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Genetic Relationships and Diversity Analysis in Turkish Laurel (Laurus Nobilis L.) Germplasm Using ISSR and SCoT Markers

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Abstract

Laurel (*Laurus nobilis* L.) has been used in the Mediterranean basin since ancient ages. Nowadays, Turkey, Mexico, Portugal, Italy, Spain, France, Algeria, and Morocco use aromatic leaves for commercial purposes, and Turkey is the largest exporter in the world. In this study, molecular characterization and genetic relationships of 94 Turkish laurel genotypes were determined by ISSR and SCoT markers. The experiment was conducted with 16 ISSR and 10 SCoT markers. While 348 of 373 bands were polymorphic with a 94.04% polymorphism rate, Nei's genetic distances ranged between 0.17 and 0.70 with 0.39 mean in ISSR. In SCoT, 175 of 227 bands were polymorphic with 76.07% polymorphism rate, and Nei's distances varied between 0.12 and 0.51. Sufficient genetic diversity determined with diversity parameters consisting the average Shannon's information index (ISSR:0.46, SCoT:0.35), the overall gene diversity (ISSR:0.19, SCoT:0.18), and the effective number of alleles (ISSR:1.52, SCoT:1.38). AMOVA (Analysis of molecular variance) revealed most of the variation was within genotypes (%96). Neighbor-joining algorithms, principal coordinate analysis (PCoA), and model-based structure resulted in harmony and clustered according to the geographical regions and provinces they collected. Genotypes were divided into two groups in ISSR and SCoT with UPGMA clustering resulting in a similar polymorphism distribution. The correlation coefficient (r) determined by marker systems' Nei's genetic distance matrices was 0.88. The results of the study put forward resources for advanced breeding techniques, and contribute to the preservation of genetic diversity, and management of genetic resources for the breeders.

1. Introduction

Medicinal and aromatic plants are mainly used in medicines to prevent diseases, maintain healthiness, or heal illnesses. Medicinal plants are widely used in body care, nutrition, cosmetics, incense, or religious ceremonies, while aromatic plants are used to maintain a pleasant smell and taste. The importance of these plants is increasing day by day with people's tendency towards nature and natural sources in the nutrition, health, and cosmetic sectors. The necessity and significance of medicinal and aromatic plants usage in routine life will never decline, and it will maintain its popularity among the scientific community [1]. Medicinal plants have become increasingly recognized especially in the last decade, particularly in the role of rural people's economies, scope in medicinal practices, use in cultural matters, and ultimately their contribution to the well-being of people [2].

Laurel is not widely recognized compared to other medicinal plants worldwide despite having a high economic value and popularity in Turkey. Currently, it has many synonyms as 'laurel,' 'sweet bay,' 'bay leaf,' and 'bay-tree.' The laurel plant originated in Anatolia and the Balkans. It naturally grows up to 600–800 m altitudes through Turkey coastline [3]. Laurel trees are generally grown in America and Europe as an ornamental plant. Besides, it is one of the most popular plants in the Mediterranean region having an enormous variety. Its aromatic leaves are grown for commercial purposes in Turkey, Mexico, Portugal, Italy, Spain, France, Algeria, and Morocco. Both essential oils and fixed oils of laurel are used in food, cosmetics, and medicine. Laurel leaves are also extensively used in folk medicine to treat rheumatism, gastrointestinal, and urinary problems. Turkey is the largest exporter in the world at laurel trade [4]. Laurel

has a lot of usage areas in Turkey. One of them is the production of essential oils with the export of a small amount [5].

In 21st-century, crop breeding programs researching natural biodiversity as a source of novel alleles to increase adaptability, yield, health properties, and nutritional value of crops have gained considerable importance. Genetic variations identification in plants was performed by physiological, morphological, and cytological features previously. However, morphological data is inadequate in determining genetic differences due to a gene affects more than one character, and that one character recognized more than one locus. The trend towards molecular methods has increased due to the insufficiency of these data in plant systematics descriptions for taxonomy studies in recent years. Hereby molecular techniques were developed concerning DNA for genotyping [6, 7].

ISSR, one of the PCR-based marker techniques containing multi-allelic loci, can quickly show the differences between closely related individuals very informatively. This is a low-cost technique developed by Zietkiewicz et al. [8] that detecting polymorphisms in microsatellites and requires a small amount of template DNA. ISSR outputs a large number of fragments and reproducibility [9]. Primers in this technique are generally based on a repeated sequence range by degenerate 3' anchor and amplify the sequence among two microsatellites [10].

Gene targeted markers, are currently very popular in developing new markers, and generate polymorphisms from the exon, intron, or promoter regions of the genes that may be directly related to gene functions [11]. SCoT, a member of gene-targeted markers, is a simple and reliable marker system developed by Collard and Mackill [12]. Compared to other marker systems, SCoT markers provide more information on biological properties and universality in plants. They also resemble inter simple sequence repeat or random amplified polymorphic DNA but have the advantage of close linkage with the target [12]. They have many benefits compared to other markers thanks to high polymorphism and reproducibility [13].

Genetic diversity of laurel plant has been investigated in some studies. In brief, limited number of molecular characterization studies have been performed related to this subject on local laurel genotypes. However, the germplasm size or/and the number of molecular markers used previously are relatively small except for the work of Karik et al. [14]. Therefore, more extensive regional studies were requiredThere are only two studies that have been conducted with ISSR markers so far [15, 16], but the research area sizes were very small compared to this study. On the other hand, SCoT has not been studied yet. In light of previous studies, the opportunity to examine the genetic diversity in laurel plants in wider areas with different marker systems has emerged for this study. On this occasion, Turkish laurel germplasm and its current variations were identified at the molecular level in this study, and the fundamental information was established for future breeding studies by utilizing these variations.

2. Materials And Methods

2.1. Plant material

Breeding materials comprised of 94 laurel genotypes, collected from 26 Turkish provinces, provided from AARI (Aegean Agricultural Research Institute), were tested in this study (Table 1, Fig. 1).

Table 1
A list of 94 Turkish laurel genotypes used in the study.

