36 00 098	S7	Sinanlı	60	N 36 05 348 E 36 04 591
B34	Batıayaz	464	N 36 09 940 E 35 59 512	ER41	Eriklikuyu	390	N 36 09 097 E 36 00 0168	SY2	Sinanlı	42	N 36 07 439 E 36 06 815
BA3	Batıayaz	438	N 36 10 810 E 35 59 341	ER42	Eriklikuyu	288	N 36 09 097 E 36 00 176	SY3	Sinanlı	43	N 36 07 441 E 36 06 813
BA9	Batıayaz	493	N 36 10 070 E 35 59 448	H1	Harbiye	170	N 36 07 718 E 35 56 423	SY5	Sinanlı	42	N 36 07 274 E 36 06 743
BA13	Batıayaz	476	N 36 09 947 E 35 59 263	H2	Harbiye	171	N 36 07 699 E 36 08 377	SY7	Sinanlı	42	N 36 07 248 E 36 06 661
D2	Döver	227	N 36 07 233 E 36 08 031	H3	Harbiye	164	N 36 07 693 E 36 08 327	SY9	Sinanlı	21	N 36 05 344 E 36 03 760
D13	Döver	232	N 36 07 208 E 36 08 060	H5	Harbiye	149	N 36 07 617 E 36 08 249	SY10	Sinanlı	21	N 36 05 338 E 36 03 752
E1	Eriklikuyu	214	N 36 09 450 E 36 00 692	H7	Harbiye	150	N 36 07 612 E 36 08 225	ŞK3	Şakşak	759	N 35 58 378 E 36 05 759
E5	Eriklikuyu	266	N 36 09 008 E 36 00 322	H11	Harbiye	138	N 36 07 564 E 36 08 177	ŞK4	Şakşak	756	N 35 58 366 E 36 05 755
E6	Eriklikuyu	261	N 36 09 012 E 36 00 184	HB7	Batıayaz	478	N 36 10 088 E 35 59 519	YY1	Yayladağı	945	N 36 00 793 E 36 07 289
E9	Eriklikuyu	253	N 36 08 988 E 36 00 090	HB8A	Batıayaz	479	N 36 10 089 E 35 59 518	YY2	Yayladağı	948	N 36 00 783 E 36 07 288
E10	Eriklikuyu	252	N 36 08 982 E 36 00 022	HB8B	Batıayaz	479	N 36 10 089 E 35 59 514	YY3	Yayladağı	949	N 36 00 764 E 36 07 2839
ER1	Eriklikuyu	258	N 36 09 006 E 36 00 049	HB10	Batıayaz	481	N 36 10 089 E 35 59 518	YY5	Yayladağı	938	N 36 00 790 E 36 07 317
ER2	Eriklikuyu	265	N 36 08 998 E 36 00 051	HB11	Batıayaz	480	N 36 10 089 E 35 59 530	YY7	Yayladağı	976	N 36 00 807 E 36 07 196
ER3	Eriklikuyu	258	N 36 08 997 E 36 00 044	HU2	Hüseyinli	79	N 36 10 464 E 36 05 952	YY8	Yayladağı	985	N 36 00 825 E 36 07 180
ER4	Eriklikuyu	265	N 36 09 018 E 36 00 040	HU3	Hüseyinli	81	N 36 10 459 E 36 05 950				

The young leaves of single plant of these selected genotypes were used as material in the experiment. The information on location, altitude and coordinates of where the bay laurel genotypes are grown is given in Table 1.

SSR Analysis

DNA was extracted from the bay laurel leaf tissue according to CTAB protocol for isolation (Doyle and Doyle, 1987), modified by Lefort et al., (1998). A total of 6 SSR markers, namely LnA2, LnD106, LnD5, LnB2, LnA106, LnB124 (Arroyo et al., 2010) were used in this study. PCR amplifications were performed as described by Selli et al. (2007) and the bonding temperatures (TM) for the 6 SSR markers are given in Table 2. Forward primers of each pair were labeled with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France). PCR products were diluted with Sample Loading Solution (SLS) in certain proportions according to the fluorescent dyes used in fluorescent primer labeling, followed by the addition of Genomelab DNA Size Standard Kit-400 and electrophoresed in CEQ 8800XL Capillary DNA analysis system (Beckman Coulter, Fullerton, CA). Allele sizes were determined for each SSR loci by using Beckman CEQ 8800 Fragment Analysis software.

