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Determination of Morphological, Pomological and Molecular Variations among Apples in Niğde, Turkey using iPBS Primers

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ABSTRACT
In addition to morphological and pomological techniques, the molecular analysis produces more information for diversity studies. Recently, the iPBS marker system is one of the techniques and a new marker system for apple studies. In this study, morphological, pomological, and molecular characteristics of local apples were investigated in 48 different samples from 29 different rural areas with varying altitudes between 1125-1726 m in Niğde, Turkey. Fruit size, fruit weight, the color of fruit peel, total soluble solids content, fruit flesh firmness characteristics are important in terms of yield, quality, storage, transportation and attractiveness. According to the pomological results from these traits, CKR2, DMR3, CLL, HCB2, YSL, ULG, ELM1, ICM have been found to superior among genotypes. In order to molecular results, the similarity of the samples varies between 0.61-1.00, under the light of this result, molecular data differentiated all individuals used in the study except one pair. Molecular data displayed that these differences were caused by genotypic differences as well as environmental conditions. This study has contributed further information about the usage of iPBS primers on apple. To protect the plant material used in the study, a collection orchard was established with genotypes. To conclude, the findings are expected to shape future breeding studies.

Keywords: *Malus domestica*, Genetic resource, Genetic diversity, Molecular analysis, Polymorphism

1. Introduction

Apple (*Malus domestica* Borhk.), which is cultivated all over the world, has great diversity and the number of known cultivars are more than 6500 and, particularly 600 cultivars in Turkey (Hancock 2012). Central Asia, Caucasus, and Turkey were the center of origin for apples. Kayseri and Ağrı provinces are two major diversity points in Turkey (Ozbek 1978). According to FAO 2020 stats, Turkey has an important position in world apple production, ranked fourth amongst China, United States, and Poland. According to TUIK 2020 stats, Niğde is one of the three major provinces in Turkey's apple production and 81.0% of Niğde's apple production is covered by Central and Bor districts. Niğde has important advantages for apple production as follows: the flat land structure of the province is suitable for establishing orchards, land prices are cheaper than other provinces producing apples in Turkey, fewer diseases have been seen such as black spots by low humidity and better-colored fruits by the temperature difference between day/night (Anonymous 2014).

The key to high yields is application of modern agricultural techniques (Demir & Doğan 2020). Although new and modern apple orchards have started to establish in Niğde recently, a lot of established orchards that are using old agricultural practices for many years. In this case, the size and color of fruits are non-uniform. The local non-registered cultivars that are widely cultivated in the province are 'Amasya', 'Orak Apple', 'Demir Apple', 'Tavşanbaşı', 'Arapkızı' and 'Hüryemez'. Also, in recent years, popular cultivars such as 'Granny Smith', 'Fuji', 'Red Chief', 'Mondial Gala', 'Super Chief', 'Scarlet Spur' have been cultivated in the newly established orchards by a dwarf and semi-dwarf rootstocks.

Morphological and pomological characteristics are environmentally affected due to the nature of it. This information is not enough to effectively differentiate individuals. On the other hand, genetic characteristics supply more stable information for this purpose. After the development of molecular techniques and utilization of plant breeding, the researcher got a chance to clarify their phenotypic data with genetic data. Thus, researchers enhance the accuracy of their studies.

The selection of parents is always an important process for plant breeders and genetic similarity data helps to make more precise decisions about this step. Numerous DNA marker systems are available to show genetic variations among plants. Simple sequence repeat (SSR) (Hokanson et al. 1998; Hokanson et al. 2001; Zhang et al. 2007, Bakır et al. 2019), inter-simple sequence repeat (ISSR) (Smolik & Krzysztoszek 2010), RAPD (random amplified polymorphic DNA) (Dunemann et al. 1994; Zhou & Li

2000) and amplified fragment length polymorphism (AFLP) (Kenis & Keulemans 2005) are just some of them. AFLP and SSR systems are considered as a substantial way to indicate genetic variations in plants but these methods demand more costly instruments, usage of advanced steps in the process, and a significant amount of time compared to others.

Inter-primer binding sites (iPBS) retrotransposon marker system was developed by Kalendar et al. (2010) for plants and as well as animal kingdoms. The importance of this marker system is not to require sequence knowledge about plant/animal of interest. Retrotransposons can relocate themselves to the genome via copying. This mechanism usually ended up with different outcomes but the main result, expansion of genome size as well as genetic variation. For this reason, retrotransposons are accepted as valuable tools among other molecular marker systems.

Other studies on fruits have been carried out using iPBS. Guo et al. (2013), conducted a study with 35 grape varieties to evaluate for their molecular diversity associated with iPBS markers. In their experiment, 99 polymorphic DNA bands were produced with 15 iPBS primers. They indicated iPBS markers suitable for genetic diversity studies on grapes. Rovna et al. (2020), carried out a study with Rosa canina fruits to determine their genome size, iPBS profiles as well as antioxidant and antimicrobial actives. Their results suggested that iPBS markers provide favorable techniques for evaluating the genetic variability of Rosa canina.

Kuras et al. (2013), performed an experiment with 5 different DNA marker techniques to distinguish five different apple cultivars and their spots. According to their results, iPBS primers produced many polymorphic DNA output that has been able to distinguish five progenitor cultivars however not many polymorphic bands were sport specific. Correct utilization of iPBS marker system has been shown the power to identify different apple cultivars.

In general, the iPBS marker system is economic compared to other marker systems (which is an important feature especially for some countries), including screening huge part of plant genomes, usage for a different living organism, and user friendly to researchers (Kuras et al. 2013; Demirel et al. 2018; Milovanov et al. 2019).

Apple has already a growing market in Turkey. The current situation can be improved by eliminating known issues and evaluating the potential of local cultivars. This is the first study conducted in Niğde with these local apple genotypes since the study of Eltez & Kaska (1985). This study aims to evaluate the situation in the manner of genetic diversity to apple on the region of interest. For archive to this goal, iPBS markers utilized as a main tool and results also shown a convenient method to apple. Some studies were conducted on apple cultivars & mutants with iPBS markers, but this is the first study to the utilization of iPBS marker system on apple genotypes. This study is also the first step of the future breeding program in the region.

2. Material and Methods

The study was carried out with 48 different apple trees in 29 rural areas of Niğde, Turkey in 2018-2019 (Table 1). Altitude values of trees vary between 1125-1726 m (Table 1). ‘Super Chief’, ‘Fuji’, ‘Granny Smith’ cultivars taken from Niğde Ömer Halisdemir University Faculty of Agricultural Sciences and Technologies Research and Application Orchard was used as a control group for pomological analysis. In addition to pomological control groups ‘Golden Delicious’, ‘Scarlet Spur’ cultivars taken from the application orchard and known local apples called ‘green sour apple’, ‘sour summer apple’, ‘red sour apple’, ‘golden seed’, ‘rabbit head’, ‘bowl apple’, ‘red summer apple’ also used in the control group of molecular analysis.

