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Characterization of Anatolian traditional quince cultivars, based on microsatellite markers

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ABSTRACT. We 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. Three pear and two apple cultivars were used as references for SSR locus data analysis and to determine allele profiles between species. Eight SSR loci that were developed from apple and pear were used, and a total of 44 alleles were found among quince cultivars. The CH01F02 locus was found to have the highest identification probability, while the CH04E03 locus had the lowest identification probability. Analysis of similarity ratios between quince cultivars showed that the lowest similarity ratio was 18% (Eşme-Bardacık), while the highest similarity ratio was 87% (Bursa-Osmancık and Osmancık-Viranyadevi). In the phylogenetic dendrogram, Eşme quince showed separate branching from other quince cultivars, and no synonymous accessions were found. These results suggest that SSR markers from pear and apple could be used to determine genetic

variation among quince cultivars. These findings can be used to guide future quince breeding and management studies.

Key words: Genetic diversity; SSR; *Cydonia oblonga*; Quince

INTRODUCTION

The genus *Cydonia*, known as quince (*Cydonia oblonga* Mill.), belongs to the family Rosaceae and the subfamily Maloideae, which includes pome fruits like apple (*Malus* spp) and pear (*Pyrus* spp). The Maloideae subfamily contains approximately 1000 species in 30 genera, all of which have 17 chromosomes (Evans and Campbell, 2002; Halász et al., 2009). Quince has been grown for over 2000 years, and its name is derived from the name of a Greek city in the town of Cydone (Sykes, 1972; Richard and Leitão, 2011). In agriculture, the quince is used mostly as a rootstock for pear cultivars and reduces maintenance and harvesting difficulties; its fruit is used in the food industry for preparing preserves, jams, jellies, and marmalades with traditional methods or modern technology. Quince is cultivated for fruit production all over the world, but most of the quince production occurs in the region where this fruit crop is supposed to have originated. The precise origin of quince is unknown, but it is thought to have originated in Northern Iran, Turkmenistan, and the far west regions of Anatolia and Greece (Sykes, 1972; Richard and Leitão, 2011).

In Turkey, quince has been cultivated for a long time, and different types and cultivars of quince are grown in different parts of Anatolia (Browicz, 1972). In 2010, Turkey was one of the most important quince producers worldwide, with production reaching 121,085 tons/year, followed by China, Uzbekistan, Morocco, Iran, Argentina, and Azerbaijan (FAO, 2012). In 1964, a germplasm collection that included different regionally developed fruit cultivars and landraces was established in Turkey (Sykes, 1972). In addition, fruit characteristics have been described for eight quince cultivars and for five other quince cultivars in Western Turkey (Sykes, 1972). Most of the economically important quince cultivars (Limon, Demir, Ekmek, and Eşme) belong to *C. vulgaris* var. *pyriformis* (Özbek, 1978).

The use of DNA-based molecular markers for identification and characterization of germplasm collections is a popular and reliable experimental method. The reason for this is that it is difficult to distinguish cultivars by morphological and phenological characterizations due to the influence of the environment and localities on phenotypes. In addition, quince tree and fruit morphological properties are very similar to each other, which makes distinction for reliable classifications difficult. General differentiation characteristics have been based on fruit shape, as apple shaped [*C. oblonga* var. *maliformis* (Mill)] and pear shaped [*C. oblonga* var. *pyriformis* (Dierb)] (Nagy-Déri, 2011).

The first attempt to use molecular markers for Turkish traditional quince cultivars (Şekergevrek, Ekmek, Limon, Tekeş) employed isoenzymes in 1988 (Sanchez et al., 1988). Eleven groups of quince and two groups of X *pyronia* (quince-pear crosses) were distinguished by isozyme patterns such as acid phosphatase, esterase, peroxidase, and phenol oxidase. As a dominant marker, random amplified polymorphic DNA (RAPD) was used (Bayazit et al., 2011) for determining genetic relationships among 13 traditional quince accessions that were selected from different parts of Turkey.

Among the DNA-based molecular markers, simple sequence repeats (SSRs) are known for being co-dominant, highly polymorphic, and having a large number of alleles per locus (Schlotterer and Tautz, 1992). SSRs have been used in pome fruit species, particularly in apple

(Gianfranceschi et al., 1998; Liebhard et al., 2002; Galli et al., 2005; Zhang et al., 2012) and pear (Kimura et al., 2002; Yamamoto et al., 2002; Bao et al., 2007; Miranda et al., 2010; Tian et al., 2012). One more advantage of SSRs is that these markers can be easily transferred between species of the same subfamily and between closely related genera. Most cross-transferability studies have reported this for the same subfamily, especially for the Rosaceae family (Yamamoto et al., 2001; Wünsch and Hormaza 2007; Halász et al., 2009; Wünsch, 2009; Mnejja et al., 2010).