Genetic Analysis

Number of alleles (N)(bp-base pair), allele frequency (alf), expected (HE) and observed heterozygosity (HO), estimated frequency of null alleles (r) and probability of identity (PI) were calculated for each loci using the program "IDENTITY 1.0" (Wagner and Sefc, 1999) according to Paetkau et al. (1995). Proportion of shared alleles was calculated by using ps (option 1-(ps) (Bowcock et al., 1994) as genetic dissimilarity in the Microsat (version 1.5) program (Minch et al., 1995). These data were then converted to a similarity matrix and a dendrogram was constructed with UPGMA (unweighted pair-group method with arithmetic mean) (Sneath and Sokal, 1973), using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (version 2.0) (Rohlf, 1988).

Nuclear DNA Content Analysis

The DNA content of the samples taken from the leaves of 95 bay laurel genotypes is analyzed at the Plant Genetics and Cytogenetics Lab of Agricultural Faculty of Namik Kemal University located in Tekirdag, Turkey. Until analysed, materials were kept at 4^{o}C between moisturized filter paper, placed in a disposable petri dish.

Absolute 2C DNA contents were determined for each genotypes using propidium iodide (PI) staining. Samples and leaf sections of Vicia sativa (2C DNA content: 3.65 pg-picogram), used as an internal standard, were simultaneously chopped and stained using the 'CyStain PI absolute P' nuclei extraction and staining kit (Partec GmbH, Munster) according to the manufacturer's instructions. Samples were analysed using a Partec CyFlow Space flow cytometer (Munster, Germany). The absolute DNA contents of bay laurel accessions were calculated based on the ratios of the G1 peak means of sample and reference standard (Tuna et al., 2001).

Results

SSR Analysis

The LnA2, LnD106, LnD5, LnB2, LnA106, and LnB124 (Arroyo et al., 2010). SSR loci used in the study (Table 2). While all other primers showed polymorphic property, only the LnD106 primer was observed monomorphic (130 bp). As a result of genetic analysis from 5 polymorphic loci, a total of 82 alleles were obtained and the average number of alleles was determined as 16.4. The highest number of alleles was observed in the LnA2 primer with 24 alleles, followed by primers LnB2 and LN B124 with 22 and 18 alleles, respectively.

The lowest number of alleles was found as 9 alleles in LnD5 and LnA106 (Table 3). The expected heterozygosity values (He) were 0.753 (LnA106) to 0.932 (LnA2), and the observed heterozygosity values (Ho) were 0.747 (LnA106) to 0.937 (LnB124). The mean values of He and Ho were 0.855 and 0.865, respectively. The highest heterozygosity value was determined in LnA2, followed by LnB2 and LnB124 loci (Table 3). PI values are inversely

correlated with the number of alleles and as the discrimination of the SSR loci gets higher, the PI values approach to zero. In addition, the PI values of 5 SSR locus are found higher than the threshold value (0.05), which is determined by Sefc et al. (2001). PI values ranged from 0.017 (LnA2) to 0.551 (LnA106).