Table 1- Information about plant materials (apple trees) and locations

Tree codes	*Name of locations*	*GPS data*	*Elevation (meter)*
KMR	Kemerhisar	37°49'56.9"N 34°35'29.3"E	1125
BHC	Bahçeli	37°50'06.7"N 34°36'39.5"E	1147
SZL	Sazlıca	37°54'04.3"N 34°38'34.8"E	1211
HLC	Halaç	37°49'39.0"N 34°41'19.3"E	1297
KRC	Karacaören	37°48'04.1"N 34°43'36.9"E	1487
KLV	Kılavuz	37°47'53.8"N 34°46'06.7"E	1571
HVZ	Havuzlu	37°46'38.0"N 34°37'59.1"E	1213
PST	Postallı	37°43'46.9"N 34°45'17.0"E	1394
DGR	Değirmenli	38°02'54.4"N 34°54'06.4"E	1494
DND	Dündarlı	38°05'28.7"N 35°09'54.4"E	1326
CKR1	Çukurbağ	37°50'09.6"N 35°03'25.8"E	1484
CKR2	Çukurbağ	37°50'08.7"N 35°03'33.2"E	1493
CKR3	Çukurbağ	37°49'60.0"N 35°03'27.7"E	1499
CKR4	Çukurbağ	37°50'07.1"N 35°03'21.4"E	1480
CKR5	Çukurbağ	37°50'07.2"N 35°03'10.9"E	1455
BDM1	Bademdere	37°55'04.7"N 35°04'14.8"E	1601
BDM2	Bademdere	37°55'01.5"N 35°04'18.1"E	1595
BDM3	Bademdere	37°54'58.9"N 35°04'24.5"E	1586
BDM4	Bademdere	37°54'53.9"N 35°04'24.2"E	1582
BDM5	Bademdere	37°54'47.8"N 35°04'26.2"E	1576
PNR1	Pınarbaşı	37°53'43.7"N 35°05'00.8"E	1574
PNR2	Pınarbaşı	37°53'36.7"N 35°05'15.9"E	1569
PNR3	Pınarbaşı	37°53'26.4"N 35°05'35.5"E	1572
PNR4	Pınarbaşı	37°53'15.0"N 35°06'02.0"E	1562
DMR1	Demirkazık	37°51'41.0"N 35°05'31.5"E	1577
PNR5	Pınarbaşı	37°53'06.4"N 35°06'24.2"E	1598
DMR2	Demirkazık	37°51'32.2"N 35°05'16.6"E	1558
DMR3	Demirkazık	37°51'28.7"N 35°05'04.8"E	1545
DMR4	Demirkazık	37°51'28.4"N 35°04'50.9"E	1556
DMR5	Demirkazık	37°51'25.4"N 35°04'43.4"E	1560
CLL	Celaller	37°48'34.6"N 34°56'09.5"E	1687
BRC	Burç	37°48'12.9"N 34°59'11.4"E	1445
ELG	Elekgölü	37°46'18.5"N 35°00'59.3"E	1365
KVL1	Kavlaktepe	37°59'29.8"N 35°05'34.0"E	1671
KVL2	Kavlaktepe	37°59'00.8"N 35°05'34.9"E	1726
HCB1	Hacıbeyli	38°07'17.7"N 35°09'19.9"E	1280
HCB2	Hacıbeyli	38°07'05.3"N 35°09'28.9"E	1283
DKL	Dikilitaş	38°06'56.9"N 35°04'25.3"E	1435
YSL	Yeşilova	38°03'31.3"N 34°49'58.3"E	1388
ULG	Uluağaç	38°02'34.6"N 34°50'20.2"E	1435
GMS	Gümüşler	37°59'56.2"N 34°45'59.7"E	1344
HMM	Himmetli	38°02'08.8"N 34°56'32.7"E	1552
ELM1	Elmalı	38°01'52.1"N 34°57'41.6"E	1603
ELM2	Elmalı	38°01'12.8"N 34°58'29.0"E	1605
KCP	Kocapınar	38°01'37.2"N 35°05'43.2"E	1571
EYN	Eynelli	37°53'51.3"N 35°03'46.9"E	1531
ICM	İçmeli	38°03'24.2"N 35°05'49.6"E	1519
YLT	Yelatan	37°40'51.6"N 35°01'14.0"E	1320

2.1 Morphological analysis

Morphological analysis was conducted before harvest (Tijskens et al. 2007). Tree habit (Upright, spreading, drooping, weeping), tree trunk diameter (Measured from 15 cm above to ground) and height of tree trunk (Measured from grafting point to first branches) (cm), one-year-old shoot length (cm), leaf blade attitude in relation to shoot (Upwards, outwards, downwards), leaf blade length and width (mm), leaf blade incisions of margin (Crenate, bicrenate, serrate type 1, serrate type 2, biserrate), petiole length (mm) and fruit general shape (Cylindrical waisted, conic, ovoid, cylindrical, ellipsoid, globose, obolid) measurements was collected for morphological analysis (UPOV 2005). All measurements except tree habit, tree trunk diameter and height of tree trunk, held with 3 repeats, each repeat subjected to 5 related plant materials in total 15.

2.2 Pomological analysis

The pomological analysis was carried out for 3 repeats, each repeat contains 5 fruit in total 15 ripe fruits for each tree. Fruit height and diameter (mm), fruit weight (g), depth of stalk cavity (mm), fruit skin color (Measured by KONICA MINOLTA CM-700d Spectrophotometer), the color of flesh (White, cream, yellowish, greenish, pinkish, reddish), number of seeds, the aperture of locules (Closed or slightly open, moderately open, fully open), firmness of flesh (Measured by handheld fruit penetrometer) (kg/cm^2), pH (Measured by VWR pHenomenal 1000L digital ph meter) and total soluble solids content (Measured by KRÜSS AR2008 digital refractometer) measurements were collected for pomological analysis (UPOV 2005).

2.3 Molecular analysis with iPBS primers

DNA extraction was conducted by the CTAB method from 3-5 young leaves taken from each tree (Dellaporta et al. 1983). The concentrations of the DNAs were then determined by the Quawell Q5000 UV-Vis Spectrophotometer and diluted to 5 ng /uL. IPBS primers were used in molecular marker analyzes (Table 4).

In total 60 apple genotypes (12 of them belong to the control group) were evaluated with 15 iPBS markers developed by Kalendar et al. (2010) to show the genetic diversity of these apple genotypes (Table 4). Diluted DNAs was amplified by PCR. The PCR was performed in a 25 µL reaction mixture containing 5 µL DNA (5ng/µL), 2.5 µL 10X DreamTaq PCR buffer, 0.375 µL dNTPs, 3 µL primer for 18 bp primers & 5 µL primer for 12-13 bp primers, 0.2 µL DreamTaq DNA polymerase.

