Journal of Applied Botany and Food Quality 89, 82 - 88 (2016), DOI:10.5073/JABFQ.2016.089.010

[1]Erciyes University Department of Horticulture, Melikgazi, Kayseri, Turkey
[2]Fruit Research Institute, Egirdir, Isparta, Turkey
[3]Plant Protection Central Research Institute, Ankara, Turkey
[4]Ataturk University Department of Horticulture, Erzurum, Turkey

Determination of genetic relatedness among Turkish apple germplasm based on ISSR markers

Aydın Uzun[1], Serif Ozongun[2], Osman Gulsen[1], Kadir Ugurtan Yılmaz[1], Suat Kaymak[3], Sezai Ercisli[4*]

(Received October 4, 2015)

Summary

Apple (*Malus domestica* Borkh.) is one of the most economically important pome fruits worldwide and Turkey is within origin center of apple. In this research, inter-simple sequence repeat (ISSR) markers were used to determine relationships among the Turkish apple accessions and some selected foreign cultivars and species. Fourteen ISSR primers produced a total of 111 fragments and 76 of them were polymorphic. The number of average polymorphic fragments per primer was 5.4. The mean polymorphism information content (PIC) was 0.37. The unweighted pair group method arithmetic average (UPGMA) analysis demonstrated that the accessions had a similarity range from 0.79 to 0.98. All accessions studied were discriminated and many subgroups were determined in the dendrogram based on the UPGMA analysis. High level of variation among the Turkish apples existed. Foreign cultivars, *M.baccata*, *M. prunifolia* and *M. sylvestris* accessions studied mix-clustered among the Turkish accessions. For sub-structuring Bayesian analysis, 71 loosely or uncorrelated markers with less than 10% missing data were used. This indicated absence of subpopulations, meaning well and equal introgression of genetic backgrounds or species available among the accessions. It can be concluded that Turkey was rich in apple genetic diversity, which may provide opportunities for apple breedind programs.

Introduction

Fruit, which contains phytochemicals that are being studied for added health benefits, has been recognized as a good source of vitamins and minerals, and for their role in preventing vitamin C and vitamin A deficiencies (BACVONKRALJ et al., 2014; ROP et al., 2014).

Among fruits, apple has special importance because it is one of the most produced fruit crops among the temperate fruits with over 75 million tons of production per year (FAO, 2012). Domesticated apples (*Malus domestica* Borkh.) have been cultivated since ancient times and are now produced in a range of area from Siberia with freezing temperatures during winter as low as -40 ºC to some equatorial locations with high temperatures (JANICK et al., 1996). Four origin centers were reported for apples including East Asia, Middle Asia, East Asia-Europe and North America. Turkey belongs to East Asia-Europe origin center and has considerable diversity (JANICK et al., 1996).

In earlier time, breeding programs were based only on selections from naturally growing apple trees. Then, hybridization became a more preferable tool for obtaining economically important new cultivars. Origin of domesticated apples was probably based on *M. sieversii* known as wild apple in central Asia (HARRIS et al., 2002; COART et al., 2006). It was argued that *M. sylvestris*, the wild apple of Western Europe, might have contributed little or even nothing to the domesticated apple gene pool at least as maternal ancestor (ROBINSON et al., 2001). Similarly, *M. sieversii* was reported as the main contributor to the genome of the cultivated apple and *M. sylvestris*, in particular, determined as the secondary contributor. Evolution of domesticated apples occurred over a long time period and involved more than one wild species (CORNILLE et al., 2012). But some findings regarding to this issue that three most frequent chloroplast haplotypes of *M. domestica* and *M. sylvestris* were nearly absent in the analysed *M. sieversii* accessions concluded complex origin of domesticated apples. Also high level of cpDNA diversity was detected among the genus *Malus* cultivars, which was explained by the hypothesis of the complex hybrid origin of *M. domestica* (COART et al., 2006). Genetic diversity and phylogenetic studies on apples have been carried out using various marker systems. Simple sequence repeats (SSRs) markers were the most reliable system for this kind of studies (PATZAK et al., 2012; GARKAVA-GUSTAVSSON et al., 2013). In addition, amplified fragment length polymorphism (AFLP) (KENIS and KEULEMANS, 2005; NING et al., 2007); chloroplast DNA (cpDNA) (COART et al., 2006) markers were used for estimating apple genetic diversity, relationships and constructing genetic maps of apples.

Inter-simple sequence repeat (ISSR) markers were reported to have higher reproducible rates due to the use of longer primers (16-25-mers) than the RAPD primers (10-mers). This marker system is cost effective because multiple loci are amplified during PCR amplification (ZIETKIEWICZ et al., 1994). It was previously used for apple genetic studies (GOULAO and OLIVEIRA, 2001; HE et al., 2011).

