

FRUIT QUALITY PARAMETERS AND MOLECULAR CHARACTERIZATION OF SOME LOQUAT ACCESSIONS

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Abstract

Loquat (*Eriobotrya japonica* (Thunb.) Lindl.), one of the firstly harvested fruit in spring is important fruit for Turkey and other Mediterranean countries because it can be supplied to market during shortage of fresh fruit. Genetic diversity within the loquats comes from seeds obtained from open pollinated trees or budspots which occur very often within the genus. The objectives of this study were to determine variations for some fruit quality characters and genetic markers among 10 foreign cultivars, 3 selected local cultivars and two change seedlings. For fruit weight the largest cultivar was 'Kanro' with 46.2 g. Concordantly with its large fruits the highest fruit length and width also were measured in 'Kanro'. Seed number of accessions studied varied between 1.22 and 3.17. The highest flesh ratio was obtained from 'Baffico' (90%) whereas 'Seedling 1' had the lowest flesh ratio (75%). There were high level of variation for total soluble solids (TSS) and acidity of accessions studied. Total soluble solids (TSS) of cultivars were determined between 14.0 and 9.9%, acidity varied between 0.39 and 1.99%. Molecular analysis, as assessed with 21 randomly amplified polymorphic DNA (RAPD) primers indicated that all of accessions were distinguished. Using unweighted pair group method arithmetic average (UPGMA) analysis of RAPD data detected that similarity values among the loquats were between 0.69 and 0.92. In the dendrogram the accessions nested in two main groups. First group consisted of 'Hafif Cukurgobek', 'Uzun Cukurgobek', 'Tanaka' and 'Sayda' whereas rest of 11 accessions nested in other group. Wide range of genetic diversity was determined in loquat accessions and it considered as advantage for breeding programs.

Introduction

Loquat (*Eriobotrya japonica* (Thunb.) Lindl.) originated in China, then it was introduced to Europe from Japan in the 18th century as ornamental tree. Later, in the 19th century, selections of cultivars with large fruits were used for fruit production. Although its specific epithet is *japonica*, the cultivated loquat in Japan was introduced from China in ancient times and its cultivation was described as early as 1180. Loquat is cultivated mainly in China, Japan, India, Pakistan, Madagascar, Reunion Island, Mauritius Island, the Mediterranean countries (Spain, Turkey, Italy, Greece, Israel), United States (mainly California and Florida), Brazil, Venezuela, and Australia. This species has adapted well to the Mediterranean climate and grows in the same areas where citrus species are cultivated. Spain is the leader in loquat production in the Mediterranean area offering more than 50% of the total Mediterranean area production. Being different from other fruit species, the blossoming period of loquat trees occurs in winter and fruits are harvested 5-6 months after full bloom in early spring. Thus, the fruits of the loquat can be sold at a higher price in spring, since there are few competitive fruits on the market except for strawberry, green plum, and green almond (Badenes *et al.*, 2000; Durgac *et al.*, 2006; Gisbert *et al.*, 2009).

It is estimated that loquat was introduced from Lebanon and Algeria to Turkey 150-200 years ago. Production of this fruit in Turkey is increasing. Recently production amount of Turkey have reached to 13000 tons. Trees which was formerly propagated by seed, is now propagated clonally with standard cultivars. The new cultivars are better accepted in the market, the demand of loquat fruit and grafted trees is increasing rapidly. The most competitive production is located in microclimatic areas in the Mediterranean region where early cultivars can be grown. In this region the highest production corresponds to Antalya province, followed by Mersin and Hatay provinces. On the other hand, the crop for

processing and fresh consumption is being increased in Aegean, Marmara region and Blacksea region (Yılmaz *et al.*, 1992; Karadeniz, 2003, Toker *et al.*, 2010).