Table 2. Characteristics of the studied SSR locus

No	SSR Loci	Repeat Motif	Primer sequence (5'-3')	Tm (C°)	Size Range (bp)*
1	LnA2 (GU344687)	$(GT)_8$ GC $(GT)_{11}$	F: TGCCCAAAAATGGTGTAG R:CGTGGTCTTAGCCTTAGTAGTC	60	256-313
2	LnD106 (GU344691)	$(ATC)_8$	F:TGCTCTACGTTTTGTGAAGATC R:CATTGGAGGGAACTTCTTTTAC	55	152-167
3	LnD5 (GU344692)	$(TGA)_8$	F: CGTTAGCACTGTCCCATCTG R: CCGAAATCACCACCTTTTTC	60	115-130
4	LnB2 (GU344693)	$(GA)_{24}$	F: TATTTGAAGGTTTCCTCTCAGA R: ATAAAGCGTGTCATTGTGAAC	55	242-293
5	LnA106 (GU344697)	$(AC)_{12}$	F: CAAATGATTTCAAGGACCAC R: AGGGGTCTTACTTCTATGAAGG	60	157-167
6	LnB124 (GU344698)	$(CT)_{16}$	F: TGGAATGTATGGCTCTGAACTC R:CCAATCACAACCAGAAAGACAG	55	223-285

* Arroyo et al., (2010)

In particular, the PI values of the primers LnA2 (0.017), LnB2 (0.018) and LnB124 (0.041) were observed to have high discriminatory power in discriminating bay laurel genotypes. Null allele values were observed generally negative in two loci (LnD5 and LnB124) and positive but close to zero in the other three loci, thus proving the low possibility of them being null alleles (Table 3). It has been observed that the allele frequencies (alf) of the 5 locus are not homogeneous (Table 4). Alleles with the highest allele frequency of the SSR loci were determined as follows: allele 250 (alf: 0.105) at LnA2, allele 93 (alf: 0.405) at LnD5, allele 125 (alf: 0.411) at LnA106, allele 254 (alf: 0.134) at LnB2 and allele 232 (alf: 0.179) at LnB124. In the presented research, 29 accessions (genotypes: AY4, B1, B23, B29, D2, ER4, O17, SY5, YY5, K2, ER29, H2, ER16, ER20, ER35, O12, YY3, ER12, ER15, ER24, H5, HU2, K4, B25, B26, H2, HB8B, K10, YY1) showing triple alleles at one SSR loci, 10 accessions (genotypes: B33, H7, D13, E1, E9, H1, S6, O4, ER24, ER14) at two SSR loci and three accessions (genotypes: A2, ER1, SY9) at three SSR loci were identified (Table 5). Genetic similarities between genotypes varied between 10% to 80%. The highest genetic similarity (80%) was determined between the E6 and O6 genotypes. The second highest genetic similarity was 70% among six genotypes (E9-O9, SY3-SY5, ER8-ER24) from different locations (Figure 1). Genotypes are divided into two major groups; Group A and Group B, as shown in the genetic relationship dendrogram (Figure 1). In Group A, 5 genotypes (B1, HB10, B6, ER3, E5) showed genetic similarity under the same main group, whereas genotypes in Group B showed genetic similarity, forming many subgroups. The highest genetic similarity in Group A was found between genotypes B1 and HB10, and between genotypes B6 and ER3 with 40%. Among 90 genotypes in Group B, the highest genetic similarities were; 50% Subgroup 1 between YY3 and H5, 60% in Subgroup 2 between S7 and ER15, 80% in Subgroup 3 between E6 and O6, 70% in Subgroup 4. between E9 and O9, and between SY3 and SY5.

Table 3. Number of alleles (bp), expected heterozygosity (He), observed heterozygosity (Ho), probability of identity (PI) and null allele frequency (r) of genotypes

SSR Loci	N	He	Ho	PI	r
LnA2	24	0.932	0.842	0.017	0.025
LnD5	9	0.767	0.863	0.132	-0.054
LnA106	9	0.753	0.747	0.551	0.003
LnB2	22	0.930	0.895	0.018	0.018
LnB124	18	0.892	0.937	0.041	-0.024
Total	82	4.273	4.326	-	-
Mean	16.4	0.855	0.865	-	-

Table 4. Allele frequencies of 5 loci. (N: number, alf: allel frequency)