Genotype Number	Geographical Region	Province/Genotype Name	District	Location	Altitude
G1	Mediterranean	Hatay1	Samandagi	Yogunoluk	317
G2	Mediterranean	Hatay2	Yayladagi	Yesiltepe	696
G3	Mediterranean	Hatay3	Antakya	Harbiye	188
G4	Mediterranean	Kahramanmaras	Andirin	Gokceli	971
G5	Mediterranean	Adana	Kozan	Eskimantas	222
G6	Mediterranean	Mersin1	Toroslar	Musali	570
G7	Mediterranean	Mersin2	Erdemli	Esenpinar	783
G8	Mediterranean	Mersin3	Silifke	Demircili	411
G9	Mediterranean	Mersin4	Anamur	Korucuk	116
G10	Mediterranean	Antalya1	Gazipasa	Demirtas	281
G11	Mediterranean	Antalya2	Manavgat	Sarilar	2
G12	Mediterranean	Antalya3	Aksu	Isparta road	22
G13	Mediterranean	Antalya4	Kemer	Goynuk	19
G14	Mediterranean	Antalya5	Kumluca	Kumluca	380
G15	Mediterranean	Antalya6	Demre	Gurses	377
G16	Aegean	Mugla1	Fethiye	Gokben	503
G17	Aegean	Mugla2	Ula	Portakallik	77
G18	Aegean	Mugla3	Marmaris	Marmaris	53
G19	Aegean	Mugla4	Milas	Kemerkoy	23
G20	Aegean	Aydin1	Kusadası	Dilek Yarımdası	59
G21	Aegean	Izmir1	Urla	Bademli	100
G22	Aegean	Izmir2	Karaburun	Ambarseki	163
G23	Black Sea	Giresun1	Merkez	Mezarlık	2
G24	Black Sea	Giresun2	Bulancak	Kucuklu	10
G25	Black Sea	Giresun3	Eynesil	Cavuslu	10
G26	Black Sea	Trabzon1	Çarsıbası	Buyukdere	170

Genotype Number	Geographical Region	Province/Genotype Name	District	Location	Altitude
G27	Black Sea	Rize1	Pazar	Liman	5
G28	Black Sea	Trabzon2	Yomra	Kasustu	129
G29	Black Sea	Trabzon3	Merkez	Degirmendere	22
G30	Black Sea	Ordu1	Fatsa	Bolaman	5
G31	Black Sea	Ordu2	Merkez	Altınordu	5
G32	Black Sea	Samsun1	Merkez	Barıncık	21
G33	Black Sea	Samsun2	Ondokuzmayıs	Geleric	2
G34	Black Sea	Samsun3	Bafra	Kelikler	8
G35	Black Sea	Samsun4	Alacam	Yukarielma	15
G36	Black Sea	Samsun5	Alacam	Esentepe	249
G37	Black Sea	Samsun6	Yakakent	Merkez	15
G38	Black Sea	Sinop1	Gerze	Merkez	7
G39	Black Sea	Sinop2	Erfelek	Merkez	169
G40	Black Sea	Sinop3	Merkez	Osmaniye	115
G41	Black Sea	Sinop4	Ayancık	Dervent	61
G42	Black Sea	Sinop5	Turkeli	Merkez	30
G43	Black Sea	Kastamonu1	Merkez	Ginolu	70
G44	Black Sea	Kastamonu2	Abana	Merkez	12
G45	Black Sea	Kastamonu3	Inebolu	Gemiciler	62
G46	Black Sea	Kastamonu4	Doganyurt	Kayran	233
G47	Black Sea	Kastamonu5	Cide	Aydincik	145
G48	Black Sea	Kastamonu6	Cide	Kuscu	16
G49	Black Sea	Bartin1	Kurucasile	Kapisuyu	147
G50	Black Sea	Bartin2	Amasra	Kalesah	145
G51	Black Sea	Bartin3	Merkez	Karasu	23
G52	Black Sea	Zonguldak1	Merkez	Gokgol	164
G53	Black Sea	Zonguldak2	Kilimli	Camlik	88

Genotype Number	Geographical Region	Province/Genotype Name	District	Location	Altitude
G54	Black Sea	Zonguldak3	Merkez	Kozlu	46
G55	Black Sea	Zonguldak4	Alapli	Merkez	20
G56	Black Sea	Zonguldak5	Eregli	Guluc	9
G57	Black Sea	Duzce	Akcakoca	Merkez	10
G58	Marmara	Sakarya1	Karasu	Yenimahalle	61
G59	Marmara	Sakarya2	Kaynarca	Merkez	48
G60	Marmara	Kocaeli1	Kandira	Agva road	51
G61	Marmara	Istanbul1	Agva	Kucukasagi	96
G62	Marmara	Istanbul2	Sile	Merkez	16
G63	Marmara	Istanbul3	Beykoz	Merkez	214
G64	Marmara	Kocaeli2	Golcuk	Basiskele	9
G65	Marmara	Kocaeli3	Karamursel	Kaytazdere	29
G66	Marmara	Yalova1	Merkez	Sogucak	29
G67	Marmara	Yalova2	Cınarcik	Merkez	5
G68	Marmara	Bursa1	Gemlik	Kursunlu	12
G69	Marmara	Bursa2	Mudanya	Merkez	4
G70	Marmara	Balikesir1	Erdek	Ocaklar	4
G71	Marmara	Balikesir2	Bandirma	Edincik	200
G72	Aegean	Izmir3	Selcuk	Sirince	339
G73	Aegean	Izmir4	Tire	Baskoy	345
G74	Aegean	Aydin2	Merkez	Efeler	59
G75	Aegean	Aydin3	Sultanhisar	Salavatli	74
G76	Aegean	Izmir5	Dikili	Kabakum	73
G77	Marmara	Balikesir3	Ayvalik	Merkez	77
G78	Marmara	Balikesir4	Altınoluk	Merkez	11
G79	Marmara	Canakkale1	Kucukkuyu	Mihli	7
G80	Marmara	Canakkale2	Merkez	Guzelyali	4

Genotype Number	Geographical Region	Province/Genotype Name	District	Location	Altitude
G81	Marmara	Canakkale3	Lapseki	Merkez	19
G82	Marmara	Canakkale4	Eceabat	Alcitepe	80
G83	Marmara	Tekirdag1	Merkez	Barbaros	15
G84	Marmara	Tekirdag2	Sarkoy	Gazikoy	25
G85	Marmara	Tekirdag3	Sarkoy	Murefte	11
G86	Marmara	Tekirdag4	Sarkoy	Merkez	11
G87	Marmara	Tekirdag5	Merkez	Degirmenalti	8
G88	Marmara	Istanbul4	Fatih	Gulhane	41
G89	Marmara	Istanbul5	Adalar	Buyukada	3
G90	Marmara	Istanbul6	Üsküdar	Fethipasa	87
G91	Marmara	Bursa3	Iznik	Hocakoy	185
G92	Marmara	Yalova3	Merkez	Elmalik	78
G93	Marmara	Bursa4	Mus.kemalpasa	Akcapinar	39
G94	Aegean	Izmir6	Menemen	AARI	10

2.2. DNA extraction

Young leaves of the plants were harvested and brought to the laboratory at the optimum time. DNAs isolated following the CTAB protocol [17] with some minor modifications [18]. İsolated DNA sampples were seperated on 0.8% agarose gel and concentrations were measured using NanoDrop (DeNovix DS-11 FX). The optimum level for PCR amplification was determined as 5 ng μL^{-1}. The stocks were diluted up to this concentration and kept at -25 ℃ until analyses.

