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[1]Department of Horticulture, Faculty of Agriculture, University of Cukurova, Adana, Turkey
[2]Ataturk Central Horticultural Research Institute, Yalova, Turkey
[3]Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum, Turkey

Genetic relatedness among quince (*Cydonia oblonga* Miller) accessions from Turkey using amplified fragment length polymorphisms

H. Topcu[1], S. Kafkas[1]*, A. Dogan[2], M.E. Akcay[2], S. Ercisli[3]

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Summary

Among fruit species cultivated in Turkey, quince shows a great deal of morphological variability and adaptability to the various environments. We attempted to study genetic relationships among 40 quince accessions using amplified fragment length polymorphisms (AFLPs) for future breeding programs. The accessions were previously characterized based on their pomological and yield characteristics and then the best ones were planted in a single collection in Ataturk Central Horticultural Research Institute, Yalova, Turkey. Six AFLP primer combinations generated a total of 746 bands, 493 of which were polymorphic (66.1%). Resolving powers of the AFLP primers ranged from 48.0 to 99.6 making a total of 421.5. Unweighted Pair Group Method with Arithmetic Average (UPGMA) clustering of the accessions showed three major clusters and 'SapancaEsme' and 'Esme-3' were the closest accessions with 95% similarity. Our study indicated that there is a high level of genetic diversity among quince accessions in Turkey and the results of this study can be used for future cultivar breeding programs in quince.

Introduction

The quince (*Cydonia oblonga* Miller) belongs the genus *Cydonia* and native to warm-temperate regions including Asia Minor (BROWICZ, 1972). The total quince production in the world is 596.532 tons, and Turkey (135.500 tons) is the leader producer country. The other important quince producer countries are China (125.000 tons), Uzbekistan (80.000 tons), Morocco (46.000 tons), Iran (36.500 tons), Argentina (27.500 tons), Azerbaijan (27.140 tons) and Spain (14.000 tons) (FAO, 2012).

The quince is a deciduous tree, growing up to 5-8 m tall and 4-6 m wide. It has very close relationships with apples and pears, and has a pear or apple shaped pome fruits, which is bright golden yellow when mature. In most quince producer countries, they are not grown in large amounts; typically several quince trees are grown in a mixed orchard with several apples and other fruit trees (WESTWOOD, 1993).

The fruits of most of quince cultivars are hard, sour and astringent in maturation time and therefore a few cultivars can be eaten raw. Due to this reason, quince fruits are mainly used to make jam and jelly or they may be peeled, then roasted, baked or stewed. The flesh of the fruit turns red after cooking (SILVA et al., 2002; 2004). The quince fruit is also known as an important dietary source due to its antioxidant, antimicrobial and antiulcerative properties (SILVA et al., 2004; HAMAUZU et al., 2006; FATTOUCH et al., 2007).

Quince trees have been used as a dwarf rootstock for pear for a long time. It forces scion to produce precocious fruits, and relatively more fruit-bearing branches, and of accelerating the maturity of the fruit (GULEN et al., 1999; MASSAI et al., 2008).

Earlier characterization of the quince genotypes were performed primarily based on phenotypic traits of the plants such as color, size, shape and other agronomical characters of fruits in Turkey (ERCAN et al., 1992; ERCISLI et al., 1999; DUMANOGLU et al., 2009; KUDEN et al., 2009). However, information from morphological and phenotypic characteristics are not sufficient to identify quince genotypes because of environmental plasticity on it. Thus, environmentally free genotypic traits are necessary for proper identification and estimation of genetic diversity among quince accessions.

Molecular characterization assays provide an efficient tool for the evaluation of genetic diversity in plants (HALASZ et al., 2010; BADRI et al., 2014). Various types of molecular markers (RFLP, RAPD, SSRs, and AFLP) have been successfully used to assess the levels of genetic diversity in fruit tree species (BELAJ et al., 2003; CHEN et al., 2005; HALASZ et al., 2006; ERCISLI et al., 2008; KAFKAS et al., 2008; KAFKAS et al., 2009). AFLP markers are highly reproducible with overall error rates of less than 2% (VOS et al., 1995), it is suitable for high-throughput genotyping, and DNA sequence information is not a prerequisite.

Although the AFLP method has been one of the most widely used marker system to identify genetic variability in many different fruit tree species, the use of this powerful and reliable method in quince is very rare. The objective of this study is to characterize 40 quince accessions of Cydonia oblonga from Turkey using AFLP markers, to determine whether AFLP markers are appropriate to discriminate quince accessions and to have a better understanding about the variability within the Turkish quince germplasm.