Turkey is both within origin center of apples and a major apple producer with 2.88 million tons of production (FAO, 2012). Hence there is considerable genetic diversity of apples in Turkey. In addition, introductions from foreign countries are available in apple genetic resources of Turkey. Conservation and characterization of this genetic pool are required for breeding programs and future utilization. In present study, genetic variation and relationships among the apple accessions collected from different parts of Turkey and some selected foreign cultivars were investigated.

Materials and methods

Plant material and DNA isolation

One hundred and fifty-eight apple accessions were used for this study including 152 *M. domestica* cultivars and local genotypes, three *M. sylvestris* accessions, two *M. baccata* and one *M. prunifola* accession (Tab. 1). The rest included common apple cultivars such as Golden Delicious, Granny Smith and Red Chief. For DNA extractions, leaf tissues of all accessions were obtained from the collection located Fruit Research Station in Egirdir of Isparta, Turkey. Total genomic DNA was extracted from young leaves by the CTAB method as described by DOYLE and DOYLE (1990). DNA concentration was measured with a spectrophotometer (BioTek Instruments, Inc. Vinooski, United States) and 10 ng/mL DNA templates were made using TE (10 mM Tris-HCl,1 mM EDTA, pH 8.0).

* Corresponding author

Tab. 1: Name of apple accessions utilized in this study

1	Lodiearlygolden	41	180887(5.2)	81	E73	121	42.bs.5(Almila)
2	Yazelmasi(2484)	42	190887(1.4)	82	E2411	122	42.e.2(Ankaraguz.)
3	Yazelmasi(2482)	43	180887(4.4)	83	E65	123	42.c.5(Yazamasya)
4	Beyazelma(2575)	44	200887(1.2)	84	E5	124	42.e.6(Kabaelma)
5	Ferik	45	Blackjon	85	E392e	125	42.ko.(Yaylapinari)
6	Karanfil(2570)	46	Daldabir	86	E24	126	42.e.4(Mayhosy.k.)
7	Beyelmasi(2477)	47	220887(3.2)	87	Batum	127	42.e.3(Hanimteni)
8	Kiselmasi(2590)	48	230887(1.2)	88	E14	128	42.e.7(Yildizkiran)
9	Sahelmasi(2600)	49	250887(1.10)	89	E13	129	42.kp.3(Karapinar)
10	Gelinelmasi(2475)	50	Yaztavsanbasi	90	Sarigobek	130	42.c.3(Tatlitavsanb.)
11	Sekerelması(2551)	51	210887(1.1)	91	Candir	131	32E1
12	Tatlielma(2511)	52	E220887	92	E25	132	Yenice
13	Sarielma	53	E180887(2.1)	93	E11	133	Orak
14	Gobek(2455)	54	E210887(1.4)	94	Uzunyumra	134	Pancarlik
15	Sogutelma(2480)	55	E170887(2.5)	95	E32	135	Samsun
16	Susuzelma(2500)	56	E210887(2.1)	96	Cigit	136	Harim
17	Mektepelmasi(2565)	57	E130887(2.3)	97	E383e	137	Gelendost
18	Altinokelmasi(2490)	58	Reinettetardiva	98	Karpuz	138	Inebolu
19	Elma(2590)	59	E70	99	Portakal	139	Golden Delicious
20	Demir(2486)	60	E42	100	E33	140	Jonagold
21	Rizedemir	61	E71	101	Gurcu	141	Ozark Gold
22	Sandik	62	E40	102	E2	142	Royal Gala
23	Petek(2577)	63	E55	103	542E	143	Elstar
24	Oltuelmasi(2594)	64	E52	104	E1	144	Melrose
25	Cincik(2471)	65	E51	105	Cidagut	145	Idared
26	Mahsusaelmasi	66	E50	106	E6	146	Fuji
27	Elma(2523)	67	E49	107	Sinap	147	Red Chief
28	Petevrekelmasi	68	E82	108	384E	148	Gloster
29	Tavsanbasi(2531)	69	E57	109	E4	149	Granny Smith
30	Tatlielma(2492)	70	E78	110	Seker	150	E72
31	Pasaelmasi	71	E66	111	E35	151	Cooper43
32	Lazelmasi(2507)	72	E47	112	E10	152	E33
33	Hüryemez	73	E56	113	Piraziz	153	*M. prunifolia*
34	Kadirhatice	74	E37	114	Gümüshane	154	*M. baccata 1*
35	Güztavsanbasi	75	E63	115	Gemlik.3	155	*M. baccata 2*
36	180887(5.1)	76	E81	116	Tokat.1	156	*M. sylvestris 1*
37	Sivanorelmasi	77	E9	117	Tokat.3	157	*M. sylvestris 2*
38	Kalkandelen	78	E76	118	Tokat.4	158	*M. sylvestris 3*
39	Karasaki	79	E48	119	42.kp.1		
40	Gurcu	80	E67	120	42.a.1		

ISSR analysis

Fourteen ISSR primers previously evaluated by FANG and ROOSE (1997) and GULSEN et al. (2010) were used for all apple accessions (Tab. 2). PCR reaction components and PCR cycling parameters were performed as described by UZUN et al. (2009). PCR products were separated on 2% agarose gel in 1 X TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 V for 2.5-3 h. The fragment patterns were photographed under UV light for further analysis. A 100 bp standard DNA ladder (GeneRuler, Fermentas) was used for estimating size of ISSR fragments.