The first genetic diversity study with SSRs for quince was undertaken with 20 quince cultivars in 2004 (Yamamoto et al., 2004), and this study assumed that SSRs from pear and apple could be transferred to quince, which belongs to the same subfamily. Many researchers have used SSR and inter-simple sequence repeat (ISSR) markers to identify quince populations and clonal variation (Dumanoğlu et al., 2009; Bassil et al., 2011; Ganopoulos et al., 2011). Although pomological data have been described for some native Turkish quince accessions (Küden et al., 2009), quince genetic germplasm has not been fully identified yet, especially using SSR markers.

To assist the breeding and germplasm management of Turkish quince germplasm, the present study aimed to generate an SSR database for traditionally cultivated Turkish quince. A total of 15 quince accessions available at the National Quince Germplasm Repository at Eğirdir-Isparta/Turkey were analyzed for variation and genetic relationships at eight SSR loci.

MATERIAL AND METHODS

Plant material

Young leaves of 15 commercial quince cultivars were collected from the Horticultural Research Institute of Egirdir in Isparta, Turkey. In addition to these 15 accessions, two apple cultivars (Florina and Golden Delicious) and three pear accessions [Passa Cnassana, Williams, and Ankara (Büyük Malatya)] obtained from the Atatürk Central Horticultural Research Institute in Yalova, Turkey, were used as references for SSR locus data analysis and to determine allele profiles among species (Table 1).

DNA isolation

DNA was extracted from leaf tissue following Lefort et al. (1998) as described in Şelli et al. (2007). The DNA concentration was estimated spectrophotometrically (NanoDrop ND-1000) and the DNA quality was checked by 1% agarose gel electrophoresis.

SSR analysis

A total of eight SSR markers, namely CH01F02, CH01H10, CH02B12, CH01D08, and CH04E03 developed from the apple genome (Gianfranceschi et al., 1998; Liebhard et al., 2002), and KA4b, KA14, and KA16 developed from pear genome (Yamomoto et al., 2001), were used in this study. Polymerase chain reaction (PCR) amplifications were performed as described by Şelli et al. (2007). 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 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 the CEQ 8800XL capillary DNA analysis system (Beckman Coulter, Ful-

lerton, CA, USA). Allele sizes were determined for each SSR loci using the Beckman CEQ Fragment Analysis software. In each run, two apple accessions (Golden Delicious, Florina) and three pear cultivars [Passa Cnassana, Wiliams, and Ankara (Büyük Malatya)] were included as reference cultivars in order to have consistent allele sizes over all runs, and these accessions enabled allele size comparison with other germplasms.

Genetic analysis

Identical cultivars, number of alleles, allele frequency, expected (H_E) and observed (H_O) heterozygosities, estimated frequency of null alleles (r), and probability of identity (PI) were calculated for each loci using the IDENTITY 1.0 program (Wagner and Sefc, 1999) according to Paetkau et al. (1995). The proportion of shared alleles was calculated 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 the unweighted pair-group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973), using the Numerical Taxonomy and Multiware Analysis System software (NTSYS-pc) (version 2.0) (Rohlf, 1988).

RESULTS

A total of eight SSR markers derived from apple and pear were used to determine the genetic diversity of 15 quince accessions, and a total of 44 polymorphic alleles were identified. The allele sizes (bp) of 20 accessions at the eight SSR loci are given in Table 1. Among the quince cultivars, the number of polymorphic alleles in each locus varied from 2 (CH04E03) to 12 (CH01F02), with an average of 5.5 (Table 2). The level of polymorphism among accessions was calculated with PI values for each of the eight SSR loci. PI values among the accessions ranged from 0.097 (CH01F02) to 0.625 (CH04E03) (Table 2). The most informative locus with regards to the PI was CH01F02 (PI = 0.097), and the least informative locus was CH04E03 (PI = 0.625).

Table 1. Allele sizes (bp) of quince and reference apple and pear cultivars at 8 SSR loci.