Materials and methods

Plant Material

Forty quince accessions (*Cydonia oblonga*) were used in the present study (Tab. 1). These accessions were previously selected for their better fruit and yield characteristics than others and vegetatively propagated, and then they were planted in the Ataturk Horticultural Research Institute in the Yalova province of Turkey. The pomological characteristics of accessions were published elsewhere (BUYUKYILMAZ, 1999).

DNA extraction and AFLP analysis

Genomic DNA was isolated from leaf tissue by the CTAB method (DOYLE and DOYLE, 1987). DNA concentration was estimated by comparing band intensity with l DNA of known concentrations, after 0.8% agarose gel electrophoresis and ethidium bromide staining. DNA was diluted to 50 ng μL^{-1} for AFLP reactions. Details of AFLP assay, adaptor and primer sequences, PCR conditions for preselective and selective amplifications, and selective primer designation were according to VOS et al. (1995). Genomic DNA was restricted with *Eco*RI/*Mse*I enzyme combination and double-stranded adaptors specific to each site were ligated. Preselective amplifications were done with primers complementary to the adaptors with an extra selective base on each primer (*Eco*RI-A/*Mse*I-C). Selective amplifications were performed using six primer combinations with three *Mse*I (M) and three *Eco*RI (E) primers (E39/M55, E39/M57,

* Corresponding author

Tab 1: The origin of quince accessions used in this study

Accession	Origin	Accession	Origin	Accession	Origin
Altin Subasi	Kocaeli	Esme 8	Sakarya	Esme 6	Kocaeli
Altin Yalova	Yalova	Havan	Yalova	Limon 2	Sakarya
Bardak	Bursa	Esme 14	Kocaeli	Viranyadevi	Yalova
Demir 1	Kocaeli	Tekkes	Yalova	Beyaz Ayva	Kocaeli
Ege-25	Izmir	27-1	Yalova	7-2	Yalova
Ekmek Keles	Bursa	Gordes	Yalova	6-3	Yalova
Esme 2	Kocaeli	Esme 10	Sakarya	10-3	Yalova
Sapanca Esme	Sakarya	Bencikli	Yalova	Ekmek Yalova	Yalova
Esme 3	Kocaeli	Ege-22	Izmir	Esme Arifiye	Sakarya
Esme 4	Kocaeli	Sekergevrek	Yalova	Esme 1	Sakarya
Limon 1	Sakarya	2-3	Yalova	Esme 7	Sakarya
19-2	Yalova	Esme 5	Kocaeli	Esme 9	Sakarya
Esme 13	Kocaeli	Esme 11	Bilecik	Esme 12	Bilecik
Limon Yalova	Yalova				

E45/M59, E42/M59, E45/M60 and E42/M60). AFLP fragments were resolved using capillary electrophoresis on an ABI 3130xl Genetic Analyzer [Applied Biosystems Inc., Foster City, Calif, (ABI)] with the data collection software 3.0 (ABI). AFLP fragments were analyzed with GeneScan analysis software 4.0 (ABI) and the data were assembled in binary format.

Data analysis

The ability of the most informative primer pairs to differentiate the genotypes was analyzed by calculating their resolving power (Rp) according to PREVOST and WILKINSON (1999) using the formula $Rp=\sum Ib$, where $Ib=1-(2 \times |0.5 - p|)$, and p is the proportion of the 40 genotypes containing the I band. Jaccard's similarity coefficients (JACCARD, 1908) were calculated for all pair-wise comparisons among the 40 quince genotypes. A dendrogram was generated using NTSYSpc version 2.11V (Exeter Software, Setauket, NY) (ROHLF, 2004) based on the un-weighted pair-group method of arithmetic average cluster analysis (UPGMA).

Results and discussion

A total of 746 fragments were amplified using six primer combinations, 493 (66.8%) were polymorphic in characterizing 40 quince accessions (Tab. 2). The number of total bands produced by each primer combination ranged from 97 (E45/M60) to 156 (E42/M59) with an average of 104.1 per primer pair. The number of polymorphic fragments per primer combination ranged from 59 to 103 with an average of 69.1. Among the six primer combinations tested, E42/M59 and E45/M60 amplified the lowest and highest number of total as well as polymorphic fragments, respectively (Tab. 1).

