Determination of genetic relationships among some cherry laurel (*Laurocerasus officinalis* Roem.) genotypes by using RAPD markers

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Abstract

A few standard fruit species such as hazelnut, chestnut and kiwifruit dominate the fruit production in Black Sea region of Turkey, while many other species for example cherry laurel (Laurocerasus officinalis Roem.) both in natural ecosystems and in rural areas could be exploited for potential new foods, valuable natural compounds and derivatives. In this study twenty-two cherry laurel genotypes, previously selected from Black Sea region in Turkey were evaluated for molecular characteristics. 63 RAPD primers were used for PCR reactions, among which 17 showed reliable polymorphic patterns. The 17 random primers generated a total of 106 RAPD bands. The similarity matrix showed that the highest genetic similarity (0.117) was between 14K03 and 54K04 and the least (0.995) was between 52K01 and 28K08 genotypes. According to the results, RAPD analysis can be used for the characterization and grouping of cherry laurel genotypes.

Keywords: Cherry Laurel, *Laurocerasus officinalis* Roem, RAPD

Introduction

The Black Sea Region of Turkey is one of the major genetic diversity centers of cherry laurel (*Laurocerasus officinalis* Roem.) and most of the cherry laurel growing in this area has arisen from seeds [1]. Cherry laurel (*Laurocerasus officinalis* Roem.) is a popular fruit (dark purple or black when mature), mainly distributed in the coasts of Black Sea region of Turkey and locally called “Taflan” or “Karayemis.” [2]. In Turkey, annual production of cherry laurel is not known because of its consumption as fresh fruit is limited use only for local markets (less used by industry at present). Besides fresh consumption, dried, pickled, and processed (into pekmez, jam, marmalade, and fruit juice) cherry laurel products are also consumed. Apart from their use for food, both fruits and seeds of cherry laurel have been used for a long time in Turkey for the treatment of stomach ulcer, digestive system complaints, bronchitis, eczemas and hemorrhoids and as a diuretic agent [3].

Turkey has a long history of cherry laurel cultivation and a wealth of cherry laurel genotypes. The cherry laurel is becoming a more popular crop in Turkey because of recent advertisement on bioactive compounds and health effects of its fruits [2]. The country has a rich gene pool of cherry laurel genotypes adapted to different local conditions in black sea region. Most trees are open pollinated seedlings of wild genotypes which vary widely in terms of productivity and fruit characteristics, such as size, shape, color, flavor and nutritional value [1]. Despite its wide usage in the Black Sea region, there have been no standard cultivars as the case for the other fruit species [1].

Earlier classifications and evaluations of the genus cherry laurel (*Laurocerasus officinalis*.) were done primarily based on phenotypic expressions of the plants such as color, shape and other agronomical characters of fruits in Turkey [4,5]. However, information from these environmentally influenced morphological and phenotypic characteristics are not sufficient to identify cherry laurel genotypes because the differences between them are often subtle and misleading. Hence, robust and environmentally little influenced genotypic traits are to be used for proper identification and estimation of genetic diversity among these genotypes.

DNA markers are independent from environmental interactions, unlimited in number and show high level of polymorphism. Therefore, they are considered invaluable tools for determining both genetic relationships and diversity. Various types of DNA markers such as RAPDs, RFLPs, SSRs, AFLPs, ISSRs are now available. Among them RAPDs gained importance due to their simplicity, efficiency, the relative ease to

perform the assay and non-requirement of DNA sequence information [6]. They have been used in studies of genetic diversity [7], phylogeny and systematics [8, 9], genetic linkage mapping [10], and gene tagging [11].

Limited studies on the genetic relationships and diversity in cherry laurel have been published. Sandalli et al. [12] reported genetic diversity within only four cherry laurel types in Turkey by using RAPD primers. However, the genetic relationships of the selected cherry laurel collection in Turkey remain unknown. The germplasm collection from Turkey shows many promising agronomic traits, including high yield capacity, pest and disease resistance, promising fruit characteristics etc. Crosses between them may lead to improved new cherry laurel varieties. In this study, we report relationships of morphological traits and molecular characteristics of 22 cherry laurel genotypes from Turkey.

Materials and methods

Sample collection

The leaf samples from twenty-two selected cherry laurel (*Laurocerasus officinalis* Roem.) genotypes were found in germplasm collection in Black Sea Agricultural Research Institute, Samsun-Turkey were collected. Twenty leaves from the top of 90-day-old primary branches of five plants per genotype were collected separately and stored immediately at –80°C for DNA extraction.