Loquats have formed various ecological types in different zones over the course of their cultivation and acclimatization. Diversity within the species comes from seeds or budspots which occur very often within the genus, although some of the mutations are not stable. Cultivation of the species has led to a large number of cultivars, due to different selection pressures applied by growers (Vilanova *et al.*, 2001). Genetic diversity is an important parameter utilized for fruit improvement, either by selection or application of various breeding methodologies. Information on genetic diversity is also valued for the management of germplasm and for evolving conservation strategies. Diversity studies would be desirable in order to allow better management and conservation of genetic resources and for planning breeding strategies. DNA markers are generally considered the best tools for determining genetic diversity, as they are unlimited in number and independent of environmental interaction, and show high polymorphism. In loquat, the usage of germplasm variability has been low to date and further studies on genetic diversity might result in more widely exploited genetic resources for cultivar improvement. The first genetic studies on loquat were based on pomological traits. However, molecular markers have been shown as a better tool for measuring genetic distances and accomplishing genetic studies (Badenes *et al.*, 2000; Badenes *et al.*, 2009; Gisbert *et al.*, 2009). To date various marker systems have been used to study genetic diversity and phylogenetic relationships in loquat such as randomly amplified polymorphic DNA (RAPD) (Vilanova *et al.*, 2001; Badenes *et al.*, 2003, Hussain *et al.*, 2009, 2011), amplified fragment length polymorphism (AFLP) (Yang *et al.*, 2009), inter simple sequence repeat (ISSR) (Xie *et al.*, 2007), simple sequence repeats (SSR) markers (Sariano *et al.*, 2005; Gisbert *et al.*, 2009) and

internal transcribed spacer (ITS) sequence (Li *et al.*, 2009). Standard sets of RAPD primers could be established to characterize most of the common genotypes that may serve as a useful supplement to the traditional morphological information (Hussain *et al.*, 2009). The objectives of this study were to determine variations of fruit quality characters and molecular profiles of some important loquat cultivars in Turkey.

Materials and Methods

Plant materials and fruit analysis: Fifteen loquat accessions consisted of 10 foreign cultivars 3 selected local cultivars and two change seedlings obtained from unknown origin (Table 1) planted to field in randomized complete block design with six replicates in 1983 in Alata Horticultural Research Institute located the Mediterranean coast where environmental conditions suited loquat requirements in Mersin province, Turkey. Tree at spacing was 7 X 7 m. The trees were managed according to standard local commercial practices and watered as needed using a drip irrigation system. Fertilization was managed based on soil and leaf analysis. Herbicides were used for weed control. Pest populations were kept under control a recommended pest management program.

For fruit analysis thirty fruits were collected per tree in beginning of May. All fruit samples were assessed for fruit weight (g), fruit length (mm), fruit width (mm), fruit index (width/ length), seed number, flesh ratio (%), flesh color, total soluble solids (%), acidity(%) and TSS/acidity for two years. All fruit samples for each tree were weighed and average fruit weight was estimated. The width and length values of each fruit were measured by a digital compass. The fruit index was calculated by dividing fruit width by fruit length. The ratio approaching unity means that fruits are round-shaped. Seed number was also counted. The flesh ratio was calculated according to the formula:

$$[(a-b)/a]*100], a= \text{mean fruit weight, } b= \text{mean seed weight}]$$

TSS was determined by a hand refractometer (Atago, Master 53, Tokyo, Japan) dropping of fruit juice. Acidity was analyzed in terms of malic acid type from the value determined by titration of this solution with 0.1N NaOH.

Molecular analysis: The total genomic DNA was extracted from young leaves by the CTAB method as described by Doyle & Doyle (1990). DNA concentration was measured with a spectrophotometer (BioTek Instruments, Inc. Vinoski, United States) and 10 ng/mL DNA templates were made using TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

A total of 21 RAPD primers (Operon Technologies, Huntsville, Alabama, USA) were used for all clones (Table 2). PCR reaction components and PCR cycling parameters for RAPD analysis were performed as described by Uzun *et al.* (2009) and DNA thermal cycler (Sensoquest Progen Scientific Ltd. Mexborough, South Yorkshire, UK) were used for PCR process. PCR products were separated on 2% agarose gel in 1X TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 volt for 2.5-3 h. The fragment patterns were photographed under UV light for further analysis. A 100 bp standard DNA ladder as the molecular standard in order to confirm the appropriate markers were used for RAPD analysis.