The percentage of polymorphic bands varied considerably among the primer combinations. The highest polymorphism ratio (72.6%) was observed in E39/M55 primer pair and followed by E39/M57 (69.9%), E42/M59 (66.0%), E42/M60 (65.7%) and E45/M59 (60.8%) and E45/M60 (60.8%) (Tab. 1). Resolving power (Rp) ranged from 48.0 (E45/M60) to 99.6 (E42/M59), with a total of 421.5. The average Rp value of the primers was found to be 70.3 (Tab. 1).

In the present study, we obtained the highest polymorphism ratio (72.6%) in E39/M55 primer pair and followed by E39/M57 (69.9%) and E42/M59 (66.0%). Screening and selection of primer combinations, which detect maximum genetic variation, are vital to establish dependable genetic relationships among accessions (KAFKAS et al., 2009).

Tab 2: Number of total and polymorphic AFLP bands, percentage of polymorphic bands, and resolving powers in the DNA-fingerprinting of 40 quince genotypes originating from Turkey.

AFLP primer combinations	Total bands (no.)	Polymorphic bands (no.)	Polymorphism (%)	Resolving powers (Rp)
E39/M55	113	82	72.6	80.9
E39/M57	123	86	69.9	57.4
E45/M59	120	73	60.8	66.5
E42/M59	156	103	66.0	99.9
E45/M60	97	59	60.8	48.0
E42/M60	137	90	65.7	69.1
Total	746	493		421.5
Mean	104.1	95	66.8	70.3

In this study, AFLP analysis successfully differentiated the quince accessions each other and it was confirmed that AFLP is a powerful marker system. AFLP markers have been previously used in the analysis of different fruit species for example mulberry (SHARMA et al., 2000), tea (KAFKAS et al., 2009), walnut (KAFKAS et al., 2005), myrtle (BRUNA et al., 2007) and pomegranate (JBIR et al., 2008) and all these results confirmed the power of AFLP technique to discriminate genotypes. Although quince is known self fertile, their level of polymorphism was comparable to those of previous studies. This is an indication of the high degree of polymorphism among the accessions tested.

In this study, Resolving power (Rp) ranged from 48.0 (E45/M60) to 99.6 (E42/M59). Rp has been found to correlate strongly with its ability to distinguish between accessions. It is usually difficult to compare the value of primers used in different investigations. The use of Rp might enable direct comparison of primers both within and between studies (PREVOST and WILKINSON, 1999).

A dendrogram constructed using UPGMA method of cluster analysis from a combined data of all primer combinations classified ac-

cessions into three main clusters (Fig. 1). Cluster I consisted of six quince accessions. The closest accessions in this cluster were 'Esme-7' and 'Esme-9' which were surrounded by the accessions 'Esme-12', 'Esme-1', 'Esme Arifiye' and 'Ekmek Yalova' respectively (Fig. 1). Cluster II include only '10-3' genotype. Cluster III contained 34 accessions and this cluster divided into two subclusters. The first subcluster included 12 accessions ('6-3', '7-2', 'Beyaz Ayva', 'Viranyadevi', 'Limon-2', 'Esme-6', 'Esme-11', 'Esme-5', '2-3', 'Sekergevrek', 'Ege 22' and 'Bencikli', whereas subcluster II contained 21 accessions ('Esme-10', 'Gordes, 27-1', 'Tekkes', 'Esme-14', 'Havan', 'Esme-8', 'Limon Yalova', 'Esme-13', '19-2', 'Limon-1', 'Esme-4', 'Esme-3', 'Sapanca Esme', 'Esme-2', 'Ekmek Keles', 'Ege 25', 'Demir-1', 'Bardak', 'Altin Yalova' and 'Altin Subasi'). The accession 'Esme-10' took place between two subclusters. In the subcluster II, 'Sapanca Esme' and 'Esme-3' were the closest accessions in this study (95.1% similarity), with the accessions 'Esme', 'Esme-4', and 'Limon-1'. The pairs of accessions 'Bardak' with 'Demir-1' and 'Ekmek Keles' with 'Esme-2' were also close to each other with 92.8% and 92.6% similarity, respectively (Fig. 1).