DNA extraction

Genomic DNA was extracted from powdered leaf materials using a modified method described by Lin et al. [12]. The purity and quantity of genomic DNA was determined spectrophotometrically and confirmed using 0.8% agarose gel electrophoresis against known concentrations of unrestricted lambda DNA.

RAPDs

Sixty-three (63) primers (Operon Technologies Inc., Alameda, CA, USA) had been used to generate RAPD profiles. PCR amplification reactions were carried out in thirty µl final volume of reaction mixture containing 10x Buffer 3.0 µl, dNTPs (10mM) 1.2 µl, magnesium chloride (25mM) 1.2 µl, primer (5µM) 2.0 µl, *Taq* polymerase (5unit) 0.4 µl, water 19.2 µl sample DNA 3.0 µl (100ng/ µl). The thermalcycler (Eppendorf Company) was programmed as 2 min at 95°C; 2 cycles of 30 sec at 95°C, 1 minute at 37°C, 2 minute at 72°C; 2 cycles of 30 sec at 95°C, 1 minute at 35°C, 2 minutes at 72°C; 41 cycles of 30 sec at 94°C, 1 minute at 35°C, 2 minute at 72°C; followed by a final 5 minute extension at 72°C then brought down to 4^{o}C.

Electrophoresis

The PCR products (27 µl) were mixed with 6x gel loading buffer (3 µl) and loaded onto an agarose (1.5% w/v) gel electrophoresis in 0.5XTBE (Tris-Borate- EDTA) buffer at 70 V for 150 min. The gel was stained in ethidium bromide solution (2 µl Etbr/100ml 1xTBE buffer) for 40 min and visualized under UV in Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK).

Data Analysis

Each gel was analyzed by scoring the present (1) or absent (0) polymorphic bands in individual lanes. The NTSYS-pc software ver. 2.02 [14] was used to estimate genetic similarities with the Jaccard's coefficient. The matrix of generated similarities was analyzed by the unweighted pairgroup method with arithmetic average (UPGMA), using the SAHN clustering module. The cophenetic module was applied to compute a cophenetic value matrix using the UPGMA matrix. The MXCOMP module was then used to compute the cophenetic correlation, i.e., to test the goodness of fit of the cluster analysis to the similarity matrix.

Results and discussion

Results of RAPD analysis are summarized in Table 1. A total of 63 decamer oligonucleotide primers were used to investigate 22 cherry laurel genotypes all belongs to *Laurocerasus officinalis.* In total, 17 out of 63 primers produced good and reproducible polymorphic bands among the 22 cherry laurel genotypes were used for further analysis. The 17 random primers generated a total of 106 RAPD bands and among them 8 fragments were monomorphic and 98 were polymorphic (Table 1).

Table 1. List of the selected primers and the degree description of the polymorphism obtained among 22 cherry laurel genotypes

Primer code	Sequence 5'→3'	Size (bp) Min-max	Polymorphic Bands	Monomorphic Bands	Total	P(%)

OPA- 2	TGCCGAGCTG	500- 1100	7	0	7	100
OPA- 4	AATCGGGCTG	800- 1250	11	0	11	100
OPA- 13	CAGCACCCAC	700- 1000	3	1	4	75.0
OPB- 10	CTGCTGGGAC	300- 700	4	0	4	100
OPH- 17	CACTCTCCTC	700- 1800	10	1	11	90.9
OPH- 19	CTGACCAGCC	550- 750	3	1	4	75.0
OPW- 1	CTCAGTGTCC	500-	1	0	1	100
OPW- 6	AGGCCCGATG	450- 1100	8	1	9	88.8
OPW- 13	CACAGCGACA	500- 1250	4	0	4	100
OPW- 18	TTCAGGGCAC	750- 1050	5	1	6	83.3
OPW- 20	TGTGGCAGCA	600- 1500	6	1	7	85.7
OPY- 1	GTGGCATCTC	450- 1100	8	1	9	88.8
OPY- 6	AAGGCTCACC	900- 1500	8	0	8	100
OPY- 7	AGAGCCGTCA	600- 650	2	0	0	100
OPY- 13	GGGTCTCGGT	500- 800	4	1	5	80
OPY- 15	AGTCGCCCTT	500- 1100	9	0	9	100
OPY- 16	GGGCCAATGT	450- 1000	5	0	5	100
Total		300- 1800	98	8	106	92.45