Following initial denaturation at 95 °C for 3 min, PCR was conducted in order of amplification for 35 cycles with denaturation at 95 °C for 15 s annealing at 50-63 (specific to primers in Table 4) for 60 s and extension at 72 °C for 2 min. Lastly, the final extension was completed in a stage of 72 °C for 7 min. Products of PCR electrophoresed at 60 volts for 2.5 hours on a 1.8% agarose gel prepared with 1X TAE, stained with ethidium bromide for 30 minutes, and then viewed with Bio-Rad Gel Doc™ XR + gel imaging system. PCR or electrophoresis process repeated if it requires to get a clearer image of gel that suitable for scoring.

2.4 Statistical analysis

The SAS program was used for statistical analysis of pomological data (SAS 2005). The Duncan's Multiple Range Test was used to differentiate the mean values of the significant values ($P<0.05$). In the evaluation of the data obtained as a result of molecular analysis, the result file was created in a binary number system according to whether the molecular markers used in the gel images were shown as (1) or not (0). From these results, a similarity matrix was created with the appropriate Jaccard method and then data clustering and TKoA analyzes were applied by using the NTSYS program (Rohlf 1998). As a result of the clustering analysis, the dendrogram was generated by the UPGMA method. Mantel's matrix correspondence test was used to test the agreement of the dendrogram with the similarity matrix. Polymorphism information content (PIC) was calculated according to the formula given by Hinze et al. (2015) due to the iPBS makers system is a dominant marker. Principal Coordinates Analysis (PCoA) was performed using PAST 4.03 software (Hammer et al. 2001).

3. Results

3.1 Morphological results

Duncan's Multiple Range Test was applied to quantitative data of morphological observations. According to the morphologic results, no 'upright' types were found in any tree habit characteristics, and 'drooping' is the most common type 20 times. The highest values (significant in statically) found in tree trunk diameter was KVL2 (46.63), the height of tree trunk was BDM1 (148.5) cm, one-year-old shoot length was BDM4 (114.22 cm). 'Downwards' type was not found in leaf blade attitude in relation to shoot characteristics and the 'upwards' type was the most common feature with 33 times. The highest values (statically significant) found in leaf blade length was ULG (87.57 cm), leaf blade width was KCP (47.59 cm). Among leaf blade incisions of margin characteristics 'crenate' and 'bicrenate' type was not found, 'serrate type 2' is the most common type with 25 times. The highest values (statically significant) found in petiole length were KVL1 (36.65cm). Results of fruit general shape indicated that only 'globose' and 'obloid' features were founded among the genotypes and 'obloid' was the most common type with 33 times (Table 2).

The results obtained from the morphological analyzes revealed the expected characteristics and values of the local Niğde, Turkey apples, and provided a basis for further characterization studies.

Table 2- Morphological analysis results

Tree codes	*Tree habit*	*Tree trunk diameter*	*Height of tree trunk*	*One year old shoot length*	*Leaf blade attitude in relation to shoot*	*Leaf blade length*	*Leaf blade width*	*Leaf blade incisions of margin*	*Petiole length*	*Fruit general shape*
KMR	Spreading	32.79	83	51.56	Upwards	71.8	45.08	Serrate type 2	30.91	Globose
BHC	Drooping	40.33	106.5	60.32	Upwards	68.25	39.58	Serrate type 2	33.51	Obolid
SZL	Spreading	13.05	92.5	30	Upwards	58.27	32.44	Serrate type 2	24.79	Obolid
HLC	Drooping	15.37	100.3	70.28	Upwards	63.52	40.1	Biserrate	27.25	Obolid
KRC	Weeping	32.75	79	83.12	Upwards	62.71	35.79	Biserrate	27.27	Obolid
KLV	Weeping	27.28	112	51.82	Upwards	70.72	40.93	Serrate type 1	33.3	Obolid
HVZ	Weeping	33.01	108	73.94	Outwards	66.56	40.87	Biserrate	31.6	Obolid
PST	Weeping	27.95	84	53.98	Upwards	74.84	38.82	Serrate type 2	26.64	Obolid
DGR	Drooping	38.13	125.7	63.8	Upwards	73.75	40.67	Serrate type 2	31.37	Obolid
DND	Drooping	22.38	108.5	44.82	Upwards	72.15	40.02	Serrate type 2	29.51	Globose
CKR1	Drooping	20.18	123	49.52	Upwards	64.56	39.09	Biserrate	32.31	Obolid
CKR2	Drooping	20.72	74.3	38.66	Upwards	70.03	36.28	Serrate type 1	27.81	Obolid
CKR3	Drooping	39.57	82.2	107.5	Outwards	71.21	36.41	Biserrate	29.17	Obolid
CKR4	Weeping	23.62	132.5	43.08	Outwards	77.86	44.15	Biserrate	35.01	Obolid
CKR5	Spreading	26.74	116	60.46	Outwards	80.93	42.16	Biserrate	34.02	Globose
BDM1	Spreading	30.46	148.5	41.24	Upwards	66.83	36.59	Serrate type 1	31.8	Obolid
BDM2	Drooping	33.61	132	71.36	Upwards	73.79	44.8	Serrate type 2	30.17	Obolid
BDM3	Drooping	36.06	50.4	65.78	Upwards	78.49	39.8	Biserrate	29.93	Globose
BDM4	Weeping	20.21	64	114.22	Outwards	73.55	41.28	Serrate type 2	34.17	Obolid
BDM5	Weeping	23.87	122	62.94	Outwards	72.47	40.4	Serrate type 2	36.21	Obolid
PNR1	Spreading	33.9	123	85.24	Upwards	69.58	41.89	Serrate type 2	29.73	Globose
PNR2	Drooping	24.67	112.4	51	Upwards	72.11	44.26	Serrate type 2	35.48	Obolid
PNR3	Spreading	28.74	97.4	81.44	Upwards	76.93	46.23	Serrate type 2	35.02	Obolid
PNR4	Drooping	32.79	83	51.56	Upwards	71.8	45.08	Biserrate	30.91	Obolid
PNR5	Weeping	40.33	106.5	60.32	Upwards	68.25	39.58	Biserrate	33.51	Obolid
DMR1	Weeping	13.05	92.5	30	Upwards	58.27	32.44	Serrate type 1	24.79	Obolid
DMR2	Weeping	15.37	100.3	70.28	Outwards	63.52	40.1	Biserrate	27.25	Obolid
DMR3	Weeping	32.75	79	83.12	Upwards	62.71	35.79	Serrate type 2	27.27	Obolid
DMR4	Drooping	27.28	112	51.82	Upwards	70.72	40.93	Serrate type 2	33.3	Obolid
DMR5	Drooping	33.01	108	73.94	Upwards	66.56	40.87	Serrate type 2	31.6	Globose
CLL	Drooping	27.95	84	53.98	Upwards	74.84	38.82	Serrate type 2	26.64	Obolid
BRC	Drooping	38.13	125.7	63.8	Outwards	73.75	40.67	Serrate type 2	31.37	Obolid
ELG	Spreading	22.38	108.5	44.82	Upwards	72.15	40.02	Serrate type 2	29.51	Obolid
KVL1	Weeping	36.48	87.3	60.7	Upwards	69.19	38.95	Serrate type 1	36.65	Obolid
KVL2	Drooping	46.63	88.1	55.76	Upwards	71.65	45.84	Serrate type 2	32.17	Globose
HCB1	Weeping	30.81	92.3	51.92	Upwards	79.4	43.73	Serrate type 1	33.44	Globose
HCB2	Drooping	40.14	79.7	57.96	Upwards	71.13	43	Serrate type 1	29.84	Globose
DKL	Spreading	35.81	101.3	61.32	Outwards	68.43	40.78	Biserrate	30.12	Globose
YSL	Drooping	23.65	62.1	67.14	Outwards	79.09	38.87	Biserrate	33.82	Obolid
ULG	Spreading	31.83	79.2	57.68	Outwards	87.57	47.36	Serrate type 2	33.48	Globose
GMS	Weeping	33.3	79.4	41.06	Outwards	80.33	44.98	Biserrate	30.49	Obolid
HMM	Spreading	21.74	60.4	76.16	Upwards	65.47	38.25	Serrate type 2	23.81	Globose
ELM1	Spreading	31.26	73.6	45.64	Outwards	69.21	41.26	Serrate type 2	23.5	Globose
ELM2	Drooping	37.97	88.6	54.36	Outwards	77.43	40.96	Serrate type 2	33.58	Globose
KCP	Spreading	33.84	120	62.08	Upwards	87.44	47.59	Serrate type 2	36.2	Globose
EYN	Spreading	38.67	98.7	71.08	Outwards	80.77	47.49	Serrate type 2	34.69	Obolid
ICM	Drooping	32.47	121.7	72.78	Upwards	82.24	40.48	Biserrate	30.66	Obolid
YLT	Spreading	35.91	117.6	81.38	Upwards	83.9	46.46	Serrate type 1	35.18	Obolid