N	LnA2	*alf*	LnD5	*alf*	LnA106	*alf*	LnB2	*alf*	LnB124	*alf*
1	230	0.005	81	0.058	123	0.032	226	0.005	212	0.005
2	236	0.068	89	0.005	125	0.411	232	0.011	214	0.132
3	238	0.021	91	0.011	127	0.032	234	0.047	216	0.026
4	240	0.089	93	0.405	129	0.179	236	0.021	218	0.037
5	242	0.026	95	0.053	131	0.037	238	0.079	220	0.137
6	244	0.053	97	0.121	133	0.011	240	0.016	222	0.011
7	246	0.047	99	0.132	135	0.105	242	0.042	224	0.068
8	248	0.100	101	0.047	147	0.016	244	0.084	226	0.026
9	250	0.105	103	0.168	149	0.179	246	0.037	228	0.105
10	252	0.095					248	0.026	230	0.042
11	254	0.074					250	0.053	232	0.179
12	256	0.026					252	0.095	234	0.037
13	258	0.053					254	0.137	236	0.132
14	260	0.084					256	0.089	238	0.032
1	262	0.021					258	0.021	240	0.011
16	264	0.021					260	0.058	242	0.005
17	266	0.005					262	0.037	246	0.011
18	268	0.021					264	0.063	248	0.005
19	270	0.011					266	0.026		
20	272	0.011					268	0.005		
21	274	0.037					270	0.032		
22	276	0.016					272	0.016		
23	278	0.005								
24	286	0.005								

Table 5. The list of third alleles of genotypes

SSR Loci	3. Allele (bp)	Genotype
LnB124	220	A2, AY4, B1, B21, B23, B29, B33, H7
	246	D2, D13, E1, E5, E9, ER1, ER4
	232	O17, SY5, H1
	226	YY5
	228	K2, S6
LnB2	244	B33, E9, ER29, H2
	250	ER1, ER16, ER20, ER35, O4, O12, YY3
	238	ER12, ER24, H7
	228	ER14, B26,SY10
LnD5	89	SK4, D13, H1
	103	E1, E9, ER15, ER24, H5, HU2, K4, SY9
LnA2	266	B25, B26, H2
	248	A2, ER1, ER14, HB8B, K10, O4, S4, S6, SY9, YY1