Data analysis

Each band was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc version 2.1) software package (ROHLF, 2000). A similarity matrix was constructed based on Dice's coefficient (DICE, 1945), which considers only one to one matches between two taxa for similarity. The similarity matrix was used to construct a dendrogram using the unweighted pair group method arithmetic average (UPGMA) to determine genetic relationships in the germplasm studied. Goodness of fit test called Mantel test was performed by using

Tab. 2: Results on ISSR primers used for apple accessions

Primers	Total Fragments	Polymorphic Fragments	Polymorphism (%)	PIC	Resolving Power
(AG)7YC	8	6	75	0.18	12.7
(AGC)6G	12	8	67	0.14	16.5
(CA)8R	6	3	50	0.06	10.1
(CAA)6	10	10	100	0.26	8.5
(CAC)3GC	6	4	67	0.26	8.4
(CT)8TG	7	6	86	0.24	5.7
(GA)8YG	5	3	60	0.04	9.8
(GACA)4	14	12	86	0.27	11.1
(GT)6GG	10	8	80	0.22	10.1
(GT)8YA	9	8	89	0.26	9.9
HVH(TCC)7	6	3	50	0.04	10.1
(TCC)5RY	8	2	25	0.03	12.3
DBDA(CA)7	6	2	33	0.02	11.9
HVH(CA)7T	4	1	25	0.02	7.9
Mean	7,9	5,4	68,5	0.15	10.3
Total	111	76	-	-	-

ultrametric distance matrix obtained from the dendrogram and similarity matrix (MANTEL, 1967). The result of this test is a cophenetic correlation coefficient, *r*, indicating how well the dendrogram represents similarity data. Polymorphic Information Content (PIC) for dominant markers was calculated as: $PIC = 1 - [f^2 + (1 - f)^2]$, where 'f' is the frequency of the marker in the data set. PIC for dominant markers is a maximum of 0.5 for 'f' = 0.5 (DE RIEK et al., 2001).

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). A Principal Coordinate Analysis (PCoA) was performed based on the variance covariance matrix calculated from ISSR data. PCoA is a computational alternative to Principle Coordinate Analysis (PCA). PCA is used for similarities and PCoA for dissimilarities. The data matrix was used to calculate distance matrix, then the distance matrix was double-centered, the double-centered matrix was then factored and a plot was made (ROHLF, 2000).

Results and discussion

ISSR analysis yielded 111 fragments and 76 of them (68.5%) were polymorphic. Number of bands scored per primer varied between 4 ($HVH(CA)_7T$) and 14 ($(GACA)_4$), with a mean of 7.9. The GenAlEx ver. 6.5 program was employed, to determine allele frequency (p and q), no of effective alleles (Ne), Shannon's information index (I), expected (He) and unbiased expected heterozygosity (uHe) (PEAKALL and SMOUSE, 2012). The PIC values for the 21 primer combinations ranged from 0.02 ($HVH(CA)_7T$ and $DBDA(CA_7)$) to 0.27 ($(GACA)_4$) with a mean of 0.15 (Tab. 2). Cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be relatively high ($r = 0.76$, $P < 0.01$).

Values for effective alleles (Ne) ranged from 1.02 ($(TCC)_5RY$) to 1.48 ($(AG)_7YC$) (average 1.28), for Shannon's information index from 0.04 ($(TCC)_5RY$) to 0.43 ($(GT)_8YA$) (average 0.28), for expected heterozygosity (He) and unbiased expected heterozygosity (uHe) from 0.02 ($(TCC)_5RY$) to 0.28 ($(GT)_8YA$) (average 0.18) (Tab. 3). A dendrogram was constructed by using the UPGMA analysis based on 111 ISSR markers. The apple accessions studied had similarity values ranging from 0.79 to 0.98 indicating a high level of variation (Fig. 1). Similarly, GOULAO and OLIVEIRA (2001) found similarity levels of ~0.75-1.00 among 41 apples according to ISSR data. On the other hand, GULSEN et al. (2010) determined higher variation among 192 apple accessions using POGP (peroxidase gene-based polymorphism) markers. In the present study, all accessions were distinguished. The dendrogram consisted of many subgroups. Foreign cultivars and three apple species studied (*M. baccata*, *M. prunifolia* and *M. sylvestris*) nested mixed with Turkish accessions. PCA was performed based on the genetic distance matrix to better

Tab. 3: ISSR primers studied, their estimated allele frequency (p & q), no of effective alleles (Ne), Shannon's information index (I), expected (He) and unbiased expected heterozygosity (uHe).