2.3. ISSR and SCoT amplification

A total of 51 ISSR and 36 SCoT markers were tested with isolated DNAs of randomly selected eight genotypes for preliminary study. As a result, 16 ISSR and 10 SCoT markers (Table 2) that have high polymorphism and clean bands were selected. PCR amplifications for ISSR had 20 µL solution with 3 µL template DNA (5 ng μL^{-1}), 2 mM dNTPs (Thermo Scientific), 1.5 µL MgCl2, 1.9 µL 1XPCR buffer (Thermo Scientific), 0.75 µL primer, 0.15 µL U Taq DNA polymerase (Thermo Scientific), and 8.5 µL ddH_2O. PCR cycle consisted of 3 min denaturation at 94 ℃, 1 min of 30 denaturation cycles at 94 ℃, 1 min of annealing temperature at 48–54 ℃ depending upon the primer, 1 min of extension step at 72 ℃, and 10 minutes of final extension at 72 ℃, and for SCoT followings were different; 2.5 µL MgCl2, 2 µL 1XPCR buffer (Thermo Scientific), 1.5 µL primer, 0.15 µL U Taq polymerase (Thermo Scientific), and 7.8 µL

ddH^2O. Reactions cycle consisted of 5 min denaturation at 94 ℃, 1 min of 35 denaturation cycles at 94 ℃, 1 min of annealing temperature at 50 ℃, 2 min of extension step at 72 ℃, and 8 minutes of final extension at 72 ℃, using BIO-RAD T100 Thermal Cycler.

Table 2
Annealing temperatures, and sequences of 16 ISSR and 10 ScoT markers that were used to explore the genetic diversity among Turkish laurel germplasm.

Primer	Sequence (5'-3')	Annealing Temp. (°C)
UBC807	AGA GAG AGA GAG AGA GT	50
UBC809	AGA GAG AGA GAG AGA GG	52
UBC810	GAG AGA GAG AGA GAG AT	50
UBC812	GAG AGA GAG AGA GAG AA	50
UBC813	CTC TCT CTC TCT CTC TT	50
UBC814	CTC TCT CTC TCT CTC TA	50
UBC815	CTC TCT CTC TCT CTC TG	50
UBC816	CAC ACA CAC ACA CAC AT	50
UBC817	CAC ACA CAC ACA CAC AA	50
UBC819	GTG TGT GTG TGT GTG TA	50
UBC825	ACA CAC ACA CAC ACA CT	50
UBC834	AGA GAG AGA GAG AGA GYT	52
UBC847	CAC ACA CAC ACA CAC ARC	52
UBC888	BDB CAC ACA CAC ACA CA	51
UBC890	VHV GTG TGT GTG TGT GT	51
UBC891	HVH TGT GTG TGT GTG TG	51
SCoT-1	CAA CAA TGG CTA CCA CCA	50
SCoT-2	CAA CAA TGG CTA CCA CCC	50
SCoT-3	CAA CAA TGG CTA CCA CCG	50
SCoT-4	CAA CAA TGG CTA CCA CCT	50
SCoT-5	CAA CAA TGG CTA CCA CGA	50
SCoT-6	CAA CAA TGG CTA CCA CGC	50
SCoT-7	CAA CAA TGG CTA CCA CGG	50
SCoT-8	CAA CAA TGG CTA CCA CGT	50
SCoT-10	CAA CAA TGG CTA CCA GCC	50
SCoT-11	AAG CAA TGG CTA CCA CCA	50

The amplified DNA products obtained were loaded onto a 2% agarose gel and separated in 4 hours (for ISSR) and 5 hours (for SCoT) at 120 V. After the gels were removed from the electrophoresis system and put into ethidium bromide for 5 minutes and passed through pure water. At the end of the process, the gels were imaged under UV and recorded for analysis.

2.4. Data Analysis

Clear, unambiguous, and strong bands were selected for scoring. The binary system is used by scanning as 1 and 0 according to the presence or absence of specific bands, among the markers according to 100 bp + DNA ladder. Polymorphism information content (PIC) was determined according to the method of Baloch et al. [19] for ISSR and SCoT. PopGene ver. 1.32 [20] was used to predict various important genetic diversity parameters like; gene diversity (H), Shannon's Information Index (I), effective alleles number (Ne), overall gene diversity (Ht), and Nei's genetic distance (GD). This study's genotypes were supplied from 4 geographical regions (Aegean, Mediterranean, Black Sea, Marmara) in Turkey. For this reason, some diversity values calculated on a regional basis using PopGene ver. 1.32 [20]. AMOVA (Analysis of molecular variance) and UPGMA (The unweighted pair group method with arithmetic mean) were performed using R software [21] to explore the level of diversity among laurel germplasm. STRUCTURE software (version 2.3.4, Stanford, CA, USA) was used to reveal the population structure of genotypes with the Bayesian clustering model's help. The cluster numbers (the number of K; the number of subpopulations) were determined following the protocol of Evanno et al. [22] was plotted and followed the K (number of clusters) against logarithm possibility relative to the standard deviation (ΔK). A total of 10 independent runs were set for each K value, and each run, the beginning burn-in period was set to 5000 with 10000 MCMC (Markov chain Monte Carlo) iterations. Correlation analysis was performed by XLSTAT [23] to determine whether a linear relationship between Nei's genetic distances of ISSR and SCoT.

3. Results

3.1. ISSR Genetic Diversity

A total of 373 clean and scorable bands were identified, with an average of 23.31 per primer. Among the scoreable bands, 348 (%93.3 of total) were polymorphic, with an average of 21.75 per primer. Primer UBC890 had the highest number of bands (46) and the highest number of polymorphic bands (42). The least scored (13) primers were UBC812 and UBC825. The average PIC (Polymorphism information content) was 0.704, and while the highest PIC was obtained from UBC825 with 0.795, the lowest PIC was obtained from UBC810 with 0.539. The mean, the highest, and the lowest NE (Effective alleles number) were 1.524, 1.416 (UBC888), and 1.728 (UBC814), respectively. The mean genetic diversity (H) was 0.308, the highest was 0.388 (UBC814), and the lowest was 0.249 (UBC888). The mean Shannon Information Index (I) was 0.464, the highest was 0.557 (UBC814), and the lowest was 0.380 (UBC888). Overall gene diversity (HT) values were between 0.064 (UBC815) and 0.285 (UBC810), with an average of 0.185 (Table 3). Nei's genetic distances were calculated to evaluate genetic diversity, with paired genetic distance measurement in 94 laurel genotypes. The mean genetic distance between the genotypes was

0.39. The highest genetic distance (0.70) was between Antalya1 and Zonguldak5. The lowest genetic distance (0.17) was between Sinop5 and Zongudak5 (Fig. 4).