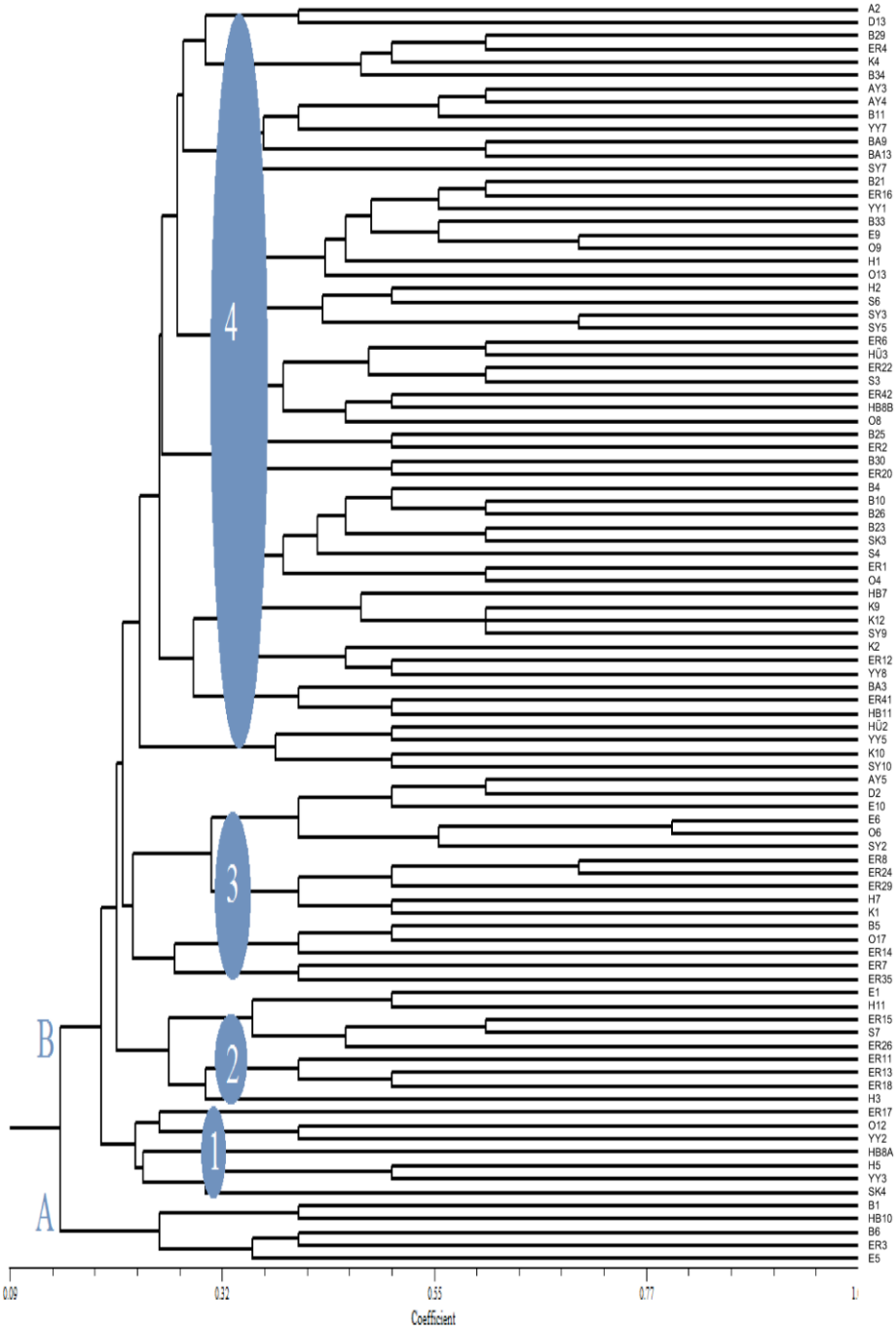

Figure 1. Dendrogram of genetic similarity among the analyzed bay laurel genotypes based on SSR markers

Flow Cytometry Analysis

The flow cytometry analysis conducted in the research show that the Nuclear DNA content values varied between 5.91 (ER20) and 6.34 (AY4), as shown in Table 6. As an example histogram of peaks were given in Figure 2. More similarities were observed on the DNA content values of the genotypes within the same location, compared to the other genotypes of the population.

Discussion

Most of the genetic characterization studies with the DNA markers of the Lauraceae family have been conducted on avocados (Mhameed et al., 1996; Mhameed et al., 1997; Fiedler et al., 1998; Davis et al., 1998;

Alcaraz and Hormaza, 2007; Borrone et al., 2007; Acheampong et al., 2008), and rarely have been conducted on bay laurel (Arroyo-Garcia et al., 2001; Marzouki et al., 2009).

Furthermore, the research conducted on genotypes of *L. azorica*, *L. novocanariensis* and *L. nobilis* using RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats) and isoenzyme molecular markers showed that the ISSR molecular markers demonstrate higher polymorphism levels compared to the other markers. Therefore it has been reported that it provides more accurate genetic discrimination (Aboel-Atta, 2009). Similarly, a study on 75 avocado genotypes collected from different geographical regions of Spain report that especially the SSR markers have high discriminatory power in genetic characterization (Alcaraz and Hormaza, 2007).

Marzouki et al. (2009), reported that bay laurel has a higher genetic differentiation than the other angiosperm and stated that *Laurus nobilis* L. may have a basic two gene pool, Western (Tunisia, Algeria and France) and Eastern Mediterranean (Turkey). For this reason, it is very important to determine the genetic characterization of Turkey's bay laurel genetic resources and to protect their alleles.