3.2 Pomological analysis

According to the Duncan's Multiple Range Test, the highest values (significant in statically) was found in fruit diameter CKR2 (72.64 mm), YSL (72.17 mm), ULG (72.07 mm), HCB2 (71.40 mm); fruit height ULG (66.40 mm); fruit weight ULG (154.44 g), YSL (147.50 g), HCB2 (145.50 g), CKR2 (144.04 g); depth of stalk cavity HCB2 (17.76 mm); the number of seeds SZL (10.33 pieces); firmness of flesh ELM1 (9.10 kg/cm^2); fruit skin color L value DMR3 (65.62), a value ICM (37.07), b value DMR3 (30.15), CLL (29.859). The most common types in flesh color were white with 31 samples and greenish with 7 samples. It was not found yellowish, pinkish, and reddish color flesh. The most observed type in the aperture of locules closed or slightly open with 28 samples and the fully open with the least common two samples. The highest value for pH was found in DMR5

(3.88) and the lowest value was in DMR3 (3.05). The highest amount of total soluble solids content was seen in ICM (14.60%) and the lowest value was found in HCB1 (10.30%) (Table 3).

Table 3- Pomological analysis results

Genoype & cultivar	FHE	FDI	FWE	DSC	FSC-dL	FSC-da	FSC-db	CFE	NSE	ALO	FFE	pH	TSSC
Fuji	71.92	58.12	152.15	14.24	50.91	17.56	18.51	2	9.07	1	10.04	3.40	13.00
Granny Smith	74.12	65.73	175.17	15.64	61.67	8.70	41.40	4	7.87	2	9.67	3.24	13.28
Super Chief	75.05	66.78	181.38	16.39	38.39	27.39	13.31	2	5.73	2	6.08	3.51	14.70
KMR	62.05	52.59	93.99	13.09	50.40	26.83	14.30	1	8.80	1	6.86	3.74	11.40
BHC	60.86	46.44	81.71	13.13	66.91	16.39	26.02	1	9.00	1	6.68	3.61	11.00
SZL	59.55	49.13	83.61	11.13	62.27	14.38	22.51	1	10.33	1	6.70	3.65	11.00
HLC	66.20	57.76	112.19	13.71	56.16	20.24	19.18	1	7.00	1	6.99	3.51	12.60
KRC	63.05	56.03	100.50	13.21	53.64	21.37	17.63	1	8.60	1	7.13	3.50	13.30
KLV	61.46	52.43	88.67	11.81	59.66	19.70	21.95	1	8.60	1	7.15	3.71	13.70
HVZ	59.60	47.78	79.16	12.35	56.20	24.00	18.19	1	8.47	1	7.64	3.50	13.80
PST	57.52	49.49	78.10	11.43	61.34	12.78	22.89	1	8.33	1	6.82	3.59	11.40
DGR	59.17	51.17	86.41	12.02	59.64	18.15	19.66	4	8.47	1	6.58	3.65	11.10
DND	58.05	51.24	81.72	11.41	63.64	9.83	24.00	1	6.87	1	6.95	3.55	10.80
CKR1	72.65	62.85	144.05	14.79	62.25	11.24	27.42	4	7.80	2	5.38	3.65	13.20
CKR2	63.33	53.22	98.21	12.94	55.20	21.17	19.75	1	6.93	2	6.97	3.61	12.40
CKR3	59.48	54.05	82.71	10.21	61.85	10.26	25.50	1	8.07	1	7.67	3.65	13.40
CKR4	62.09	52.86	91.88	16.51	58.57	19.37	20.69	1	8.47	1	7.17	3.56	11.00
CKR5	59.53	50.42	84.80	11.48	57.29	17.06	20.86	1	8.67	2	8.03	3.55	11.30
BDM1	64.12	56.40	103.81	13.14	50.67	27.50	14.59	1	9.53	1	7.32	3.59	12.30
BDM2	68.01	62.89	126.44	16.31	50.37	28.00	15.33	1	8.67	2	7.41	3.57	13.80
BDM3	58.56	50.71	81.83	12.02	59.89	16.80	21.16	1	8.20	1	7.25	3.41	12.40
BDM4	61.35	50.00	87.12	12.66	53.50	27.11	15.82	1	8.67	1	7.07	3.48	12.40
BDM5	58.21	50.06	80.69	11.84	54.94	23.58	17.54	1	10.27	1	7.52	3.50	11.40
PNR1	62.69	52.89	96.39	13.21	55.90	19.05	19.69	1	7.73	1	7.63	3.53	12.20
PNR2	65.03	55.70	103.93	13.01	52.92	26.32	16.23	1	7.33	1	7.49	3.43	13.60
PNR3	63.60	55.56	103.04	13.39	56.03	23.38	18.32	1	8.67	1	7.07	3.46	13.20
DMR1	63.62	55.73	103.78	12.94	52.31	27.68	20.96	1	8.00	1	6.76	3.76	13.30
PNR5	63.47	54.60	99.96	13.83	40.90	35.47	17.95	1	7.73	1	7.42	3.42	11.40
DMR2	67.33	59.32	124.54	13.92	53.66	21.52	23.55	1	8.60	3	6.49	3.42	13.80
DMR3	63.45	56.90	104.16	15.47	65.63	14.18	30.16	1	8.27	1	7.16	3.05	13.10
DMR4	63.10	56.01	104.91	13.03	46.94	30.96	17.12	1	9.47	1	6.83	3.52	13.10
DMR5	60.65	53.41	95.71	11.86	54.06	21.63	22.13	4	8.07	1	6.24	3.88	11.80
CLL	52.49	45.32	58.86	9.44	58.97	12.30	29.86	4	8.93	2	7.80	3.62	14.20
BRC	58.85	50.47	82.65	13.14	50.72	28.06	18.88	1	7.87	1	6.55	3.57	13.90
ELG	68.67	57.47	119.44	14.53	50.17	27.67	18.27	1	8.53	1	6.16	3.42	12.20
KVL1	66.14	56.47	110.46	14.36	53.73	18.25	25.96	2	6.73	1	6.65	3.35	13.60
KVL2	67.94	58.10	126.55	13.36	54.24	16.66	26.16	2	6.87	2	6.36	3.65	13.40
HCB1	60.32	53.73	92.63	12.92	51.90	25.35	20.11	1	6.73	1	6.90	3.47	10.30
HCB2	71.41	61.94	145.51	17.77	43.83	33.90	13.92	1	8.53	1	5.44	3.44	12.20
DKL	65.77	62.93	125.60	15.00	45.31	30.67	16.58	1	6.07	1	6.74	3.66	14.00
YSL	72.18	63.64	147.50	16.30	42.91	29.89	16.45	2	8.67	2	6.31	3.56	13.60
ULG	72.07	66.40	154.44	16.56	52.50	21.04	22.15	2	6.40	2	5.83	3.73	12.80
GMS	63.03	54.97	106.98	12.65	51.79	19.06	22.06	4	8.07	2	6.72	3.51	11.00
HMM	63.36	57.24	108.67	12.19	55.26	11.45	27.58	4	8.80	2	8.09	3.47	13.80
ELM1	52.75	48.59	66.20	8.51	53.45	17.56	24.83	4	7.40	2	9.11	3.56	14.40
ELM2	66.94	59.96	121.23	14.33	48.71	25.21	19.74	1	6.47	2	7.82	3.53	12.30
KCP	65.24	60.97	116.30	14.68	59.36	18.02	26.52	2	6.53	2	7.23	3.63	13.20
EYN	64.80	57.19	108.34	12.10	53.39	26.24	21.18	2	9.67	2	7.78	3.57	13.10
ICM	63.02	54.07	100.94	12.41	43.40	37.07	15.77	2	8.53	2	7.87	3.71	14.60
YLT	62.97	54.26	100.55	13.51	56.43	12.81	24.89	2	8.60	3	7.29	3.63	12.40