Species	No.	Cultivar	CH01F02	CH01H10	CH01D08	CH02B12	CH04E03	KA14	KA4b	KA16
Quince	1	Alaycık	122:138	93:95	271:271	108:120	188:200	159:159	110:136	159:159
	2	Bardacık	122:132	93:95	271:271	108:120	188:200	159:159	102:132	159:175
	3	Bencikli	146:184	93:95	259:275	120:136	200:200	159:159	110:136	135:159
	4	Bursa	166:182	93:95	259:271	108:120	200:200	139:149	110:136	135:159
	5	Çengelköy	148:182	93:95	271:271	108:120	188:188	151:159	110:136	141:159
	6	Eşme	162:180	97:97	261:275	116:138	200:200	151:159	110:136	141:159
	7	Havan	122:134	93:95	271:271	108:120	188:200	151:159	102:132	147:159
	8	İskilip	162:166	93:93	271:271	108:128	188:188	159:159	110:136	135:139
	9	İstanbul	166:182	85:87	271:271	108:120	188:200	159:159	110:136	135:159
	10	Kalecik	182:182	93:95	275:275	108:120	188:200	135:159	110:136	135:139
	11	Limon	166:182	93:95	275:275	108:120	188:200	159:159	110:136	135:159
	12	Osmancık	166:182	93:95	271:271	108:120	188:200	139:149	110:136	135:159
	13	Şekergevrek	178:182	93:95	261:271	108:120	188:200	135:159	110:136	135:139
	14	Tekeş	182:182	95:95	261:271	108:120	188:188	151:159	110:136	141:159
	15	Viranyadevi	166:182	93:95	271:271	108:120	188:200	151:159	110:136	135:159
Apple	16	Golden Delicious	168:178	93:111	249:271	140:140	198:198	167:167	136:138	143:147
	17	Florina	182:206	93:113	253:277	126:140	196:196	167:167	136:136	143:147
Pear	18	Passa Cnassana	166:174	105:105	279:283	154:154	178:178	179:187	80:94	117:127
	19	Ankara (Büyük M.)	156:164	99:109	281:295	134:134	178:178	179:185	94:94	123:133
	20	Wiliams	160:174	103:103	243:279	114:134	178:204	177:185	94:94	131:135

Table 2. Number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), probability of identity (PI), and the frequency of null alleles (r) of 15 quince cultivars analyzed at 8 SSR markers.

SSR loci	N_A	H_E	H_O	PI	r
CH01F02	12	0.802	0.866	0.097	-0.035
CH01H10	5	0.617	0.800	0.372	-0.112
CH01D08	4	0.544	0.333	0.342	0.136
CH02B12	6	0.620	1.000	0.366	-0.234
KA4b	4	0.615	1.000	0.379	-0.237
KA14	5	0.557	0.600	0.297	-0.027
KA16	6	0.700	0.933	0.229	-0.137
CH04E03	2	0.500	0.600	0.625	-0.066
Total	44	4.952	6.132		
Average	5.5	0.619	0.766		

The mean H_O ranged from 0.333 (CH01D08) to 1.000 (CH01F02, KA4b), with an average of 0.766, whereas H_E ranged from 0.500 (CH04E03) to 0.802 (CH01F02), with an average value of 0.619 (Table 2). The H_O values were found to be high for CH01F02, CH01H10, CH02B12, KA4b, KA14, KA16, and CH04E03, while the H_E value was relatively high for the CH01D08 locus.

The frequency of one or more alleles in each SSR locus were found to be higher than that of other alleles at the same loci: allele 182 in CH01F02 (0.3000), allele 93 in CH01H10 (0.3750), allele 275 in CH01d08 (0.1500), alleles 108 and 120 in CH02B12 (0.3250), allele 159 in KA14 (0.4750), allele 136 in KA4b (0.400), allele 159 in KA16 (0.3250), and alleles 188 and 200 in CH04E03 (0.3750) (Table 3). As for differences in allele size (bp) among reference cultivars and quince cultivars, comparison of apple and quince cultivars found no common allele size in CH02B12, CH04E03, and KA14, whereas comparison of pear and quince cultivars showed no common allele in CH02B12, CH04E03, CH01D08, CH01H10, KA14, and KA4b loci (Table 1 and Table 3). The frequency of null alleles at CH01D08 (r = 0.136) loci was positive (Table 2), but these low values showed the absence of null alleles.

Table 3. Allele frequencies of 8 SSR loci of quinces and reference apple and pear cultivars.