Arroyo et al. (2010) scanned a total of 63 genotypes belonging to species of *L. nobilis* and *L. azorica* with newly designated 20 polymorphic SSR markers. 196 alleles were found in 37 genotypes belonging to the *L. nobilis* species, with an average of 9,7 alleles per primer. In the 26 genotypes belonging to the *L. azorica* species, 222 alleles, with an average of 14,8 alleles per primer were found. The highest number of alleles in the research in other plants such as olive (Bandelj et al.,2004).

Nuclear DNA content value (2C DNA) of *Lauris nobilis* L. (diploid) is reported to be between 6.1 and 6.8 (Zonneveld et al., 2005; Bennett and Leitch, 2011), which is similar to results of the conducted research. This proved that there is no polyploidy in the 95 bay laurel genotypes. The genetic relationship dendrogram showed heterogeneous branching. Genotypes taken from the same locations generally showed alterations at different levels of the dendrogram. Genetic relationship dendrogram showed genetic similarity of more than 55% in some genotypes (AY4-AY3, B26-B10, BA13-BA9, SY3-SY5, K12-K9) that grow in the same region. However, although some genotypes grow in different regions, they are observed to have the highest genetic similarity in the dendrogram, proving that there may be a natural gene flow in the region.

conducted by Arroyo et al. (2010) was observed with 18 alleles in primer LnB106a for *L. nobilis*, and the highest alleles with 26 alleles in primers LnB116 and LnA2 for *L. azorica*. In our research, LnA2 and LnB2 loci were identified as the most polymorphic loci with 24 and 22 alleles, respectively. In this respect, LnA2 locus is proved to have an effective discrimination power in both *L. azorica* (Aroyo et al., 2010) and *L. nobilis* L. genotypes.

In another study carried out in 66 laurel genotypes collected from 7 different Mediterranean locations, a total of 34 alleles were detected in 4 polymorphic SSR, with a mean of 9 alleles per primer (Marzouki et al.,2009). In our study, a total of 82 alleles were found in 95 genotypes taken from different locations of the same province. The average number of alleles was 16.4, suggesting allele of Hatay province. The He and Ho values of 5 SSR loci were determined to be between 0.747 and 0. 937, and these values were found to be similar to the He and Ho values (0.729-0.995) from the study (Aroyo et al. 2010) with the same locus.

In the presented study, triallelic pattern was observed in some *L. nobilis* L. genotypes. This condition, which is also determined in a total of 4 SSR loci (LnB124, LnB2, LnD5 and LnA2), can be attributed to the chimerism seen in leaf layers (no plant polyploidy condition) (Hocquigny, et al. 2004). Chimerism refers to at least two genetically different cell layers resulted from a mutation in the apical meristem (Burge et al., 2002). The genetic variation in these layers may cause more than two alleles to be seen in the co-dominant SSR locus. Triallelic SSR loci have also been found