FHE: fruit height; FDI: fruit diameter; FWE: fruit weight; DSC: depth of stalk cavity; FSC-dL FSC-da FSC-db: fruit skin color; CFE: the color of flesh; NSE: number of seeds; ALO: aperture of locules; FFE: firmness of flesh; TSSC: total soluble solids content; CFE: white=1, cream=2, yellowish=3, greenish=4, pinkish=5, reddish=6; ALO: closed or slightly open=1, moderately open=2, fully open=3

3.3. Molecular results

Fifteen different iPBS primers were used in the study (Table 4) to the determination of total of 60 samples for molecular analysis. Gel images of the iPBS 2392 which one of the primers used in the study, are given in Figure 1. As a result of molecular analyzes, 143 polymorphic bands were obtained.

Table 4- Description of used iPBS primers with names, sequence, annealing temperature, polymorphism rates and PIC values

Primer names	Primer sequences	Temperatures of annealing (°C)	Polymorphism rate (%)	PIC value
2075	CTCATGATGCCA	50°C	94%	0.34
2381	GTCCATCTTCCA	50°C	79%	0.28
2382	TGTTGGCTTCCA	50°C	88%	0.34
2400	CCCCTCCTTCTAGCGCCA	50°C	52%	0.24
2398	GAACCCTTGCCGATACCA	51°C	71%	0.29
2252	TCATGGCTCATGATACCA	52°C	71%	0.39
2277	GGCGATGATACCA	52°C	55%	0.23
2375	TCGCATCAACCA	52°C	60%	0.16
2392	ATCTGTCAGCCA	52°C	88%	0.36
2085	ATGCCGATACCA	53°C	47%	0.21
2095	GCTCGGATACCA	53°C	15%	0.11
2232	AGAGAGGCTCGGATACCA	55°C	17%	0.13
2237	CCCCTACCTGGCGTGCCA	55°C	17%	0.10
2079	AGGTGGGCGCCA	60°C	13%	0.10
2081	GCAACGGCGCCA	63°C	5%	0.10

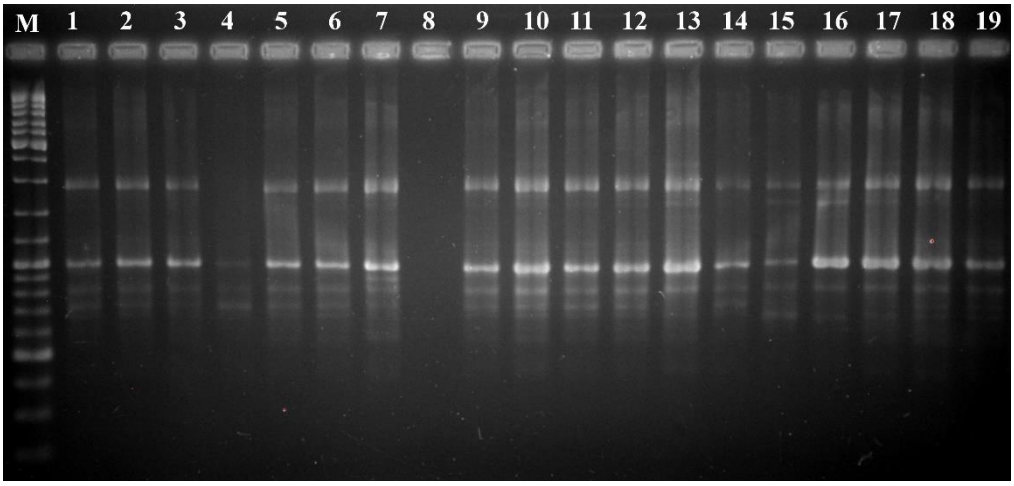

Figure 1- Gel image of iPBS 2392 primer, samples 1-19. 'M' stand for ladder (1 kb DNA ladder)

The main 48 samples were divided into 4 main branches and the similarity rates varied between 0.61-1.00 (Figure 2), with control groups these results extend to 7 main branches and the 0.54-1.00 similarity rates. Polymorphism rates of primers vary between 94%-5% and their PIC values vary between 0.10-0.34 (Table 4). The results of PCoA were similar to those of the cluster analysis. The first, second and third dimensions explained 23.6, 14.3 and 11.1% of the total variation making a total of 49% (Figure 3).