Table 1. Fruit quality parameters with statistically assigned groups of 15 loquat accessions.

Cultivars	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit index (width/length)	Seed number	Flesh ratio (%)	Flesh color	TSS (%)	Acidity (%)	TSS/ Acidity
Taza	29.7 b-e ^x	42.44 ab	34.63 b	0.82 abc	3.01 a	79 cd	Orange	13.1 b	0.57 g	23.0 c
Hafif Cukurgobek*	37.6 b	27.78 d	26.17 cd	0.94 a	1.22 e	85 abc	Light yellow	13.2 b	1.14 b	11.5 f
Akko XIII	27.2 e	38.13 bc	31.78 b	0.83 abc	1.68 dc	85 abc	Orange	12.1 c	0.83 de	14.6 c
C. de Grasse	29.2 cde	39.54 bc	31.22 bc	0.80 abc	1.41 de	85 abc	Light orange	12.0 cd	1.07 bc	11.2 f
Ottaviani	32.1 b-e	37.06 bc	32.49 b	0.88 ab	2.13 bcd	80 bcd	Orange	13.1 b	0.39 h	33.7 a
Sayda	32.8 b-e	40.16 abc	34.03 b	0.85 abc	3.04 a	79 cd	Light orange	12.9 b	0.66 fg	19.7 d
Uzun Cukurgobek*	36.8 bcd	45.66 ab	33.98 b	0.75 bc	2.78 ab	79 bcd	Orange	11.5 de	1.08 b	10.6 fg
Victor	27.2 e	38.01 bc	32.50 b	0.86 abc	1.82 cde	81 bcd	Cream	13.9 a	0.46 h	30.2 b
Kanro	46.2 a	49.07 a	45.66 a	0.93 a	2.48 abc	85 abc	Orange	10.5 fg	0.98 c	10.7 fg
Tanaka	31.8 b-e	44.04 ab	30.81 bc	0.70 c	1.68 dc	86 ab	Orange	9.9 g	0.41 h	24.3 c
Bafico	29.0 de	37.97 bc	30.40 bc	0.80 abc	1.26 e	90 a	Cream	12.9 b	0.87 d	14.8 e
Yuvarlak Cukurgobek*	29.3 b-e	38.90 bc	31.17 bc	0.81 abc	2.20 bcd	86 ab	Cream	10.9 ef	0.74 ef	14.9 e
Gold Nugget	37.5 bc	45.46 ab	34.41 b	0.76 bc	3.17 a	82 bcd	Orange	10.2 g	1.07 bc	9.6 g
Seedling 1 ⁺	14.6 f	26.67 d	25.13 d	0.94 a	1.60 de	75 d	Cream	14.0 a	1.96 a	7.1 h
Seedling 2 ⁺	29.2 cde	33.19 cd	30.27 bcd	0.91 ab	2.92 ab	79 bcd	Light orange	12.3 c	1.99 a	6.2 h
P value	8.35	9.24	5.26	0.167	0.79	7.13	-	0.55	0.09	1.45

* Turkish selections, +change seedling from Turkey

^x Mean separation within columns by Tukey's multiple range test. P<0.05 each value is the average of 2 years with six replicates

TSS= Total soluble solids

Table 2. List of RAPD primers, their numbers of total and polymorphic fragments and percentage of polymorphism range and polymorphism information contents (PIC) used in this study.