The UPGMA dendrogram formed from AFLP bands successfully separated the closely-related quince accessions (particularly 'Esme' genotypes). It is clear that there is a large clonal variability within 'Esme'. This genotype was grown in different parts of Western Anatolia (Tab. 1). This fact points to cultivation since remote times in quince growing areas in Anatolia and finally the numerous 'Esme' types would subsequently have been generated through accumulation of somatic mutations during the long period of mutations (ERCISLI, 2004). Local people groups would have moved over long distances in the Anatolia over time and carried with them the core of quince variation, while further *ad hoc* cultivation would have caused additional and location-specific variation. Moreover, previous study indicating 'Esme' genotypes showed a certain degree of morphological and phenological variability within the population of individuals (BUYUKYILMAZ, 1999). Most fruit tree species, including quince, are vegetatively propagated to maintain agronomically valuable genotypes. However, after many propagation cycles, clones accumulate phenotypic differences in agronomic traits and clonal diversity appears (ORIVE, 2001). This diversity can then be used to select the best clones or a new improved cultivar within a given variety. A recent study of the molecular polymorphisms generated along vegetative propagation (CARRIER et al., 2012) through a genome-wide comparison of spontaneous grape 'Pinot noir' clones showed that only a small number of SNP and indel events are at the origin of clonal variation, while mobile elements of many families are involved in most polymorphisms, displaying the highest mutational event. In general, our results were in agreement with those of DUMANOGLU et al. (2009) who indicated a large genetic diversity within different 'Kalecik' quince clones in Turkey (average polymorphism ratio with 65%) by using SSR markers. YAMAMATO et al. (2004) used a total 118 SSR markers (developed for apple and pears) on 20 quince cultivars and they obtained relatively high discrimination capacity from 39 SSR markers. YUKSEL et al. (2013) conducted simple sequence repeat (SSR) analyses of 15 traditional quince (*Cydonia oblonga*) cultivars from Anatolian gene sources for molecular characterization and investigation of genetic relationships. They used eight SSR loci that previously developed from apple and pear and they found similarity ratio between 18-87% among cultivars.

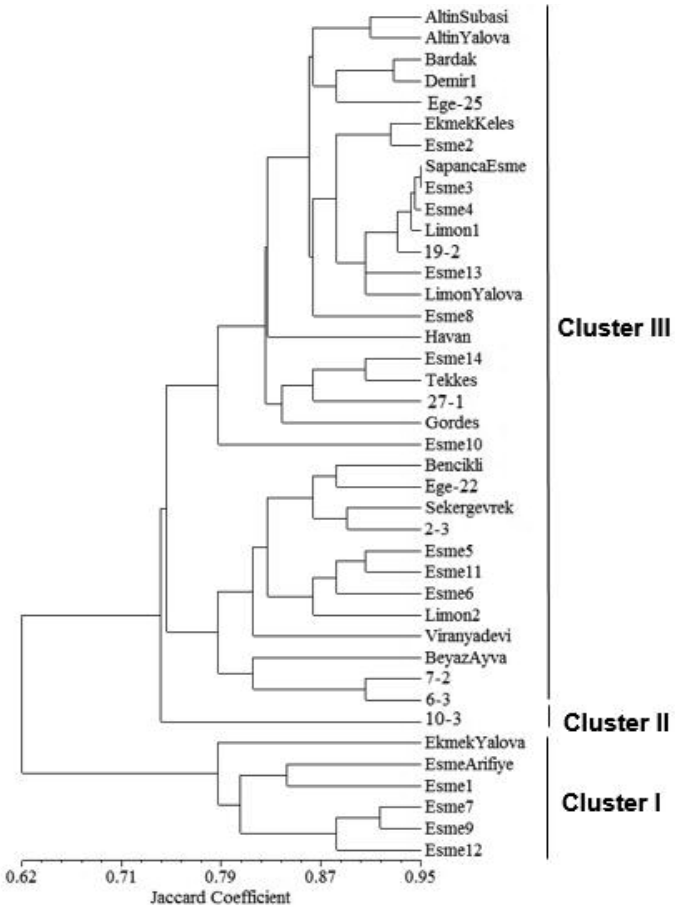

Fig. 1: Dendrogram of 40 quince genotypes originating from Turkey resulting from the unweighted pair-group method of arithmetic mean cluster analysis based on Jaccard similarity coefficient obtained from 746 AFLP markers.

