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Research Article / Araştırma Makalesi

Distinguishing the Protected Designation of Origin Apricot (*Prunus armeniaca* L. cv. Şalak) from Closely Related Cultivars by High Resolution Melting

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Abstract: The apricot cultivar *Prunus armeniaca* cv. Şalak (registered as "Iğdır Kayısısı") is a Protected Designation of Origin (PDO) apricot and produced in Aras Basin (Iğdır, Turkey) region. Authenticating the special products, which has adulteration potential, by DNA based methods is reliable and cost-effective. The aim of the current study is to distinguish the PDO apricot from closely related cultivars by High Resolution Melting. We tested 12 SSR markers previously validated for *Prunus* spp. by means of distinguishing the ability of five closely related apricot cultivars that are Şalak (AS), Hasanbey (HB), Hacıhaliloğlu (HH), Kabaaşı (KB), and Şekerpare (SK) produced in Turkey. Capillary electrophoresis validation showed 11 of 12 markers amplified unique fragments for the cultivars. HRM analysis combined with the Principal Component Analysis (PCA) successfully distinguished the PDO AS from closely related cultivars. HRM analysis combined with PCA can be a cost-effective and reliable authenticating method for PDO food products.

Keywords: Genotyping, microsatellites, authenticity, food adulteration, SSR-HRM.

Coğrafi İşarete Sahip Şalak Kayısı Çeşidinin (*Prunus armeniaca* L. cv. Şalak) Yakın İlişkili Çeşitlerde Yüksek Çözünürlüklü Erime Yöntemi ile Ayırt Edilmesi

Öz: Şalak kayısı çeşidi (*Prunus armeniaca* cv. Şalak) Aras Havzası'nda üretimi yapılan ve coğrafi işarete sahip bir kayısı çeşididir. Tescilli ismi Iğdır Kayısısı olarak belirlenmiştir. Özellikle gıda aldatmacasına maruz kalma potansiyeli olan özel gıda ürünlerinin DNA temelli yöntemler ile tanımlanması güvenilir ve görece ucuz maliyetlidir. Bu çalışmanın amacı Şalak kayısı çeşidinin, yakın ilişkili kayısı çeşitlerden Yüksek Çözünürlüklü Erime (HRM) yöntemi kullanarak ayırt edilmesi için bir protokol geliştirmektir. Çalışmada, daha önceden *Prunus* türleri için doğrulanmış 12 adet SSR belirtecinin, Türkiye'de üretimi yapılan Şalak (AS), Hasanbey (HB), Hacıhaliloğlu (HH), Kabaaşı (KB) ve Şekerpare (SK) kayısı çeşitlerini ayırt etme başarısı test edilmiştir. Çalışmada ayrıca HRM verilerinden Temel Bileşenler Analizi yapılabilmesi için R yazılımında kullanılabilecek bir komut dosyası oluşturulmuştur. Kılcal elektroforez ile doğrulanmış 12 SSR belirtecinden 11 tanesinin, her kayısı çeşidi için farklı fragmentler çoğalttığı tespit edilmiştir. Temel Bileşenler Analizi ile desteklenmiş HRM sonuçlarının Şalak kayısı çeşidini diğer çeşitlerden başarılı bir şekilde ayırt ettiği belirlenmiştir.

Anahtar kelimeler: Genotipleme, mikrosatelitler, genetik doğrulama, gıda aldatmacası, SSR-HRM.

1. Introduction

Apricot is an important drupe fruit and it has been cultivated in Asia since 2000 BC. The plant is cultivated in warm to subtropical regions throughout the world. *Prunus armeniaca* L. is the commonly cultivated apricot species and thousands of genotypes are cultivated. Turkey is the leading country in apricot production with 677,000 tons of average production (FAOSTAT, 2020). With this production capability, Turkey supplies approximately 20% of the world's total apricot production. In Turkey, apricot production is specialized in Malatya, Erzincan, and Iğdır regions (Ercişli, 2004). According to the Turkish Statistical Institute 2019 data, Malatya province is leading the apricot production with 391,000 tons in Turkey (Fig. 1) (TÜİK, 2020). Turkey has 28 registered apricot cultivars (Turkish Apricot Research Institute, 2019) and numerous genotypes. Iğdır is also an important province for apricot production. *P. armeniaca* cv. Şalak, which is cultivated in Iğdır province is awarded the mark of Protected Designation of Origin (PDO) and named as "Iğdır Kayısısı" (Iğdır Apricot) by the Turkish Patent and Trademark Office (Registration number 385, dated 17 September 2018).

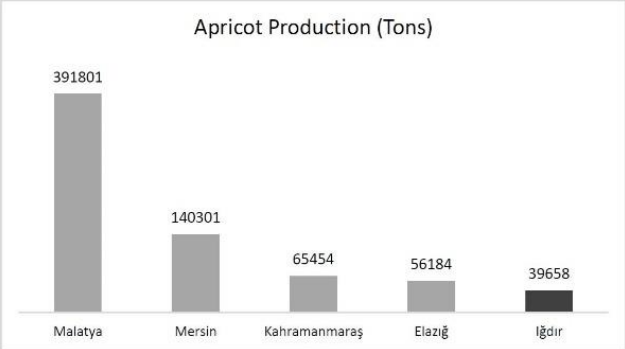

Figure 1. Top five apricot producing regions in Turkey for the 2019 year (TÜİK, 2020).