RAPD primers	TFN	PFN	PP(%)	PIC
OPB 06	11	8	73	0.60
OPF 08	15	14	93	0.83
OPF 10	12	7	58	0.47
OPF 12	14	11	79	0.60
OPH 02	10	8	80	0.59
OPI 01	7	7	100	0.78
OPI 03	5	3	60	0.18
OPI 04	7	7	100	0.62
OPI 07	6	2	33	0.32
OPI 11	7	2	29	0.27
OPI 15	15	8	53	0.42
OPI 16	12	12	100	0.77
OPL 04	12	6	50	0.33
OPM 04	10	8	80	0.50
OPM 05	11	10	91	0.57
OPM 10	8	6	75	0.45
OPM 11	14	12	86	0.58
OPM 16	15	13	87	0.61
OPM 19	12	12	100	0.62
OPW 15	9	7	78	0.48
OPW 19	12	9	75	0.59
Mean	10.6	8.2	77	0.53
Total	224	172	-	-

TFN: Total fragment number

PFN: Polymorphic fragment number

PP: Polymorphism percent

Data analysis: For fruit characters, data were analyzed using JMP 5.0 (SAS Institute Inc., Cary, NC, USA) and means were separated and grouped (a, ab, abc, abcd, b, . . . , etc.) using Tukey's test ($p < 0.05$). Molecular analysis was performed as follows: each band was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf, 2000). A similarity matrix was constructed using RAPD data based on Dice's coefficient (Dice 1945). Then, the similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the accessions studied. The genetic similarity matrix and ultrametric distance matrix produced from UPGMA- based dendrogram with COPH module nested in the same software was compared using Mantel's matrix correspondence test (Mantel, 1967). The result of this test is a cophenetic correlation coefficient, r , that indicates how well dendrogram represents similarity data. Polymorphism information content (PIC) values were calculated for RAPD markers according to Smith *et al.* (1997), using the following formula for all primer:

$$PIC = 1 - \sum f_i^2,$$

where f_i^2 is the frequency of the i th allele. PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles (Smith *et al.*, 1997). PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies).

Results and Discussion

Fruit quality: Fruit quality parameters of the loquat cultivars were evaluated. There were significant differences at $p < 0.05$ among the cultivars for all traits studied (Table 1). For fruit weight 'Kanro' was the best cultivar for fruit weight (46.2 g) whereas the lowest fruit weight was determined in 'Seedling 1' (14.6 g). Fruit weight is one of the important factors for marketing. Fruit weight of 13 loquat cultivars was determined between 39.1 g ('Dr. Trabut') and 22.5 g ('C. de Grasse') in previous study carried out in Adana province (Paydas *et al.*, 1992). In our study there was higher variation in fruit weight because some cultivars were obtained from open pollinated source in this study. Also 'Kanro' had the highest fruit length (49.07 mm) and width (45.66 mm) concordantly with its large fruits. Fruit index values of cultivars varied between 0.70 ('Tanaka') and 0.94 ('Hafif Cukurgobek' and 'Seedling 1'). Fruit shape of loquats varied from pear shape to round generally (Caldeira & Crane 1999). When fruit index value closer to 1.00 it is accepted as round shape. The highest seed number per fruit was found in 'Gold Nugget' (3.17) and the lowest was in 'Hafif Cukurgobek' (1.22). It was previously reported that seed numbers of loquats were between 1 and 6 and 'Gold Nugget' had the highest seed number (3–6) in agreement with our results (Caldeira & Crane 1999). On the other hand, Llacer *et al.* (2003) also notified that seed number of 'Gold Nugget' was 3.2 that similar to our present study. Flesh ratio of cultivars studied varied between 75% (Seedling 1) and 90% ('Baffico'). High level of flesh ratio of 'Baffico' was result from its low number (1.26) of seeds. Flesh ratio was reported in loquats as 66.6–92.5% by Caldeira and Crane (1999) and as 77.3%–85.1% by Yilmaz *et al.*, (1992), which is consisted with our study. Flesh color of cultivars varied from cream to orange. It was previously reported that flesh color of loquat cultivars changed from white to orange (Caldeira & Crane 1999).