In present study, in most cases, quince accessions within the same cluster did not have similar morphological fruit characteristics. For example, in the Cluster I, the closest accessions 'Esme-7' and 'Esme-9' have different tree and fruit characteristics. 'Esme7' has light yellow fruit skin color at maturation and Esme9 has greenish yellow skin color. Esme7 has oval with neck fruit shape, whereas 'Esme9' has neck less oval fruits. The yields of these accessions are also very variable (BUYUKYILMAZ, 1999). In addition, in the Cluster I, 'EkmekYalova' has yellow skin color. The genotype 'Esme-1' has medium fiber in its fruits; however the other accessions had low fiber levels. Fruit weight of six accessions in Cluster I varied from 350 to 396 g and yield per tree varied from 62 kg ('Esme-7') to 110 kg ('EsmeArifiye') (BUYUKYILMAZ, 1999).

In the Cluster II, the accessions 'SapancaEsme' and 'Esme-3' were the closest accessions with 95% similarity ratio. However, these two accessions had different fruit characteristics. For example, 'SapancaEsme' had oval with neck fruit shape, however 'Esme-3' had oval with light neck fruit shape. The other closest pairs of accessions 'Bardak'-'Demir-1' and 'EkmekKeles'-'Esme-2' have also different fruit characteristics: 'Bardak' has greenish yellow, long neck fruits while 'Demir-1' has light yellow oval with light neck fruits. 'Bardak' has more fiber characteristics than 'Demir-1' (BUYUKYILMAZ, 1999).

The accessions from the same province were assigned in the different cluster. For example, the selections from Esme cultivar in Kocaeli province, 'Esme-1' placed in Cluster I and the other accessions ('Esme-2', 'Esme-3', 'Esme-4', 'Esme-5' and 'Esme-6') distributed in Cluster II. Likewise, the Esme accessions from Sakarya province were also assigned in different clusters ('Esme-7' and 'Esme-9' in Cluster I and 'Esme-8' and 'Esme-10' in Cluster II (Fig. 1). The closest pair of accessions in this study, 'SapancaEsme' and 'Esme-3', were collected from the different provinces (Sakarya and Kocaeli) in Northwestern parts of Turkey. This could be at-

tributed to the unrestricted movement of planting materials from region to region, high self-pollination habit and perennial nature of the crop. On the contrary, the accessions 'Esme-11' and 'Esme-12' were collected from Bilecik province in Turkey, however they were genetically far from each other. The low genetic similarity between these accessions collected from the same region could be attributed to the vast agro-ecological diversity present within each region that can contribute to the development of genetically diverse accessions through natural selection. Therefore, similarity in collection locality may not necessarily imply genetic similarity in quince, in Turkey.

Dissimilarities in groupings using molecular markers or phenotaxonomic characteristics were also reported in strawberry (Garcia et al., 2002), mulberry (Orhan et al., 2007) and olive plants (Hagidimitriou et al., 2005). These discrepancies were also previously reported by Zamani et al. (2007), who compared data from the genetic distance matrices obtained from RAPD markers and from fruit characteristics. Their correlation coefficient, for comparison of morphological and RAPD data, was only 23%. These results support the view that morphological characteristics are not reliable for estimating genetic relationships among large and diverse groups of accessions, and should be used mainly for discrimination.

Results of the present study as well as studies by Yamamato et al. (2004) in Japan Dumanoglu et al. (2009) in Turkey, Halasz et al. (2009) in Hungary and Bassil et al. (2011) in USA indicated the presence of genetic variation both genotypic and clonal level among quince genotypes. This confirms the importance of conservation of the Turkish quince gene pool for the quince industry. Since quince is a tree and a perennial crop, it demands large area and year round management which is expensive. Moreover, morphological markers require evaluation over long periods of time and the present study indicated the inadequacy of morphological characters for characterization of closely related accessions. Hence, genetic diversity analysis using DNA-based marker techniques is recommended for cost effective and efficient conservation of quince germplasm.

The results of the present study may benefit breeders in selecting the most diverse accessions to begin crossing and selection programs. This may result in increased quince growing for better fruit production. This report is also demonstrates the presence of mutations of agronomical relevance within a monoclonal cultivar of Esme.

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Addresses of the authors:
H. Topcu, S. Kafkas, Department of Horticulture, Faculty of Agriculture, University of Cukurova, 01330, Adana, Turkey
E-mail corresponding author: skafkas@cu.edu.tr
A. Dogan, M.E. Akcay, Ataturk Central Horticultural Research Institute, Yalova, 77102, Turkey
S. Ercisli, Department of Horticulture, Faculty of Agriculture, Ataturk University, 25240, Erzurum, Turkey