Primers	P	q	Ne	I	He	uHe
(AG)7YC	0.66	0.34	1.48	0.39	0.27	0.27
(AGC)6G	0.60	0.40	1.26	0.27	0.17	0.17
(CA)8R	0.80	0.20	1.13	0.14	0.09	0.09
(CAA)6	0.30	0.70	1.40	0.41	0.26	0.26
(CAC)3GC	0.57	0.43	1.42	0.37	0.25	0.25
(CT)8TG	0.39	0.61	1.37	0.37	0.23	0.23
(GA)8YG	0.90	0.10	1.24	0.26	0.16	0.16
(GACA)4	0.29	0.71	1.34	0.33	0.21	0.21
(GT)6GG	0.38	0.62	1.38	0.38	0.24	0.24
(GT)8YA	0.41	0.59	1.47	0.43	0.28	0.28
HVH(TCC)7	0.79	0.21	1.13	0.17	0.10	0.10
(TCC)5RY	0.76	0.24	1.02	0.04	0.02	0.02
DBDA(CA)7	0.94	0.06	1.14	0.15	0.10	0.10
HVH(CA)7T	0.93	0.07	1.15	0.19	0.11	0.11
Mean	0.62	0.38	1.28	0.28	0.18	0.18

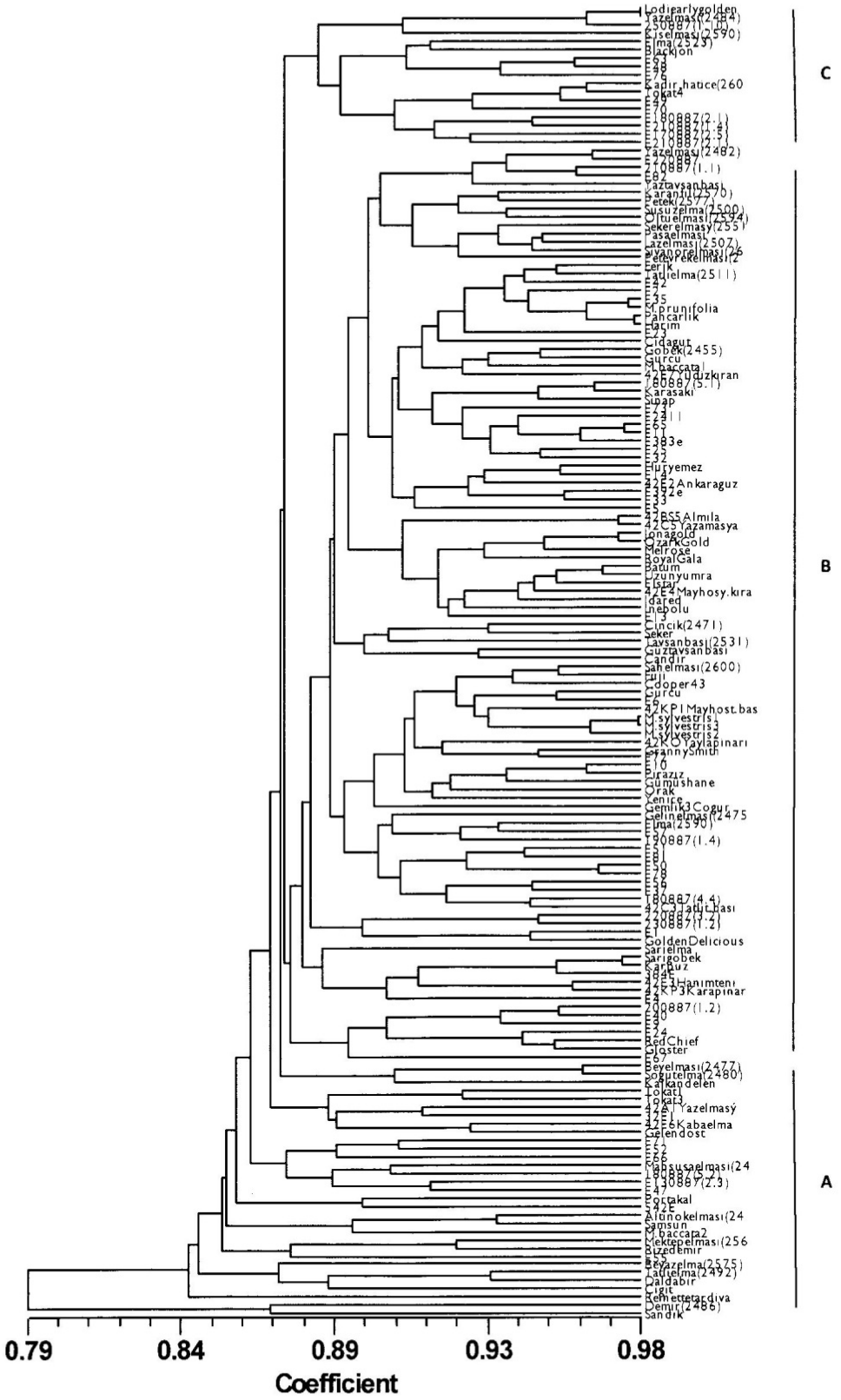

Fig 1: Dendrogram of 158 apple accessions based on the ISSR markers and the UPGMA method.

understand genetic relationships. Fig. 2 presents the distribution of different genotypes according to two principal axes of variation using PCoA, which revealed the variation among the accessions similar to the UPGMA analysis.

Among the apples studied 'Sandik' and 'Demir (2486)' were the most distinct accessions with similarity value of 0.79. These two apples were collected from Northeast Anatolia and East Black Sea region of Turkey (CETINER, 1981). In another study, 'Demir (2486)' was clearly separated from the other apples (GULSEN et al., 2010). 'Reinette Tardiva' was also apart from other apples with similarity