The highest number of polymorphism (100.0%) was observed with primer OPA2, OPA4, OPB10, OPW1, OPW13, OPY6, OPY7, OPY15 and OPY16 while the lowest (75.0%) was observed with OPA13 and OPH19 primers, respectively (Table 1). The size of amplified fragments ranged between 300 and 1800 bp for all primers. The similarity matrix showed that the highest genetic similarity (0.117) was between 14K03 and 54K04 and the least (0.995) was between 52K01 and 28K08. This result indicates that the genotypes 14K03 and 54K04 are genetically closer than the other genotypes. Similarly, the lowest genetic similarity between 52K01 and 28K08 points to the possibility of utilizing them for heterosis breeding. The average genetic similarity among the genotypes was 0.539, which clearly shows significant genetic diversity among the selected genotypes. Hence, these genotypes are to be preserved as valuable genetic resources for breeding. Earlier studies using RAPD in different fruit species such as mulberry [9], sea buckthorn [15] and grape [16] techniques showed large genetic variations present among different cultivars.

The dendrogram realized from the RAPD markers grouped the genotypes into four clusters, containing 17, 2, 1 and 2 accessions, respectively (Figure 1). Cluster 1 also divided 2 subclusters. These high genetic distances present among these genotypes clearly suggest that they may have originated from genetically divergent parents or have a long history of adaptation to their respective micro-climatic regions. Moreover, continuous seed propagation for thousands of years could result the occurrence of a diversity of cherry laurel genotypes. This genetic diversity is an important resource that could be used to contribute to cherry laurel breeding program for different aims.

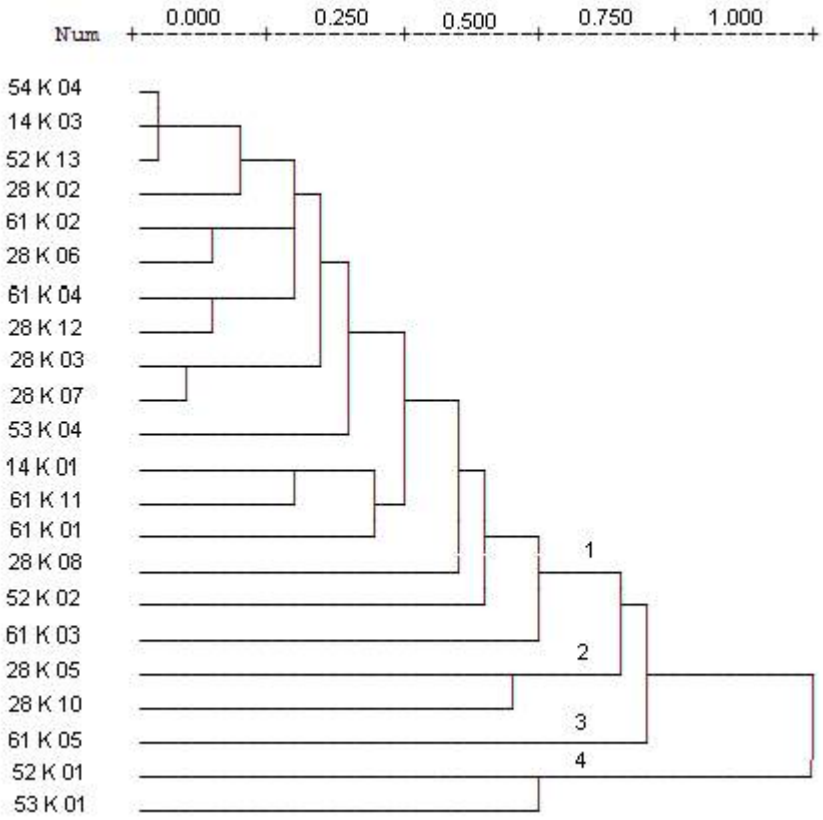

Figure 1. UPGMA dendrogram of 22 cherry laurel genotypes based on 17 random RAPD primers

The cophentic correlation coefficient indicated a correlation of r = 0.93 between the similarity matrix and the UPGMA dendrogram, indicating a good representation of the relationships among the accessions.

This is one of the first attempt to use molecular markers to investigate the genetic relationships of wide number cherry laurel genotypes belongs to *Laurocerasus officinalis* grown in Turkey. RAPD technique could be useful tool for the management of plant collections. This data provides a scientific basis for future selection and management of germplasm. Results of this study indicate that in Turkey the level of polymorphism among cherry laurel genotypes is appreciably high and these genotypes can be used in breeding programs and the information generated in this study is of much use in the improvement of cherry laurel through breeding. These results are of particular interest given the current and future benefits of local crop varieties in improving production diversification and food security, as well as in securing traditional and sustainable crops utilization. In addition, Black Sea region has proved to be a large reservoir of agro biodiversity wealth with potential to enhance the aesthetic value of the natural and rural landscape.

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