3.2. SCoT Genetic Diversity

A total of 227 clean and scorable bands were identified, with an average of 22.7 per primer. Among the 227 scorable bands, 175 (%77.1 of total) were polymorphic, with an average of 17.5 polymorphic bands per primer. Primer SCoT-2 had the highest number of bands (34). The least scored (16) primers were SCoT-7, SCoT8, SCoT10, and SCoT11. Primer SCOT-1 had the highest polymorphic bands (28). The average PIC was 0.454, the highest PIC was obtained from SCoT-5 with 0.629, and the lowest PIC was obtained from SCoT-8 with 0.154. The mean, the highest, and the lowest NE were 1.383, 1.132 (SCoT-8), and 1.522 (SCoT-6/SCoT-11). The mean H was 0.230, the highest was 0.306 (SCoT-10), and the lowest was 0.086 (SCoT-8). The mean I was 0.352, the highest was 0.458 (SCoT-6), and the lowest was 0.113 (SCoT-8). HT values were between 0.082 (SCoT-8) and 0.241 (SCoT-1) with an average value of 0.177 (Table 3). The mean genetic distance between genotypes was 0.26. The highest genetic distance (0.51) was detected between Antalya5 and Kastamonu2. The lowest genetic distance (0.12) was between Bartin2 and Istanbul2 (Fig. 5).

Table 3
Genetic diversity parameters were identified to evaluate 94 laurel genotypes using 16 ISSR and 10 SCoT primers.

Primer	Name	TAB	NPB	PPB (%)	PIC	NE	H	I	HT
ISSR	UBC807	29	27	93.10	0.722	1.573	0.333	0.495	0.187
	UBC809	18	17	94.44	0.714	1.471	0.279	0.426	0.168
	UBC810	18	16	88.89	0.539	1.568	0.313	0.457	0.285
	UBC812	13	12	92.31	0.722	1.497	0.297	0.450	0.204
	UBC813	14	13	92.86	0.574	1.496	0.286	0.431	0.241
	UBC814	17	16	94.12	0.767	1.728	0.388	0.557	0.234
	UBC815	18	17	94.44	0.730	1.509	0.302	0.458	0.064
	UBC816	25	25	100	0.756	1.470	0.292	0.454	0.250
	UBC817	21	21	100	0.715	1.578	0.340	0.510	0.169
	UBC819	10	9	90	0.678	1.536	0.320	0.481	0.183
	UBC825	13	13	100	0.795	1.499	0.306	0.471	0.038
	UBC834	16	16	100	0.661	1.496	0.310	0.473	0.113
	UBC847	34	33	97.06	0.763	1.444	0.273	0.423	0.157
	UBC888	43	35	81.40	0.676	1.416	0.249	0.380	0.181
	UBC890	46	42	91.30	0.698	1.495	0.293	0.445	0.270
	UBC891	38	36	94.74	0.753	1.613	0.351	0.519	0.214
	Mean	23.31	21.75	94.04	0.704	1.524	0.308	0.464	0.185
	Total	373	348						
SCoT	SCoT-1	32	28	87.50	0.478	1.497	0.289	0.434	0.241
	SCoT-2	34	27	79.41	0.518	1.388	0.224	0.342	0.208
	SCoT-3	23	18	78.26	0.432	1.376	0.220	0.335	0.168
	SCoT-4	28	15	53.57	0.389	1.281	0.172	0.263	0.166
	SCoT-5	22	21	95.24	0.629	1.420	0.270	0.425	0.227
	SCoT-6	24	22	91.67	0.640	1.522	0.304	0.458	0.222

TAB, Total amplified bands **NPB**, Number of polymorphic bands **PPB**, Percentage of polymorphic bands **NE**, Effective alleles number **PIC**, Polymorphism information content **H**, Gene diversity **I**, Shannon's information index **HT**, Overall gene diversity

Primer	Name	TAB	NPB	PPB (%)	PIC	NE	H	I	HT
	SCoT-7	16	13	81.25	0.463	1.400	0.247	0.381	0.161
	SCoT-8	16	5	31.25	0.154	1.132	0.086	0.137	0.082
	SCoT-10	16	14	87.50	0.527	1.522	0.306	0.457	0.161
	SCoT-11	16	12	75	0.307	1.290	0.181	0.286	0.140
	Mean	22.70	17.50	76.07	0.454	1.383	0.230	0.352	0.177
	Total	227	175						

TAB, Total amplified bands **NPB**, Number of polymorphic bands **PPB**, Percentage of polymorphic bands **NE**, Effective alleles number **PIC**, Polymorphism information content **H**, Gene diversity **I**, Shannon's information index **HT**, Overall gene diversity

3.3. Comparative analyses of ISSR and SCoT results

STRUCTURE software divided 94 laurel genotypes into two main populations by the Bayesian clustering model according to ISSR and SCoT data (Fig. 2). Populations were classified according to their existence in 4 geographical regions (Black Sea Region, Marmara Region, Aegean Region, and Mediterranean Region) to detect better analyze the variation. From this point, to understand the population structure more clearly, laurel germplasm was divided into two populations according to the Bayesian-based clustering algorithm. Population A included mostly genotypes from the Mediterranean region. Population B had mostly genotypes from the Aegean and Marmara regions. A high rate of the Black Sea region's genotypes were present in population A (Fig. 3).

Although few differences were detected between ISSR and SCoT, a high level of consistency was found between population structures (Fig. 2, Fig. 3).

As a result of AMOVA, the variation within the population was higher than the variation among populations. The majority of genetic variations occurred as 96% in the population sets in AMOVA for both markers (Table 4).

Table 4
Analysis of molecular variance to determine the genetic diversity of Turkish Laurel genotypes.

ISSR Source	Df	SS	MS	Est. Var.	%
Among Pops	3	35.848	11.949	0.271	4
Within Pops	90	533.724	5.930	5.930	96
Total	93	569.573		6.202	100
SCoT Source	**Df**	**SS**	**MS**	**Est. Var.**	**%**
Among Pops	3	21.963	7.321	0.157	4
Within Pops	90	346.023	3.845	3.845	96
Total	93	367.986		4.001	100

Df, Degrees of freedom **SS**, Sum of squares **MS**, Mean square **Est. Var.**, Estimated variance

Genetic diversity was calculated according to the collection regions (Mediterranean, Aegean, Marmara, and Black Sea) of 94 laurel genotypes. Genotypes collected from the Black Sea region showed a higher number of gene diversity (0.28), effective alleles (1.49), expected heterozygosity (0.20), and Shannon's information index (0.43) according to ISSR analysis. Besides, genotypes of Mediterranean showed higher values except for Ht in SCoT analysis (Table 5)

Table 5
A geographical region based diversity parameters assessment for Turkish Laurel germplasm.

Primer	Region	NE	H	I	HT	GD
	Mediterranean	1.41	0.24	0.37	0.14	0.37
	Aegean	1.45	0.26	0.40	0.19	0.40
ISSR	Black Sea	1.49	0.28	0.43	0.20	0.39
	Marmara	1.46	0.27	0.41	0.17	0.38
	Mediterranean	1.38	0.23	0.35	0.13	0.30
	Aegean	1.34	0.21	0.31	0.12	0.25
SCoT	Black Sea	1.35	0.22	0.33	0.18	0.26
	Marmara	1.33	0.20	0.30	0.16	0.24

NE, Effective alleles number **I**, Shannon's information index **H**, Gene diversity **HT**, Overall gene diversity **GD**, Nei's genetic distance

The neighbor-joining clustering analysis conducted to determine relationships among 94 genotypes was given in Fig. 4 and Fig. 5. The distribution of laurel genotypes with PCoA on biplot according to geographical regions was shown in Fig. 6.