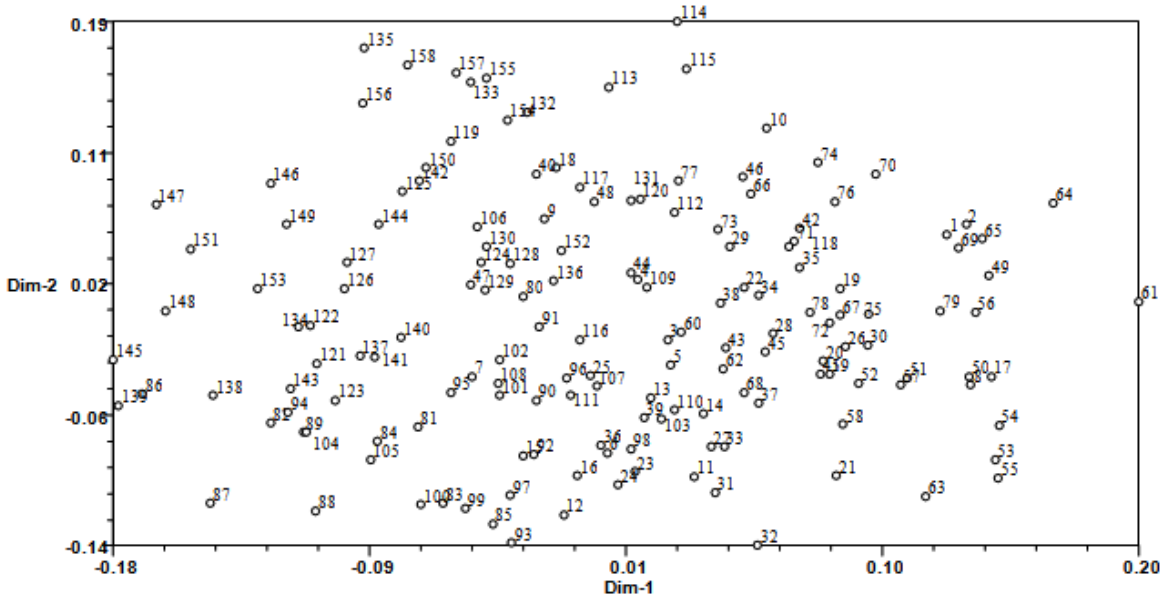

Fig. 2: Principal coordinate analysis diagram showing the relationships among 158 apple accessions (numbers of accessions were identifed in Tab. 1).

of 0.84. 'Cigit', 'Daldabir', 'Tatlielma (2492)', 'Beyazelma (2575)' were nested in the same subgroup while 'Mektep' apple, 'Rizedemir' and 'E55' apples were in the same cluster. '*M. baccata* 1' was clustered with 'Altinok' and 'Samsun' accessions.

Two *M. baccata* accessions studied in present research were distinguished from each other and clustered with the Turkish local accessions. 'Portakal' and '542E', two little acidulated accessions were in the same subgroup. 'Mahsusa elmasi', 'E47', '130887', '180887', 'E66', 'E52' and 'E71' were clustered closely. Another subgroup consisted of '42E6 Kabaelma', '42A1 Yazelması' sampled from Konya province, '32E1', 'Gelendost' sampled from Isparta province and 'Tokat1', 'Tokat3'. The UPGMA clustering grouped the genotypes into meaningful clusters. Three apples 'Kalkandelen', 'Sogutelma (2486)' and 'Beyelmasi (2477)' having similar fruit characteristics with sourish flavour were clustered together. In the dendrogram describing the accessions above generated many different groups (A).