N	CH01f02	Freq.	CH01h10	Freq.	CH01d08	Freq.	CH02b12	Freq.	KA14	Freq.	KA4b	Freq.	KA16	Freq.	CH04e03	Freq.
1	122	0.0750	85	0.0250	243*	0.0250	108	0.3250	135	0.0500	80*	0.0250	117*	0.0250	178	0.1250
2	132	0.0250	87	0.0250	249*	0.0250	114*	0.0250	139	0.0500	94*	0.1250	123*	0.0250	188	0.3750
3	134	0.0250	93	0.3750	253*	0.0250	116	0.0250	149	0.0500	102	0.0500	127*	0.0250	196*	0.0500
4	138	0.0250	95	0.3250	259	0.0500	120	0.3250	151	0.1250	110	0.3250	131*	0.0250	198*	0.0500
5	146	0.0250	97	0.0500	261	0.0750	126*	0.0250	159	0.4750	132	0.0500	133*	0.0250	200	0.3750
6	148	0.0250	99*	0.0250	271	0.0500	128	0.0250	167*	0.1000	136	0.4000	135	0.2500	204*	0.0250
7	156*	0.0250	103*	0.0500	275	0.1500	134	0.0750	177*	0.0250	138	0.0250	139	0.0750	-	-
8	160*	0.0250	105*	0.0500	277*	0.0250	136	0.0250	179*	0.0500	-	-	141	0.0750	-	-
9	162	0.0500	109*	0.0250	279*	0.0500	138	0.0250	185*	0.0500	-	-	143*	0.0500	-	-
10	164*	0.0250	111*	0.0250	281*	0.0250	140*	0.0750	187*	0.0250	-	-	147	0.0750	-	-
11	166	0.1750	113*	0.0250	283*	0.0250	154*	0.0500	-	-	-	-	159	0.3250	-	-
12	168*	0.0250	-	-	295*	0.0250	-	-	-	-	-	-	175	0.0250	-	-
13	174*	0.0500	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	178	0.0500	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	180	0.0250	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	182	0.3000	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	182	0.0250	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	206*	0.0250	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Freq. = allele frequency. *Alleles were not found among quince genotypes.

Analysis of genetic similarity indices between quince cultivars showed that the lowest similarity was 18% (Eşme-Bardacık), while the highest similarity was approximately 87% (Bursa-Osmancık and Osmancık-Virandevi) (Table 4). In the dendrogram, reference cultivars and quince cultivars grouped in three main branches. Among the quince cultivars, Eşme showed significant distinction, being grouped in a different branch than the other quince cultivars (Figure 1). No synonymous accessions were found among the quince cultivars.

Table 4. Genetic similarities (%) between quince cultivars.

Quince cultivar	Alaycık	Bardacık	Bencikli	Bursa	Çengelköy	Eşme	Havan	İskilip	İstanbul	Kalecik	Limon	Osmancık	Şekergevrek	Tekeş	Viranyadevi
Alaycık	1														
Bardacık	0.750	1													
Bencikli	0.562	0.438	1												
Bursa	0.562	0.438	0.625	1											
Çengelköy	0.688	0.562	0.438	0.562	1										
Eşme	0.312	0.188	0.438	0.312	0.375	1									
Havan	0.688	0.812	0.375	0.438	0.625	0.250	1								
İskilip	0.562	0.438	0.375	0.438	0.562	0.250	0.375	1							
İstanbul	0.688	0.562	0.500	0.625	0.625	0.312	0.500	0.625	1						
Kalecik	0.562	0.438	0.562	0.562	0.562	0.312	0.438	0.500	0.562	1					
Limon	0.688	0.562	0.688	0.688	0.625	0.375	0.500	0.562	0.750	0.812	1				
Osmancık	0.688	0.562	0.500	0.875	0.688	0.250	0.562	0.562	0.750	0.625	0.750	1			
Şekergevrek	0.625	0.500	0.500	0.625	0.625	0.312	0.500	0.562	0.625	0.812	0.688	0.688	1		
Tekeş	0.562	0.438	0.375	0.500	0.812	0.438	0.500	0.438	0.562	0.562	0.562	0.562	0.625	1	
Viranyadevi	0.750	0.625	0.562	0.750	0.812	0.375	0.688	0.625	0.812	0.688	0.812	0.875	0.750	0.688	1