The linear relationship of ISSR and SCoT in Nei's genetic distances was calculated as an average of 0.881, a very high correlation ratio. The result meant a very high correlation between the two analyzes (ISSR and SCoT) in the entire data set.

4. Discussion

4.1. Determining polymorphism status of Turkish Laurel Germplasm using 16 ISSR and 10 SCoT markers

A total of 16 ISSR and 10 SCoT markers were used to determine the genetic diversity of the Turkish laurel germplasm (Table 3). Total and polymorphic band numbers were higher than Karik et al.'s [14] iPBS-Retrotranspozon (195/164), Sevindik's [16] ISSR (46/22), and Arroyo-Garcia et al.'s [24] AFLP (167/100) studies on laurel. Also, Mohamed et al. [15] worked widely on the *Lauraceae* family using ISSR markers (209/165), and findings were lesser than ours. The polymorphism mean in this study was 94,04% for ISSR markers and 76.07% for SCoT markers. The rate of ISSR was higher than reported by Karik et al.'s [14] 83.98% and Sevindik [16]'s 49.2%. From this perspective, ISSR markers obtained larger variations in the Turkish laurel germplasm compared to other marker systems. PIC, which is the value for the exploration of discriminating power marker among genotypes [25], and efficiency of polymorphic loci [26], was the mean of 0.704 in ISSR and 0.454 in ScoT. These values were higher than reported by Karik et al.'s [14] PIC value (0.361) in laurel accessions. When the marker systems were compared, ISSR and ScoT had a higher value than iPBS-Retrotrasnposon in terms of PIC in Turkish laurel germplasm. Additionally, PIC values in some previous ISSR and SCoT studies, 0.35 in rice with ISSR [27], 0.35 in safflower with SCoT [28], 0.32 in sesame with ISSR [29], in salvia 0.38 and 0.40 with ISSR and SCoT respectively [30], 0.30 in olive with ISSR [31], and 0.448 in safflower with ISSR [32] didn't exceed the results of this study. Besides, Agarwal et al.'s [33] PIC value (0.79) of SCoT exceeded the PIC of SCoT in this study. In another study with both marker systems (ISSR and SCoT), Igwe et al.'s [34] PIC values in *Vigna unguiculata* L. (Walp) accessions were higher than the PIC values of this study. In comparison to previous studies, the findings of this study mostly had higher PIC values. This information proves the discriminative power between genotypes and the efficiency of polymorphic loci.

Many studies have proven ISSR and SCoT to be strong, reliable, and reproducible markers [28, 30, 32, 33]. Also, to test the DNA profiles' reproducibility and reliability for both marker systems, the PCR process was repeated in both primers. The same number of polymorphic bands were detected as a result. For this reason, both marker systems were extremely reproducible and reliable at the point of diagnosis.

4.2. Genetic diversity status of Turkish Laurel Germplasm using 16 ISSR and 10 SCoT markers

Local genotypes underwent genetic changes over time due to biotic and abiotic factors, and they have been the main source of agricultural production for centuries [35]. Therefore, it is important to collect and preserve local genetic resources and to evaluate them appropriately. Diagnosing of laurel genetic sources

at the molecular level is important in terms of laurel breeding activities to be carried out in Turkey. Turkish laurel germplasm's genetic diversity identified using ISSR and SCoT markers (Table 3). The maximum number of effective alleles is always an indispensable parameter for detecting genetic variation since it reveals more genetic variation. The average number of effective alleles in this study was 1.524 in ISSR, and 1.383 in SCoT, which was higher than Agarwal et al. [36] (1.364) with ISSR, Xu et al. [37] (1.372) with ISSR, and Gogoi et al. [38] (1.33 and 1.19) with SCoT and ISSR respectively. In another study, Dilipan et al. [39] obtained a 1.44 Ne value from SCoT and exceeded ours. Mean gene diversity results (ISSR:0.308; SCoT:0.230) higher than reported in other studies [40–43], and the germplasm in this study appears to have higher diversity. Shannon's information index (I) is a crucial parameter for understanding genetic diversity variation by integrating equality and abundance [44]. The mean Shannon information indexes in this study (ISSR: 0.464; SCoT:0.352) were also higher than some previous studies [38, 40, 45]

Genetic distances were calculated among 94 Turkish laurel genotypes. According to ISSR results, Nei's genetic distances ranged between 0.17 and 0.70, and the mean was 0.39. The highest genetic distance was between Zonguldak5 and Antalya1, with a value of 0.70. Genotypes with the closest genetic distance with a value of 0.10 were between Sinop5 and Zonguldak5. In the SCoT analysis, Nei's genetic distances ranged between 0.12 and 0.51, and the mean value was 0.26. Kastamonu2 and Antalya5 had the highest genetic distance value (0.51). Istanbul2 and Bartin2 had the lowest one (0.12). The most important information obtained from genetic distances in testing plant breeding methods is selecting genotypes with the furthest distance from each other since the gene pool is desired as large as possible. In this perspective, according to ISSR results, Zonguldak5 and Antalya1 genotypes, according to the SCoT results, Kastamonu2 and Antalya5 genotypes, can be the most recommended genotypes as parents for laurel breeding. Various diversity parameters were also calculated according to the geographical regions where the genotypes were collected to elucidate the genetic variations in more detail (Table 5). According to ISSR results, The Black Sea region and according to the SCoT results, the Mediterranean region showed a higher level of diversity than other regions. These regions' common feature is that they are superior to the other two regions in geographical distances between genotypes. On this occasion, it clearly seems that expanding the geographical boundaries will expand the gene pool gathered.

According to AMOVA, higher variations were identified within the laurel genotypes as 96% (in both analyses). Higher variations within the genotypes may be due to genetic drift, pollination, gene flow, adaptation, and ecotypes variation [46]. Also, environmental transformations and human activities may be responsible for this situation [47]. Neighbor clustering analysis aims to group genotypes according to their geographical regions and provinces, was performed for 94 Turkish laurel germplasm. Apart from the Aegean region, the same regions' genotypes were generally together. In Turkey, laurel plants are native to Hatay, Mersin, Adana, and Kahramanmaras provinces (Mediterranean Region) [4]. Over time, with the farmers' attraction to this plant, it spread to Marmara and Black Sea regions. The mixture in this region is very high since it passes from all regions to the Aegean region after these regions. Genotypes were divided into geographical regions where they collected with PCoA (Fig. 6). The conflict regarding the Aegean region's laurel gene pool was proved by this study, which gave results to the degree that supports this hypothesis. Aegean region genotypes appeared as mixture of 3 regions' genotypes, supporting the

gene flow hypothesis. According to the model-based structure algorithm, genotypes were divided into two population groups to their provinces and corresponding geographical regions (Fig. 2). Genetic distances of 94 laurel genotypes in 4 geographical regions were separated as Population A and B. Population A contained mostly genotypes of the Mediterranean region except for three genotypes. Population B contained mainly genotypes from the Aegean and Marmara regions. A high rate of the Black Sea's genotypes were present in population A (Fig. 3). Gene escapes were observed between these two populations, albeit in small amounts, due to the possibility of seed changes and some crossbreeding.