Total soluble solids of evaluated cultivars were between 9.9% ('Tanaka') and 14% ('Seedling 1'). Yilmaz *et al.* (1992) found TSS of loquats between 9.28% and 13.30% and the lowest TSS was determined in 'Tanaka' in agreement with our study. On the other hand 'Tanaka' was reported as a cultivar which had lower TSS content than most of cultivars in study performed in Adana conditions (Paydas *et al.*, 1992). Also in previous study carried out in Italy 'Tanaka' was found to have the lowest TSS content as 11.1% (Insero *et al.*, 2003). Acidity of fifteen accessions varied from 0.39% ('Ottaviani') to 1.99% ('Seedling 2'). Acidity of two accessions, not previously characterized, were higher (1.96 and 1.99%) than the others. Acid content of loquats was reported between 0.28 and 1.08 (Paydas *et al.*, 1992) and 0.48–1.05 (Yilmaz *et al.*, 1992) which is consistent with our study. There was high level of variation for ratio of TSS/acidity of cultivars. 'Ottaviani' had the highest ratio (33.7) whereas 'Seedling 1' (7.1) and 'Seedling 2' (6.2) had the lowest. It is considered that cultivars had lower TSS/acidity ratio could be harvested later than the others.

Molecular analysis: For molecular analysis, a total of 21 RAPD primers were used for PCR amplification and 224 fragments with high intensity were scored. The amplified fragments per primer varied from 5 (OPI 3) to 15 (OPF 08, OPI 15 and OPM 16) with an average of 10.2 (Table 2). Average polymorphic fragments per primer were found as 8.2. These fragment number was higher than reported previously (1.26) by Vilanova *et al.*, (2001) and was similar to the results (9.4) found by Hussain *et al.*, (2009) based on their RAPD data. Maximum number of polymorphic fragments was 14 which were obtained from OPF 08. Ratio of polymorphic RAPD markers was very high (77%). The PIC values for the 21 primers ranged from 0.18 (OPI 03) to 0.83 (OPF 08) in our study.

The data obtained from RAPD analyses were used to perform genetic similarity analysis among the 15 loquat cultivars. The similarity matrix was calculated using 224 RAPD fragments according to Dice's coefficient (Dice, 1945). Then, the similarity matrix was used to perform UPGMA cluster analysis. Cophenetic correlation between ultrametric similarities of tree and the similarity matrix was $r = 0.79$, $p < 0.01$. This means that the dendrogram moderately represents the similarity. All of accessions used in this study were distinguished. The loquat samples studied had a similarity levels ranging from 0.69 to 0.92 (Fig. 1). Based on the UPGMA analysis, 15 loquat accessions were divided into two main groups: Group A and B. The first group (A) included four cultivars that 'Tanaka', 'Sayda', 'Uzun Cukurgobek' and 'Hafif Cukurgobek'. Two of them which 'Uzun Cukurgobek' and 'Hafif Cukurgobek' are local selections in Turkey and 'Hafif Cukurgobek' was reported as suitable for commercial production (Aksoy, 1995). Group B separated into two subgroups (B1 and B2) at the similarity level of 0.75. Subgroup B1 consisted of 'Ottaviani', 'Baffico', 'Yuvarlak Cukurgobek', 'Gold Nugget', and 'Seedling 1'. As two universal cultivars 'Tanaka' originated from Japan and 'Gold Nugget' originated from USA also was found in distinct groups in previous study carried out with SSR

markers in agreement with our results (Soriano *et al.*, 2005). Similarly, 'Tanaka' and 'Ottaviani' were determined in different groups based on SSR data (Gisbert *et al.*, 2009). Rest of six cultivars 'Taza', 'Akko XIII', 'Champagne de Grasse', 'Victor', 'Kanro' and 'Seedling 2' nested in subgroup B2. In the dendrogram the highest similarity value was determined between 'Akko XIII' and 'Champagne de Grasse', as 0.92. These two cultivars originated in Japan (Morton, 1987).