In the dendrogram, the rest of 127 apple accessions were grouped into two main groups, B and C. Group B had more accessions and were separated into two subgroups. Two foreign red skin cultivars ('Gloster' and 'Red Chief') and five local Turkish accessions were clustered in the smaller group of B. Foreign cultivars were not grouped apart from the Turkish accessions. They were mixed clustered with local apples, indicating similar genetic backgrounds. Accordingly, Sonmezoglu and Kutuk (2014) found that 23 local apple genotypes collected from Karaman, Turkey and three foreign cultivars were divided into two major groups and numerous subgroups, revealing a rich variation among the apple genotypes. On the other hand, Pereira-Lorenzo et al. (2008) found genetic difference between Spanish apple accessions and non-native cultivars. Similarly, Gasi et al. (2010) found that traditional Bosnia and Herzegovina cultivars were differentiated quite clearly from foreign cultivars, except for few genotypes. Differences between the present study and others may be because of genetic backround of local apple accessions studied. Large subgroup of group B divided into eight subclusters. One of them consisted of sourish flavour accessions including 'Sarielma', 'Sarigobek', 'Karpuz', 'Hanimteni (42E3)', 'Karapinar (42KP3)'. Last two apples also found in the same cluster in previous study based on POGP markers (Gulsen et al., 2010). These two accessions were collected from the same region of Turkey.

One of the foreign cultivars, 'Golden Delicious' was apart from the other common cultivars and clustered with three local apples. In group B, the largest subcluster consisted of 30 apple accessions. Similarity of these accessions was between 0.89 and 0.98. In this subcluster, three foreign cultivars, 'Granny Smith', 'Cooper' and 'Fuji', three *M. sylvestris* accessions and many local accessions were nested. Some of the local apples, 'Gelin', '42KO1 Yaylapinari' and '42KP1 Mayhostavsanbasi' shared the same fruit characteristics such as soury flavor. 'Granny Smith' and 'Fuji' were closely related based on ISSR data. Similarly, these two cultivars were grouped closely according to SSR (Gasi et al., 2010) and POGP markers (Gulsen et al., 2010). On the other hand, Goulao and Oliveira (2001) found that 'Granny Smith' and 'Fuji' were distinct based on ISSR data. Three *M. sylvestris* accessions studied nested in this subcluster and were nearly identical. They were closely related to the Turkish local apple accessions belong to *M. domestica*. Coart at al. (2006) found high levels of haplotype sharing between *M. sylvestris* and *M. domestica* and assumed an interspecific gene flow, which is probably bidirectional and brought about by the use of (local) wild *Malus* genotypes for the (local) cultivation process of apple. Four local apples, 'Candir', 'Güztavsanbasi', 'Tavsanbasi', 'Seker' and 'Cincik' were grouped closely in the dendrogram. Two of them 'Güztavsanbasi' and 'Tavsanbasi' were collected from the same province of Turkey (Cetiner, 1981).

Most of foreign cultivars studied in the present study ('Idared', 'Elstar', 'Royal Gala', 'Melrose', 'Ozark Gold' and 'Jonagold') were grouped together. Five local Turkish accessions were also nested in this group. 'Elstar', 'Jonagold' and 'Gala (Galaxy)' were clustered based on SSR data (Gasi et al., 2010). In another study, 'Royal Gala', 'Jonagold' and 'Ozark Gold' were clustered closely but 'Idared' and 'Melrose' nested in the same group and slightly distinct from these three cultivars (Goulao and Oliveira, 2001). Two apple species (*M. baccata* (No:1) and *M. prunifolia*) were closely related. They were clustered with several Turkish accessions such as 'Huryemez', 'Karasaki', 'Gurcu', 'Cidagut', 'Harim', 'Pancarlik' and 'Ferik'. Two *M. baccata* accessions studied were apart from each other. The similar results reported by Hokanson et al. (2001) and Yao et al. (2010) indicated variation among the *M. baccata* samples. Only one *M. prunifolia* accession was used in this study and it was highly similar to *M. baccata* (No:1) but not identical to

M. baccata (No:2). *Malus prunifolia* and *M. baccata* were found in diverse clusters in a previous study (FORTE et al. 2002). Similarly, HARRIS et al. (2002) concluded that these two species were apart from each other according to nuclear ribosomal internal transcribed spacer gene. In addition, ZHOU and LI (2000) assumed that *M. prunifolia* originated from hybridization between *M. sieversii* and *M. baccata*. The last subcluster of large subgroup of group B consisted of 14 apple accessions including 'Petevrekelmasi', 'Sivanora', 'Lazelmasi', 'Pasaelmasi', 'Sekerelmasi', 'Oltuelmasi', 'Susuzelma', 'Petek', 'Karanfil', 'Yaztavsanbasi', 'Yazelmasi', 'E82', 'E220887' and '210887 (1.1)'. In the dendrogram, 16 Turkish accessions and two foreign cultivars ('Blackjon' and 'Lodi Early Golden') were clustered in group C. 'Lodi Early Golden' was very similar to 'Yazelmasi (2484)' and both were early maturing cultivars.