The correlation coefficient was 0.881 (r) in the correlation analysis performed to check whether there is a linear relationship between both data sets (ISSR and SCoT) according to Nei's genetic distances. In the molecular characterization study conducted by Etminan et al. [30] on Salvia species using 22 ISSR and 20 SCoT primers, the correlation coefficient was 0.83 (r). Compared to the mentioned study, our correlation coefficient was higher, and there was a high consistency between ISSR and SCoT in our study.

One of the most important steps towards breeding activities is genetic diversity assessment, as it provides a new source of variation [48]. The genetic diversity parameters obtained with this study will create an excellent database for laurel breeding.

5. Conclusions

Determining the degree and distribution of genetic diversity is crucial for future studies of the breeding material to be improved. In this study, ISSR and SCoT markers, which are very effective markers to detect laurel plants' genetic diversity, have been tried. As previously mentioned, no studies were conducted with the SCoT markers on the laurel. On the other hand, this large-scale study in the laurel plant will provide more information to breeders than the other studies made in small regions.

According to genetic distance values, ISSR study revealed Zonguldak5 and Antalya1 genotypes, SCoT study revealed Kastamonu2 and Antalya5 genotypes to be the most recommended genotypes as parents for laurel breeding. A remarkable genetic diversity was achieved among 94 laurel populations. In general, there was nearly no difference between the two marker systems in evaluating genetic distances of the laurel populations. However, when 94 laurel genotypes are assessed in terms of all genetic diversity parameters (Total amplified bands, Number of polymorphic bands, Percentage of polymorphic bands, Polymorphism information content, Effective alleles number, Overall gene diversity, Shannon's information index, Gene diversity), the ISSR marker system resulted in more informative. Besides, since the SCoT markers are generated from the genome's functional region, it will be more useful to use these markers in QTL mapping, construction of linkage maps, considering genetic diversity and genotype identification. Because of the high genetic diversity and adequate geographic boundaries of the study area, after this study, the real aim is to develop new varieties to obtain more information about genetic diversity and useful breeding studies.

Declarations

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Writing – review & editing: Vahdettin Ciftci

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

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Figures



Figure 1

The locations of 94 laurel genotypes used in the study Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

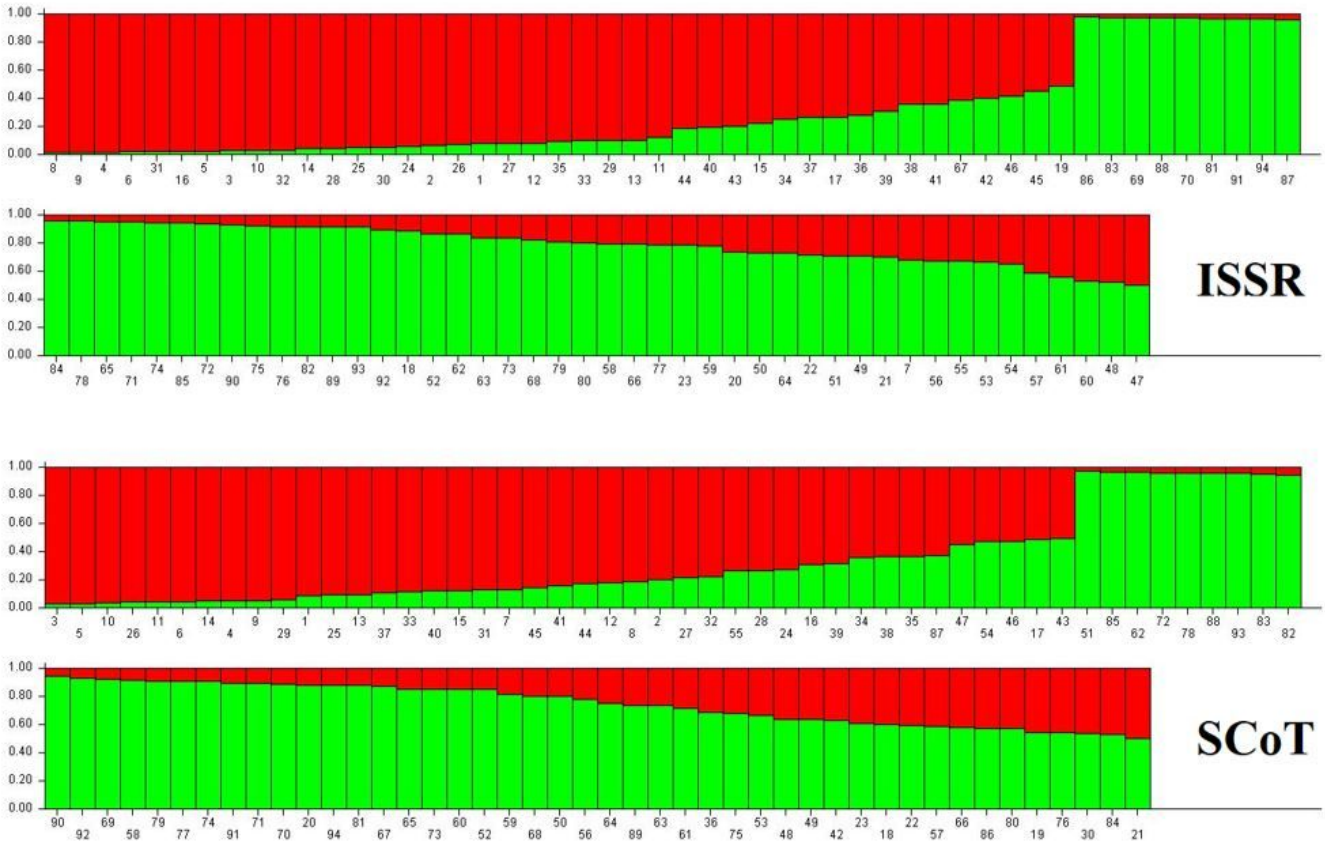


Figure 2

Structure-based clustering of 94 laurel genotypes using 16 ISSR and 10 SCoT markers.