Loquat cultivars are generated by natural hybridization (seeds) and selection of budspots. It is difficult that distinguish of cultivars originated from mutation by different marker patterns. However, all cultivars that came from natural hybridization, even if they were from the same country or area, were identifiable (Vilanova *et al.*, 2001). Materials used in this study might be originated from natural hybridization or selfing. Five of the genotypes used ('Hafif Cukurgobek', 'Yuvarlak Cukurgobek', 'Uzun Cukurgobek', 'Seedling 1' and 'Seedling 2') were selected from orchards as chance seedlings in Turkey and rest of cultivars were introduced from Mediterranean countries. Therefore, all are distinguishable. In the dendrogram there was no clear grouping based on geographic origin of loquats cultivars. It was previously notified that loquat was introduced to France and England at the end of 1780s, and after it was distributed throughout Mediterranean countries (Badenes *et al.*, 2009). Thus, clustering cultivars analyzed in present study might be explained under this assumption. For fruit quality characters there were also significant differences among the cultivars. It was reported that loquat cultivars selected by natural mutation might show different pomological traits therefore closely genetically related (Gisbert *et al.*, 2009). High level of variations determined in loquat cultivars might be adequate for breeding of new cultivars. RAPD markers successfully distinguished accessions studied consequently their fruit quality differences. This technique was adequate for studies on genetic diversity and relationships in loquats.

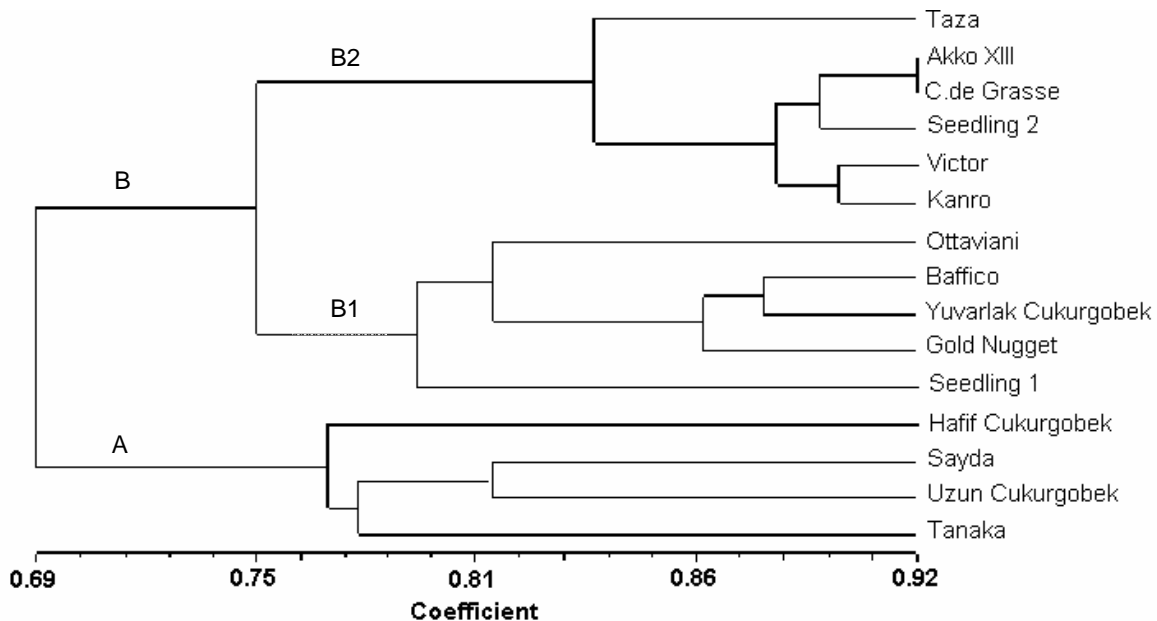


Fig. 1. Dendrogram of the 15 loquat accessions using UPGMA method obtained from 224 RAPD markers.

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