Conclusions

Several significant results were obtained from this study. ISSR markers confirmed efficiency for characterization of apple germplasm and cultivar identification. All of 158 apple accessions were distinguished from each other. The Turkish apple accessions were closely related to the other known species, *M. sylvestris*, *M. prunifolia* and *M. baccata*. These three species were clearly separated from each other and they were mixed grouped with the Turkish accessions. This verified gene flow among apple species and local apple genotypes. Turkey has considerable morphological and molecular diversity in its apple genetic resources. Turkey is located in East Asia-Europe origin center for apple and the middle of three important continents (Asia, Africa and Europe). This region including Turkey and Iran was important in apple domestication and their transfer from Central Asia to the western countries (GHARGANI et al., 2009). The accessions studied in present study are maintained in the germplasm plots and are being investigated for important agronomic characters to exploit potential interest.

Acknowledgements

The authors thank Scientific Research Projects Unit of Erciyes University for funding and supporting the project with the project number of FBA-10-3299.

References

BACVONKRALJ, M., JUG, T., KOMEL, E., FAJT, N., JARNI, K., ZIVKOVIC, J., MUJIC, I., TRUTIC, N., 2014: Effects of ripening degree and sample preparation on peach aroma profile characterization by headspace solid-phase microextraction. Turk. J. Agric. For. 38, 676-687.

CETINER, E., 1981: Turkish plant genetic resources fruit and vineyard inventory. Ege Bölge Zirai Ars. Enst. Yay. No: 19. (In Turkish).

COART, E., VAN GLABEKE, S., DE LOOSE, M., LARSEN, A.S., ROLDAN-RUIZ, I., 2006: Chloroplast diversity in the genus *Malus*: New insights into the relationship between the European wild apple (*Malus sylvestris* (L.) Mill.) and the domesticated apple (*Malus domestica* Borkh.). Mol. Ecol. 15, 2171-2182.

CORNILLE, A., GLADIEUX, P., SMULDERS, M.J., ROLDAN-RUIZ, I., LAURENS, F., LE CAM, B., NERSESYAN, A., CLAVEL, J., OLONOVA, M., FEUGEY, L., GABRIELYAN, I., ZHANG, X.G., TENAILLON, M.I., GIRAUD, T., 2012: New insight into the history of domesticated apple: Secondary contribution of the european wild apple to the genome of cultivated varieties. PLOS Genet. 8 (5), e1002703. doi:10.1371/journal.pgen.1002703.

DE RIEK, J., CALSYN, E., EVERAERT, I., VAN BOCKSTAELE, E., DE LOOSE, M., 2001: AFLP based alternatives for the assessment of distinctness, uniformity and stability of sugar beet varieties. Theor. Appl. Genet. 103, 1254-1265.

DICE, L.R., 1945: Measures of the amount of ecologic association between species. Ecology 26, 297-302.

DOYLE, J.J., DOYLE, J.L., 1990: Isolation of plant DNA from fresh tissue. Focus 12, 13-15.

FANG, D.Q., ROOSE, M.L., 1997: Identification of closely related *Citrus* cultivars with inter-simple sequence repeat markers. Theor. Appl. Genet. 95, 408-417.

FAO, Food and Agricultural Organization of the United Nations., 2012. http://faostat.fao.org

FORTE, A.V., IGNATOV, A.N., PONOMARENKO, V.V., DOROKHOV, D.B., SAVELYEV, N.I., 2002: Phylogeny of the *Malus* (Apple Tree) species, inferred from the morphological traits and molecular DNA analysis. Russ. J. Genet. 3, 1150-1160.

GARKAVA-GUSTAVSSON, L., MUJAJU, C., SEHIC, J., ZBOROWSKA, A., BACKES, G.M., HIETARANTA, T., ANTONIUS, K., 2013: Genetic diversity in Swedish and Finnish heirloom apple cultivars revealed with SSR markers. Sci. Hortic. 162, 43-48.

GASI, F., SIMON, S., POJSKIC, M., KURTOVIC, N., PEJIC, I., 2010: Genetic assessment of apple germplasm in Bosnia and Herzegovina using microsatellite and morphologic markers. Sci. Hortic. 126, 164-171.

GHARGANI, A., ZAMANI, Z., TALAIE, A., ORAGUZIE, N.C., FATAHI, R., HAJNAJARI, H., WIEDOW, C., GARDINER, S.E., 2009: Genetic identity and relationships of Iranian apple (*Malus domestica* Borkh.) cultivars and landraces, wild *Malus species* and representative old apple cultivars based on simple sequence repeat (SSR) marker analysis. Genet. Res. Crop Evol. 56, 829-842.