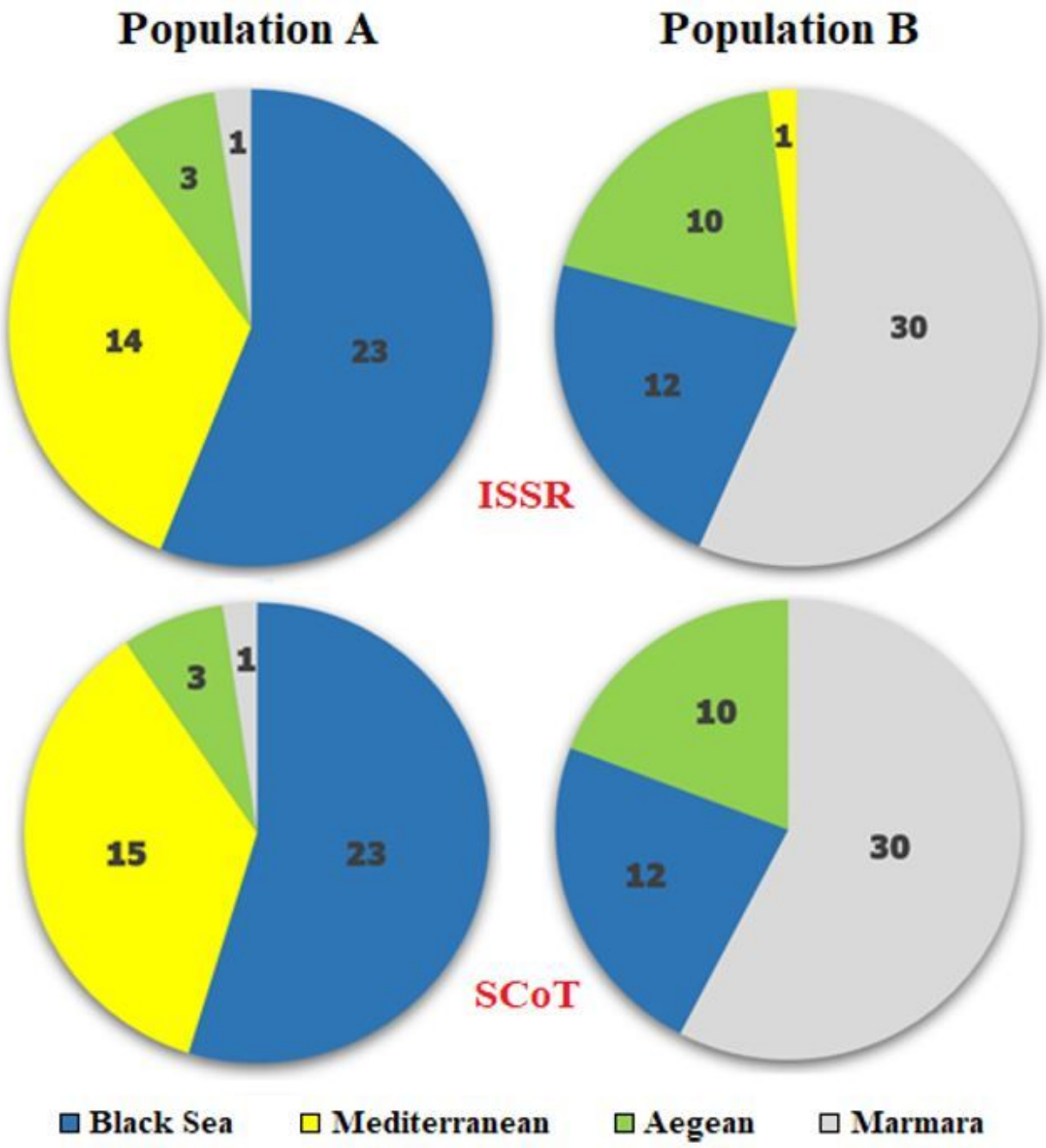


Figure 3

Separating 94 laurel genotypes to structure-based clustering using 16 ISSR and 10 SCoT markers.

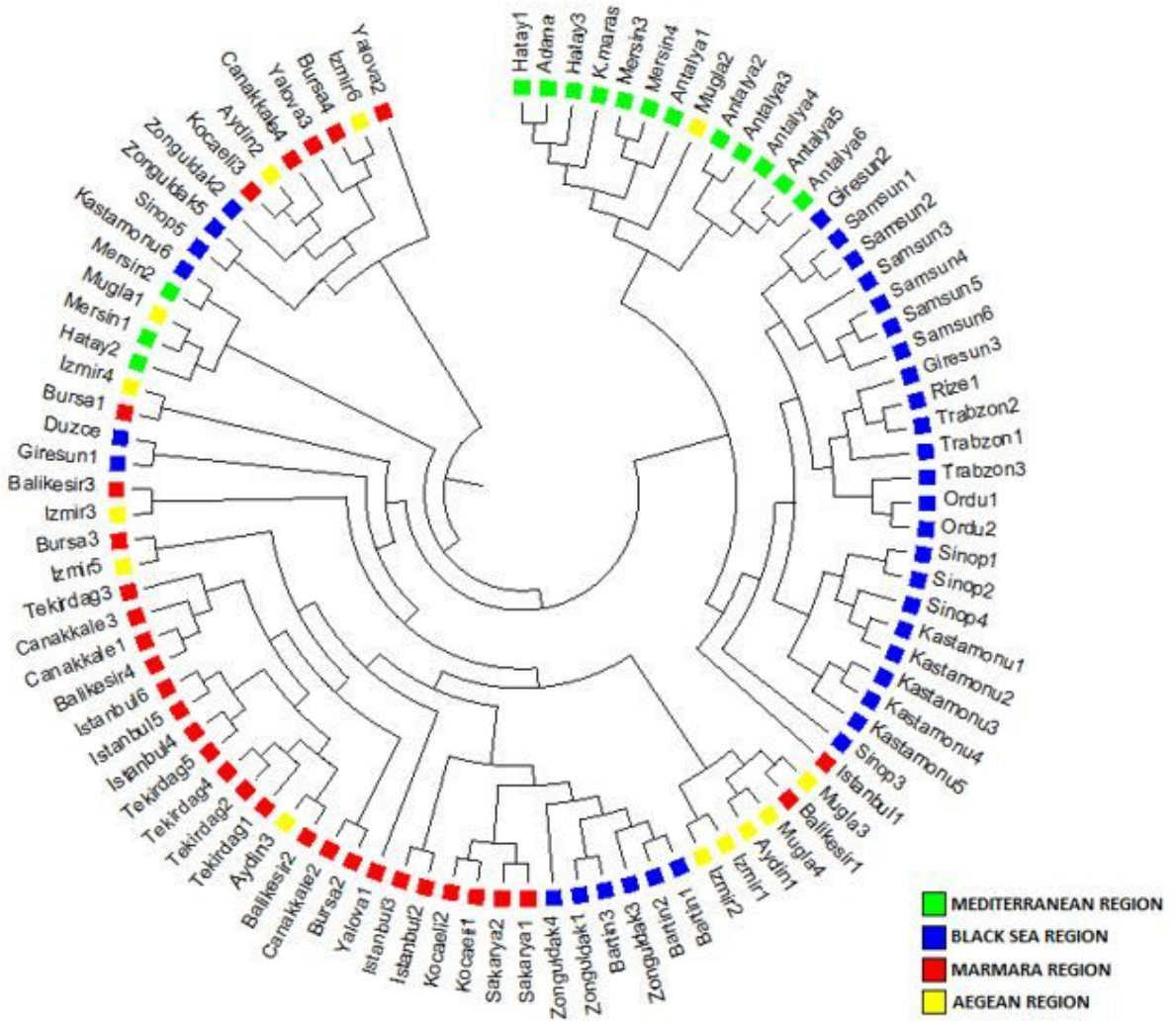


Figure 4

UPGMA based clustering of 94 laurel genotypes using 16 ISSR markers.