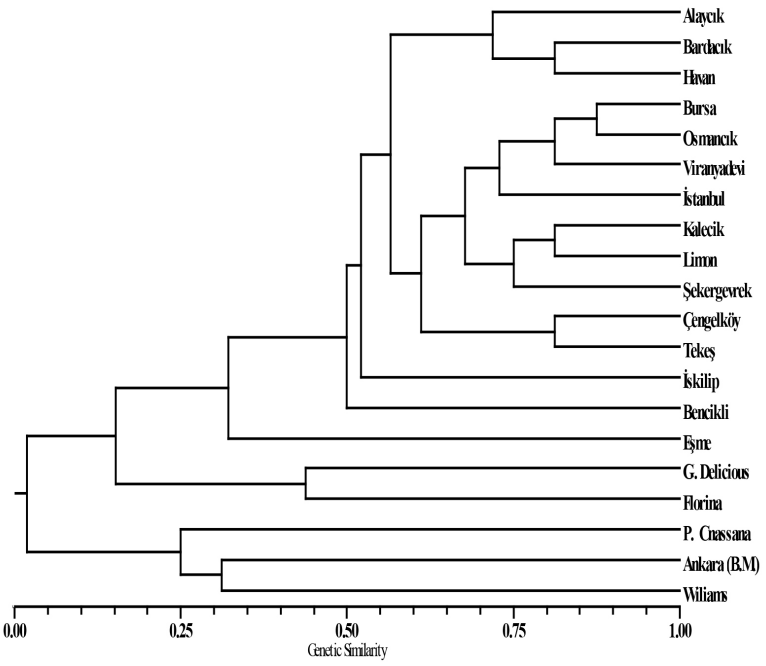

Figure 1. UPGMA cluster analysis of the SSR data from quince and reference pear and apple cultivars based on Jaccard's coefficient of genetic similarity.

DISCUSSION

SSR markers developed from other *Prunus* species (apple and pear) were used successfully for the genetic characterization of quince cultivars in this study, as has been observed previously (Dumanoğlu et al., 2009; Halász et al., 2009; Bassil et al., 2011).

In a study carried out by Halász et al. (2009) for the characterization of 36 quince cultivars using seven SSR loci developed from apple, it was reported that the loci CH04E03 and CH01F02 gave lower allele numbers when compared to other SSR studies (Liebhard et al., 2002; Galli et al., 2005) conducted on apple cultivars. In the present study, although the CH04E03 locus also gave the lowest number of alleles, the CH01F02 locus was surprisingly found to produce the highest number of alleles of all loci. In addition, in a study (Bassil et al., 2011) to identify 91 quince cultivars from different countries along with Turkish cultivars such as Ekmek, Limon, Şekergevrek, and Tekeş with SSR markers, polymorphism degrees determined in CH01F02, CH01D08, and CH01H10 loci were found to be low. However, in our study, among the 15 quince cultivars, these loci gave 12, 4, and 5 alleles, respectively, and showed a significant level of polymorphism.

Yamamoto et al. (2004) reported that SSR loci originating from apple and pear could be used in genetic distinction and identification studies for quince; however, they also reported that KA4b, KA14, and KA16 loci from pear could not be used for identification, as they showed more than two bands. In our study, these loci showed adequate polymorphism and played an active role in identification and distinction of the 15 quince cultivars analyzed.

In addition, Dumanoğlu et al. (2009) found that in addition to CH01F02, CH01H10, CH02B12, and CH01D08, the loci KA14 and KA16 showed a clear distinction in clones analyzed, and clonal differences emerged, indicating that these loci are indeed effective in quince cultivar identification.

Due to the lack of any common allele between species, three loci (CH02B12, CH04E03, and KA14) and six loci (CH02B12, CH04E03, CH01D08, CH01H10, KA14, and KA4b) can preferably be used for comparisons of pear-quince and apple-quince cultivars, respectively.

In our study, the H_O values were found to be high for CH01F02, CH01H10, CH02B12, KA4b, KA16, and CH04E03 loci (Table 2), and previous reports also found relatively high H_O values for CH01F02, CH02B12, and KA16 (Dumanoğlu et al., 2009) and for CH01H10 (Dumanoğlu et al., 2009; Bassil et al., 2011) in some quince cultivars.

Similarity ratios between 'Limon-Kalecik' and 'Limon-İstanbul' cultivars that were based on RAPD markers used in a previous study (Bayazit et al., 2011) were similar with those obtained in the present study. The similarity ratio between Kalecik-Osmancık was found to be the same (62%) in both studies. Similarity ratios between Çengelköy-Limon and Çengelköy-İstanbul cultivars were not equivalent between studies; however, and there was instead a 20% difference. The fact that we observed no synonymous cultivars among quince species, and that genetic similarity ratios between 15 local quince species were very high indicate the richness of quince gene sources in Anatolia.

Although germplasms of fruit species (grape, plum, apricot, etc.) from Turkey have been genetically characterized with SSR markers and other molecular markers (Ayanoğlu et al., 2007; Akpınar et al., 2010; Bayazit et al., 2011; Yilmaz et al., 2011), no SSR-based genetic identification study has been conducted on Turkish quince germplasm without clonal variation study of Kalecik quince cultivar by Dumanoğlu et al. (2009). The findings of this study will

help to identify Turkey's quince germplasm, and will assist in the improvement of agricultural practices, such as quince propagation, quince breeding, and promote better management strategies for quince cultivars.

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