GOULAO, L., OLIVEIRA, C.M., 2001: Molecular characterisation of cultivars of apple (*Malus* × *domestica* Borkh.) using microsatellite (SSR and ISSR) markers. Euphytica, 122, 81-89.

GULSEN, O., KAYMAK, S., OZONGUN, S., UZUN, A., 2010: Genetic analysis of Turkish apple germplasm using peroxidase gene-based markers. Sci. Hortic. 125, 368-373.

HARRIS, S.S., ROBINSON, J.P., JUNIPER, B.E., 2002: Genetic clues to the origin of the apple. Trends Genet. 18, 426-430.

HE, P., LI, L., LI, H., WANG, H., YANG, J., WANG, Y., 2011: Genetic analysis of wild apple resources in Shandong province based on inter-simple sequence repeats (ISSR) and sequence-specific amplification polymorphism (S-SAP) markers. Afr. J. Biotechnol. 10, 9501-9508.

HOKANSON, S.C., LAMBOY, W.F., SZEWC-MCFADDEN, A.K., MCFERSON, J.R., 2001: Microsatellite (SSR) variation in a collection of *Malus* (apple) species and hybrids. Euphytica, 118, 281-294.

JANICK, J., CUMMINS, J.N., BROWN, S.K., HEMMAT, M., 1996: Apples. In: Janick, J., Moore, J.N. (ed.), Fruit Breeding, Tree and Tropical Fruits, 1, 1-78.

KENIS, K., KEULEMANS, J., 2005: Genetic linkage maps of two apple cultivars (*Malus domestica* Borkh.) based on AFLP and microsatellite markers. Mol. Breed. 15, 205-219.

MANTEL, N., 1967: The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209-220.

NING, Z., HONG-XIANG, S., XIA-HONG, G., YUN-CONG, Y., YANG, W., YONG-QING, F., 2007: Relationship between ornamental and wild species of *Malus* in China. Acta Hortic. Sinica 34**,** 1227-1234.

PATZAK, J., PAPRSTEIIN, F., HENYCHOVA, A., SEDLAK, J., 2012: Genetic diversity of Czech apple cultivars inferred from microsatellite markers analysis. Hortic. Sci. 39, 149-157.

PEAKALL, R., SMOUSE, P.E., 2012: GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatic. 28, 2537-2539.

PEREIRA-LORENZO, S., RAMOS-CABRER, A.M., GONZALEZ-DIAZ, A.J., DIAZ-HERNANDEZ, A.B., 2008: Genetic assessment of local apple cultivars from La Palma, Spain, using simple sequence repeats (SSRs). Sci. Hortic. 117, 160-166.

ROBINSON, P., HARRIS, S.A., JUNIPER, B.E., 2001: Taxonomy of the genus Malus Mill. (Rosaceae) with emphasis on the cultivated apple, *Malus domestica* Borkh. Plant Syst. Evol. 226, 35-58.

ROHLF, F.J., 2000: NTSYS-pc, numerical taxonomy and multivarite analysis

system, version 2.11. New York, Exeter Setauket.

ROP, O., ERCISLI, S., MLCEK, J., JURIKOVA, T., HOZA, I., 2014: Antioxidant and radical scavenging activities in fruits of 6 sea buckthorn (*Hippophae rhamnoides* L.) cultivars. Turk. J. Agric. For. 38, 224-232.

SMITH, J.S.C., CHIN, E.C.L., SHU, H., SMITH, O.S., WALL, S.J., SENIOR, M.L., MITCHEL, S.E., KRESORICH, S., TIEGLE, J., 1997: An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor. Appl. Genet. 95, 163-173.

SONMEZOGLU, O.A., KUTUK, A., 2014: Determination of genetic diversity among Karaman apple landraces. J. Food Agric. Environ. 12, 52-54.

UZUN, A., GULSEN, O., KAFA, G., SEDAY, U., 2009: Field performance and molecular diversification of lemon selections. Sci. Hortic. 120, 473-478.

YAO, L., ZHENG, X., CAI, D., GAO, Y., WANG, K., CAO, Y., TENG, Y., 2010: Exploitation of Malus EST-SSRs and the utility in evaluation of genetic diversity in Malus and Pyrus. Genet. Res. Crop Evol. 57, 841-851.

ZIETKIEWICZ, E., RAFALSKI, A., LABUDA, D., 1994: Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics 20, 176-183.

ZHOU, Z.Q., LI, Y.N., 2000: The RAPD evidence for the phylogenetic relationship of the closely related species of cultivated apple. Genet. Res. Crop Evol. 47, 353-357.

Address of the corresponding author:
E-mail: sercisli@gmail.com