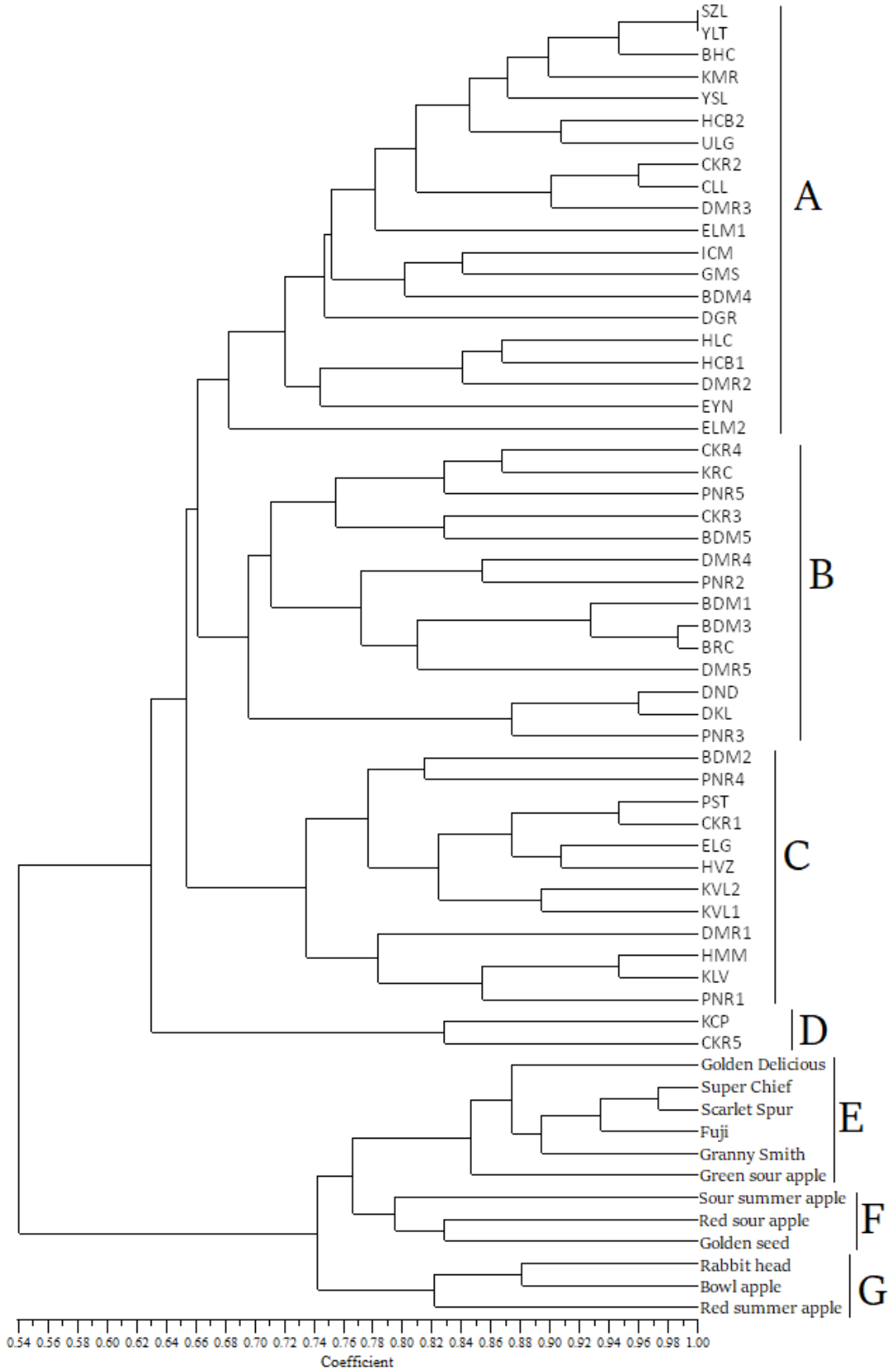

Figure 2- UPGMA dendrogram that shows the genetic diversity of samples with control groups using iPBS primers

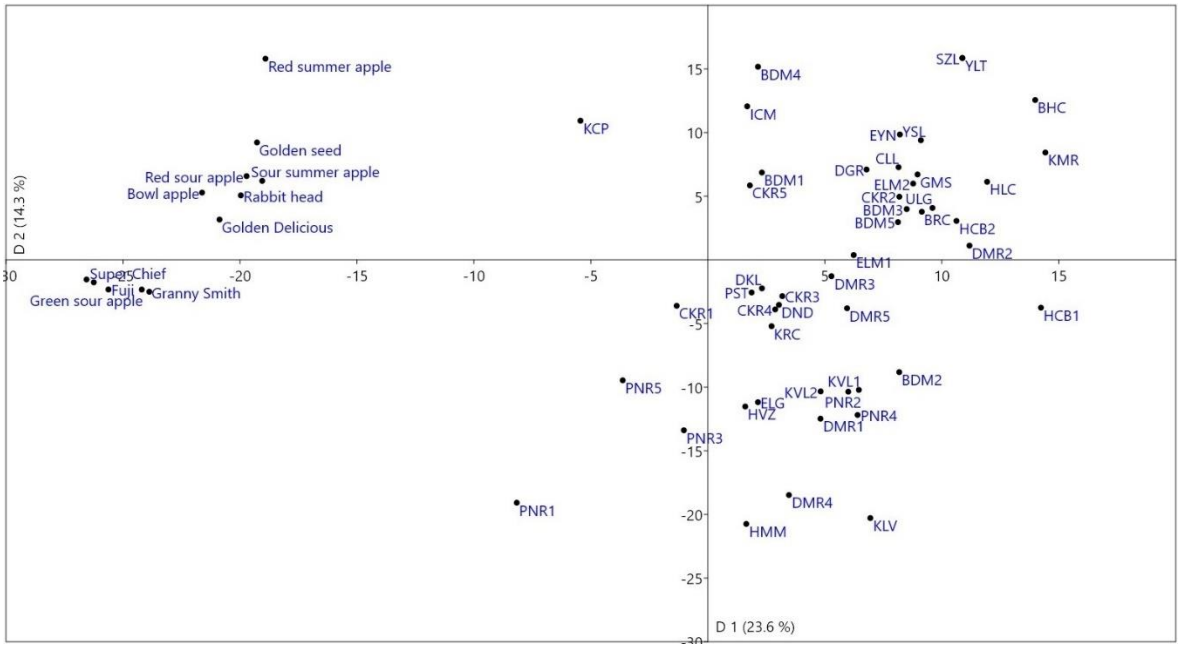

Figure 3- PCoA scatter plot showing the genetic diversity of a total 60 sample on first 2 dimension