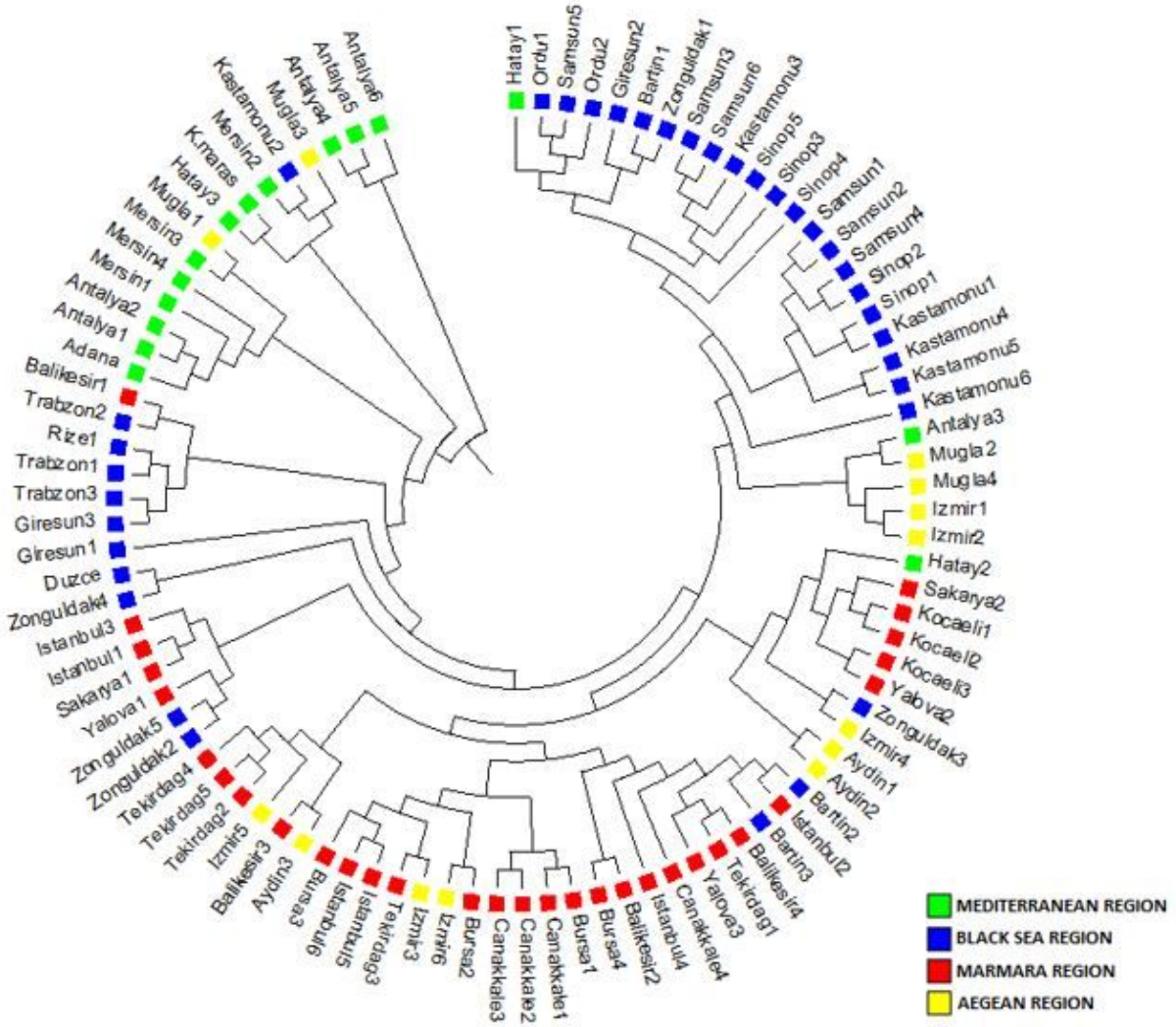


Figure 5

UPGMA based clustering of 94 laurel genotypes using 10 SCoT markers.

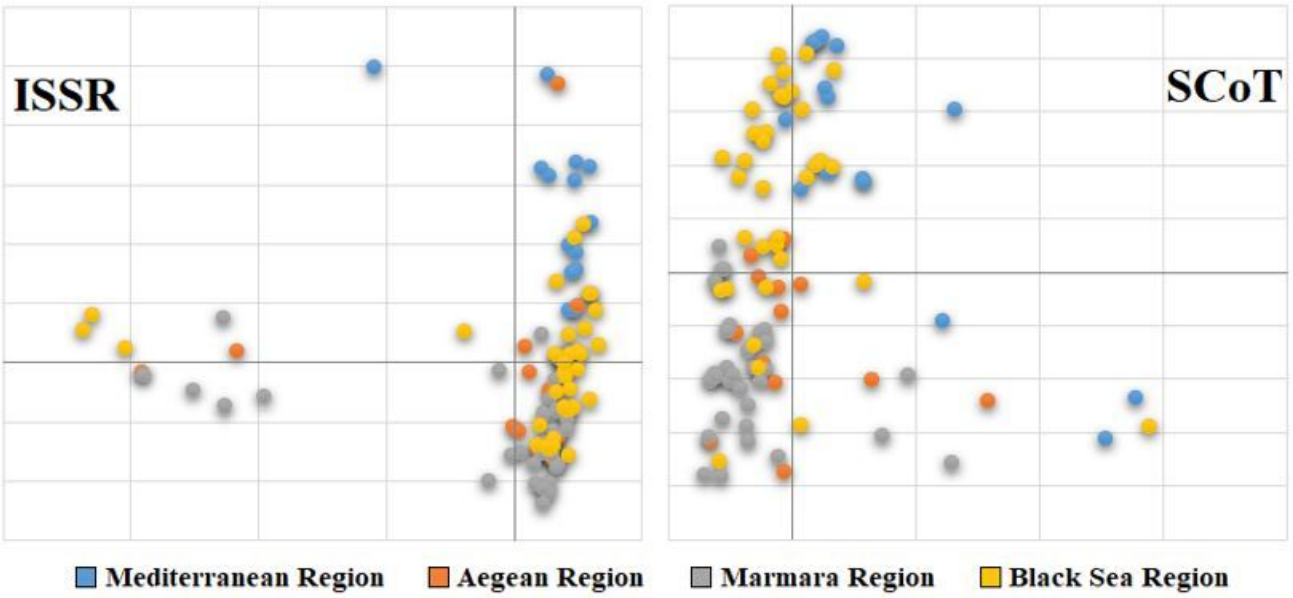


Figure 6

Principal coordinate analysis of Turkish laurel germplasm using 16 ISSR and 10 SCoT markers.