Table 6. Nuclear DNA content (picogram) of 95 bay laurel genotypes

No	Genotype	2c-Value	No	Genotype	2c-Value	No	Genotype	2c-Value
1	A2	6.33	**33**	ER-6	6.28	**65**	K-1	6.21
2	AY3	6.16	**34**	ER-7	6.19	**66**	K-2	6.04
3	AY-4	6.36	**35**	ER-8	6.14	**67**	K-4	6.28
4	AY-5	6.19	**36**	ER-11	6.19	**68**	K-9	6.18
5	B-1	5.97	**37**	ER-12	6.10	**69**	K-10	6.25
6	B-4	6.24	**38**	ER-13	6.11	**70**	K-12	6.17
7	B-5	6.32	**39**	ER-14	6.15	**71**	O-4	6.19
8	B-6	6.27	**40**	ER-15	6.18	**72**	O-6	6.17
9	B-10	6.22	**41**	ER-16	6.12	**73**	O-8	6.25
10	B-11	6.05	**42**	ER-17	6.28	**74**	O-9	6.24
11	B-21	6.00	**43**	ER-18	6.17	**75**	O-12	6.19
12	B-23	6.18	**44**	ER-20	5.91	**76**	O-13	6.14
13	B-25	6.22	**45**	ER-22	6.25	**77**	O-17	6.17
14	B-26	6.17	**46**	ER-24	6.27	**78**	S-3	6.16
15	B-29	6.22	**47**	ER-26	6.16	**79**	S-4	6.21
16	B-30	6.14	**48**	ER-29	6.14	**80**	S-6	6.14
17	B-33	6.25	**49**	ER-35	6.24	**81**	S-7	6.12
18	B-34	6.15	**50**	ER-41	6.34	**82**	SY-2	6.23
19	BA-3	6.14	**51**	ER-42	6.30	**83**	SY-3	6.23
20	BA-9	6.23	**52**	H-1	6.18	**84**	SY-5	6.25
21	BA-13	6.22	**53**	H-2	6.17	**85**	SY-7	6.20
22	D-2	6.21	**54**	H-3	6.28	**86**	SY-9	6.26
23	D-13	6.35	**55**	H-5	6.24	**87**	SY-10	6.12
24	E-1	6.27	**56**	H-7	6.23	**88**	SK-3	6.16
25	E-5	6.24	**57**	H-11	6.14	**89**	SK-4	6.20
26	E-6	6.08	**58**	HB-7	6.10	**90**	YY-1	6.09
27	E-9	6.21	**59**	HB-8A	6.27	**91**	YY-2	5.96
28	E-10	6.20	**60**	HB-8B	6.13	**92**	YY-3	6.16
29	ER-1	6.14	**61**	HB-10	6.28	**93**	YY-5	6.03
30	ER-2	6.05	**62**	HB-11	6.25	**94**	YY-7	6.27
31	ER-3	6.11	**63**	HU-2	6.26	**95**	YY-8	6.28
32	ER-4	5.98	**64**	HU-3	6.33			

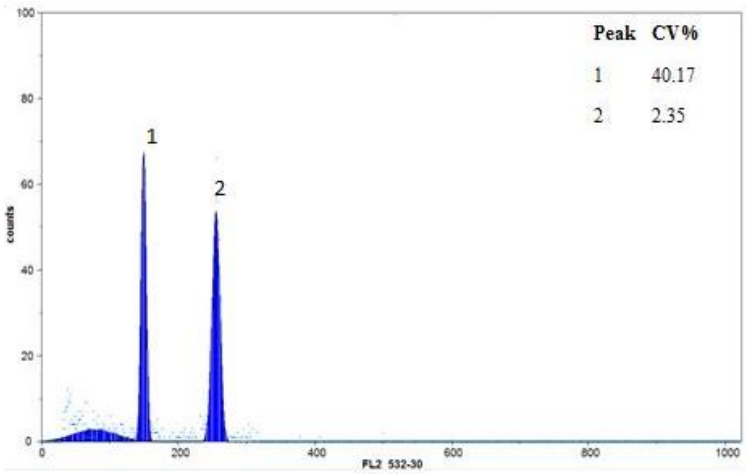

Figure 2. In histogram the following peaks are marked: 1-nuclei at G1 phase of internal standart (*Vicia sativa*, 2C=3.65 pg DNA); 2-nuclei at G1 phase of laurel sample (E9) Coefficient of variation value (CV %) of each peak are also given

Because the success of natural or cultural reproduction with cuttings are very low for bay laurel plant, the variation seen in the levels of genetic similarities depending on the regions where the genotypes are grown is thought to originate from hybridization due to insect activities. The significance of the variations among the genotypes carried out by this study offers the importance of a detailed examination and registration of the gene resources in the Hatay region.

This is the first study, which performed SSR analysis of 95 genotypes growing in Hatay province of Turkey. Also this study is important for the genetic characterization of bay laurel genotypes with commercial value and also for the identification and preservation of bay laurel populations already under threat. The significant difference among the genotypes point out that new species can be found in future studies.

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