4. Discussion

Eltez & Kaska (1985) stated that fruit length 56.25-31.30 mm, fruit width 60.25-34.25 mm, fruit weight 180.20-55.45 gr, depth of stalk cavity 19.00-4.66 mm, total soluble solids content 16.65-11.20, firmness of flesh of 10.05-6.48 kg/cm^2, the number of seeds 10.60 in their study with Amasya apples in Niğde. Although compared with the results of this study were shown prominent values among samples of the fruit diameter and fruit length, the overall values of the samples overlap with the value range. This is especially important for showing the consistency of the results, as the location and material of the two studies are similar. Under this information and with molecular results of our study, the diversity of area clarified. Coskun & Askın (2016) stated that fruit length 64.70-53.90 mm, fruit width 76.60-64.90 mm, fruit weight 184.30-96.90 gr, total soluble solids content 14.20-11.30, firmness of flesh 8.73-6.43 kg/cm^2 in their study on local apple cultivars in Eğirdir. In this study, prominent samples were seen as fruit flesh hardness when pomological results compared with the previous study. Fruit flesh hardness important for crispness (De Belie et al. 2000). Although the two studies carried out different locations as materials both apples local to their location. Senyurt et al. (2015) stated that fruit weight of Amasya cultivar 96.43 g, fruit length 54.95 mm, fruit diameter 61.73 mm, stalk pit depth 8.86 mm, fruit flesh hardness 7.92 kg/cm^2, the weight of fruit 210.60-72.50 g, fruit width 79.70-57.40 mm, fruit length 71.15-50.68 mm, fruit flesh hardness 10.00-6.80 kg / cm^2, total soluble solids content % 14.20- 11.90, pH 4.24-3.08 values in their study in Egirdir different Amasya types. Compared to the result with earlier studies, although the samples in the total soluble solids content are prominent, the values overlapped.

The fruit size and weight are important for higher yield in production and also a wanted feature for most of the consumers. The color of the fruit skin is from the consumer's initial assessment and appeal of the product. Fruit flesh firmness is important to less damage during storage and transportation and also important for the crunchiness which is a trait consumer seeks when eating apples. Total soluble solids content was used in the selection and comparisons were made based on pomological results in this study because of its unique taste, sensation, and saturation (Arıkan et al. 2015). Since the color of the fruit skin increases the attractiveness of the product for the consumer and high total soluble solids content value increases the taste of fruit and saturation of the fruit, it is important that the situation of samples used in the study show superiority (Chagne et al. 2014). Pomological results show that CKR2, DMR3, CLL, HCB2, YSL, ULG, ELM1, ICM samples stand out among other samples according to fruit size, fruit weight, fruit shell color, fruit flesh hardness, total soluble solids content.

Günes & Durgac (2018) stated that the similarity rate between 0.39-0.72 as a result of the analysis using RAPD markers on local apples in the Gülnar region. Kaya et al. (2015) stated that the similarity ratio between 0.38-0.79 as a result of the analysis using RAPD markers on local apple sources in Lake Van Basin. Masum et al. (2014) stated that the highest similarity between Marmara and Black Sea Region samples was 92.4%, the lowest similarity between Black Sea-Central Anatolia region samples was 70.5% in the study conducted with local apples belonging to Marmara, Black Sea, Aegean, and Central Anatolian regions at Atatürk Central Horticultural Research Institute.

In this study, phenotypic results indicated diversity among collected genotypes as well as their distinction to the control group. But some of these results may be influenced environmentally due to genotypes collected from 29 different rural areas. Under these circumstances, the most coherent way to validation of phenotypic results is the utilization of molecular techniques

to achieve more correct information about samples. In this study, iPBS is the chosen technique to manage this goal. The similarity ratio of this study 0.61-1.00 was compared with the previous studies and most of the samples shown higher similarities probably caused by location's effects on diversity but the results of molecular analysis have shown a variation among collected genotypes as well as their distinction to the control group. Although environmental influences can't be denied, both phenotypic and molecular results consistent with each other. Likewise, each sample that distinguishes the other can be used as a genetic source.

The study was conducted in an area where the total coverage approximately 1 853 km^2. Locations of BDM, PNR, DMR and CKR are relatively close to each other and contain 10 of 14 genotypes that under braches 'B'. On the other hand, this kind of phenomenon couldn't be seen for other main branches. Especially branches 'A' spread the whole area as well as 'B' on the UPGMA dendrogram. Branches 'D' only contain two genotypes and the distance between these two genotypes 26.6 km. In account of the most distance between two genotypes 59.72 km (HBC1 and HVZ), 26.6 km nearly the half of the distance between most distance genotypes. The location where the genotypes collected may affect to structure of braches on UPGMA dendrogram in some cases but the only reason underlying diversity among genotypes (Figure 2).

5. Conclusions

Morphological, pomological, and molecular differences were due to both location and genetics sources as the trees were found at varying heights in the elevation range of 1125-1726 m and sampled from the different parts of the province. Currently, limited studies have been carried out on apple with iPBS markers. Some studies were conducted on apple cultivars and mutants as well as other fruit species but this study is the first utilization of the iPBS markers system on apple genotypes. This research also supplied valuable information about this field via expanding the information about it. The current status can be improved by increasing and improving apple production in these rural areas with the use of modern agricultural techniques in production and storage stages, establish orchards with dwarf or semi-dwarf rootstocks and narrow plant spacing, perform agricultural practices on time with correct methods. According to information gathered from local peoples, these local apples in the province have decreased from the past and they are in danger of disappearing in the future. To preserve the plant material used in the study, 4 scions were taken from all of the trees and grafted on MM106 rootstocks and have been established a collection orchard in Niğde Ömer Halisdemir University Faculty of Agricultural Science and Technologies Faculty. Thus, in addition to the conservation of these resources, it will be possible to make controlled breeding for future studies.

References

Anonymous (2014). Researches on Determining the Current Situation of the Agri-Food Sector and Research-Development, Innovation Needs in Niğde and Surrounding Provinces. Ayhan Şahenk Faculty of Agricultural Sciences and Technologies (In Turkish)

Arıkan Ş, İpek M & Pırlak L (2015). Determination of phenological and pomological characteristics of some apple varieties in Konya ecological conditions. *Turkish Journal of Agriculture - Food Science and Technology* 3(10): 811-815 DOI: 10.24925/turjaf.v3i10.811-815.402 (In Turkish)

Bakır M, Dumanoğlu H, Erdoğan V, Ernim C & Macit T (2019). Characterization of wild apricot (Prunus armeniaca L.) genotypes selected from Cappadocia region (Nevşehir-Turkey) by SSR markers. *Journal of Agricultural Sciences* 25(4): 498-507 DOI: https://doi.org/10.15832/ankutbd.457850

Chagne D, Dayatilake D, Diack R, Oliver M, Ireland H, Watson A, Gardiner SE, Johnston J W, Schaffer J R & Tustin S (2014). Genetic and environmental control of fruit maturation, dry matter and firmness in apple (Malus × domestica Borkh.). Horticulture Research 1(1): 1-12. DOI: 10.1038/hortres.2014.46

Coskun S & Askın M A (2016). Determination of Pomological and Biochemical Properties of Some Local Apple Varieties. *SDU Faculty of Agriculture Journal* 11(1): 120-131 (In Turkish)

De Belie N, De Smedt V & De Baerdemaeker J (2000) Principal component analysis of chewing sounds to detect differences in apple crispness. Postharvest Biology and Technology 18(2): 109-119. DOI: 10.1016/S0925-5214(99)00070-8

Dellaporta S L, Wood J & Hicks J B (1983). A plant DNA minipreparation: version II. Plant molecular biology reporter 1(4): 19-21 DOI: 10.1007/BF02712670

Demir Z & Doğan I (2020). The Impact of Different Cover Crops, Mechanical Cultivation and Herbicide Treatment on The Soil Quality Variables and Yield in Apple (Malus domestica Borkh.) Orchard with a Coarse-Textured Soil. *Journal of Agricultural Sciences* 26(4): 452-470 DOI: https://doi.org/10.15832/ankutbd.527445

Demirel U, Tindas I, Yavuz C, Baloch F S & Caliskan M E (2018). Assessing genetic diversity of potato genotypes using inter-PBS retrotransposon marker system. Plant Genetic Resources 16(2): 137 DOI: 10.1017/S1479262117000041

Dunemann F, Kahnau R & Schmidt H (1994). Genetic relationships in Malus evaluated by RAPD 'fingerprinting'of cultivars and wild species. Plant Breeding 113(2): 150-159 DOI: 10.1111/j.1439-0523.1994.tb00717.x

Eltez M & Kaska N (1985). Selection of Highly Special Kaşel-Amasya Apple Types That Give Fruit Every Year in Niğde Region. *Natural Science Journal* 1(9) (In Turkish)

FAO (2020). FAOSTAT. Retrieved in May 2020 from http://www.fao.org/faostat/en/#data/QC

Günes A & Durgac C (2018). Morphological, Phenological, Pomological and Molecular Identification of Apple Genotypes Grown in Gülnar Region. *Journal of Moleculer Biology and Biotechnology* 2(1): 28-33 (In Turkish)

Hammer Ø, Harper D A & Ryan P D (2001). PAST: Paleontological statistics software package for education and data analysis. Palaeontologia electronica 4(1): 9

Hancock J (2012). Plant evolution and the origin of crop species 3rd Edition. CABI

Hinze L L, Fang D D, Gore M A, Scheffler B E, Yu J Z, Frelichowski J & Percy R G (2015). Molecular characterization of the Gossypium diversity reference set of the US National Cotton Germplasm Collection. Theoretical and Applied Genetics 128: 313-327 DOI: 10.1007/s00122-014-2431-7

Hokanson S C, Szewc-McFadden A K, Lamboy W F & McFerson J R (1998). Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a Malus× domestica Borkh. core subset collection. Theoretical and Applied Genetics 97(5-6): 671-683 DOI: 10.1007/s001220050943

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(3): 281-294 DOI: 10.1023/A:1017591202215

Kalendar R, Antonius K, Smýkal P & Schulman A H (2010). iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theoretical and Applied Genetics 121(8): 1419-1430 DOI: 10.1007/s00122-010-1398-2

Kaya T, Balta F & Sensoy S (2015). Fruit quality parameters and molecular analysis of apple germplasm resources from van lake basin, Turkey. *Turkish Journal of Agriculture and Forestry* 39(6): 864-875 DOI: 10.3906/tar-1406-24

Kenis K & Keulemans J (2005). Genetic linkage maps of two apple cultivars (Malus× domestica Borkh.) based on AFLP and microsatellite markers. Molecular Breeding 15(2): 205-219 DOI: 10.1007/s11032-004-5592-2

Kuras A, Antonius K, Kalendar R, Kruczy´nska D & Korbin M (2013). Application of five DNA marker techniques to distinguish between five apple (Malus× domestica Borkh.) cultivars and their sports. *The Journal of Horticultural Science and Biotechnology* 88(6): 790-794 DOI: 10.1080/14620316.2013.11513040

Masum B, Ergul A, Kazan K, Akcay M E, Yuksel C, Bakır M, Mutaf F, Akpınar A E, Yaşasın A S & Ayanoğlu H (2014). Genetic analysis of Anatolian apples (Malus sp.) by simple sequence repeat. *Journal of Systematics and Evolution* 52(5): 580-588 DOI: 10.1111/jse.12099

Milovanov A, Zvyagin A, Daniyarov A, Kalendar R & Troshin L (2019). Genetic analysis of the grapevine genotypes of the Russian Vitis ampelographic collection using iPBS markers. Genetica 147(1): 91-101 DOI: 10.1007/s10709-019-00055-5

Ozbek S (1978). Special Fruit (Deciduous Fruit Species In Winter). Çukurova University Publication of Agriculture Faculty (In Turkish)

Rohlf F J (1998). NTSYS-pc, numerical taxonomy and multivariate system Version 2.0. Exet. Software. New York

SAS Institute Inc (2005). SAS User Guide; Sas/Stat, Version 8. SAS Inst. Inc.

Senyurt M, Kalkısım O & Karadeniz T (2015). Pomological characteristics of some standard and local apple (Malus communis L.) varieties grown in Gümüşhane region. *Academic Agriculture Journal* 4(2): 59-64 (In Turkish)

Seymen T & Polat M (2015). Determination of Phenological, Pomological Properties and Morphological Characterization of Some Amasya Apple Types (In Apple). *Harran Journal of Agricultural and Food Science* 19(3): 122-129. DOI: 10.29050/harranziraat.647181

Smolik M & Krzysztoszek O (2010). Evaluation of genetic variability in choosen apple (Malus× domestica Borkh.) cultivars by ISSR-PCR analysis. *Russian Journal of Genetics* 46(7): 819-827 DOI: 10.1134/S1022795410070069

TUİK (2020). Retrieved in May 2020 from https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr

Tijskens L M M, Herold B, Zude M & Geyer M (2007). How to choose criteria to harvest apples. The dynamics of maturity. In International Conference on Ripening Regulation and Postharvest Fruit Quality (796): 197-200. 10.17660/ActaHortic.2008.796.25

UPOV (2005). Apple, Guidelines For The Conduct Of Tests For Distinctness, Uniformity And Stability. TG/14/9

Zhang C, Chen X, He T, Liu X, Feng T & Yuan Z (2007). Genetic structure of Malus sieversii population m Xinjiang, China, revealed by SSR markers. *Journal of Genetics and Genomics* 34(10): 947-955 DOI: 10.1016/S1673-8527(07)60106-4

Zhou Z Q & Li Y N (2000). The RAPD evidence for the phylogenetic relationship of the closely related species of cultivated apple. Genetic Resources and Crop Evolution 47(4): 353-357 DOI: 10.1023/A:1008740819941