The PDO products have higher economic value than the non-PDO ones due to their relatively smaller

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production scale and harder production processes, globally. Therefore, it is necessary to ensure that consumers trust PDO authenticity. This causes the emergence of the "traceability of PDO" term. The quantitative traceability of PDO originated food products are important for consumer's protection against food adulteration.

DNA-level tracing of authenticity is quite reliable since it is a stable macromolecule and exists in each tissue and organs and not affected by environmental factors (Li et al., 2018). There are various molecular methods that have the capability of traceability of PDO products such as DNA barcoding, SSR genotyping, and etc. Nevertheless, due to both having specialized downstream processes needed (i.e. bioinformatics skills and computing skills) and being relatively expensive, cheaper and faster methods are needed. High Resolution Melting (HRM) is a technique based on monitoring the DNA denaturation and quantification by a Real-Time PCR instrument. Its advantages can be listed as being cost-effective, having high throughput, requiring less laboratory duty, and no complicated software knowledge needed compared to other marker methods. Another advantage of HRM is detecting the sequence variation without sequencing or hybridization processes (Tindall et al., 2009). Either microsatellite markers or barcoding regions have been employed for HRM analysis successfully (Druml & Cichna-Markl, 2014). HRM was successfully applied to the detection of herbal medicine products (Li et al., 2018), genotyping of peach genotypes (Chou et al., 2020), authenticity testing of sweet cherry products (Ganopoulos et al., 2011), and species identification of ginseng (Osathanunkul & Madesis, 2019). In a previous study, researchers developed novel HRM markers to detect plum pox virus (PPV) resistance by targeting PPV resistance locus in *Prunus armeniaca*. According to the results, PPV resistance locus could be detected by using a reliable and user-friendly method HRM (Passaro et al., 2017).

This study aims to develop an HRM based protocol, which distinguishes the PDO Şalak cultivar from closely related cultivars, and validate the Şalak at the cultivar level.

2. Material and Methods

2.1. Plant materials and DNA extraction

In this study, we chose closely related Turkish apricot cultivars Şalak (AS), Hasanbey (HB), Hacıhaliloğlu (HH), Kabaaşı (KB), and Şekerpare (SK). According to the information we obtained from the local community and Apricot Research Institute authority, those cultivars are rather close in terms of morphology and taste. Therefore, we would like to test the HRM performance on those cultivars. We obtained AS, HB, HH, KB, and SK cultivars from the Republic of Turkey Ministry of Agriculture and Forestry Apricot Research Institute (Malatya, Turkey).

We extracted total DNA from 100 mg leaf tissue of the samples using the modified CTAB protocol as described in the literature (Aydın et al., 2018). We confirmed the DNA concentration and integrity by NanoDrop (Maestrogen) and agarose gel electrophoresis and stored the DNA samples at -20°C.

2.2. Primers mining

Twelve sets of SSR markers that were previously validated for apricot were chosen (Table 1), and checked the amplification success and optimized the PCR conditions for all the primers on each apricot cultivar. The optimum PCR reaction was carried out in a total volume of 20 µl containing 2X Reaction Buffer (Thermo Scientific, USA), 0.1 mM dNTPs, 0.2 µM both primers, 1 U Taq DNA polymerase (Thermo Scientific, USA), 1 mM Mg^{+2}, 10 ng total DNA and nuclease-free water. Thermal cycling (Sensoquest Labcycler Gradient, Germany) condition was 95°C 3 min first denaturation, 35 cycles of 95°C 30 s denaturation, 55°C 30 s annealing, 72°C 1 min extension, and thermal cycling was finalized by 72°C 10 min extension step. PCR products were validated by capillary electrophoresis (CE) (Qiaxcel Advanced, Germany) with QIAxcel DNA High Resolution Kit (Qiagen, Germany). The software settings were used as follows; Process profile: Default High Res v2.0; Method: 0H1200; Size marker: GeneRuler 100bp Plus, Thermo, USA (run by side the samples); Alignment marker: QX 15bp-3kb. The results were visualized and analyzed by the ScreenGel 1.2 software.

Table 1. SSR primers used in the study and specifications. Lower / Upper limits are starting and ending points of melting which is filtered for PCA analysis in the R script.

Primer	Sequence (Forward and Reverse, 5′→3′)	Melting Temperature	Temperature Optimization for Principal Component Analysis		Expected Size (bp)	Reference
			Lower Limit	Upper Limit		
pchgms1	GGGTAAATATGCCCATTGTGCAATC GGATCATTGAACTACGTCAATCCTC	55°C	71.22	78.39	~194	Sosinski et al., 2000
pchgms2	GTCAATGAGTTCAGTGTCTACACTC AATCATAACATCATTCAGCCACTGC	55°C	72.93	79.42	~163	
pchgms4	ATCTTCACAACCCTAATGTC GTGGAGGCAAAAGACTTCAAT	55°C	73.16	80.71	~174	
UDP96-001	AGTTTGATTTTCTGATGCATCC TGCCATAAGGACCGGTATGT	57°C	76.92	82.7	~120	Cipriani et al., 1999
UDP96-003	TTGCTCAAAAGTGTCGTTGC ACACGTAGTGCAACACTGGC	57°C	76.92	82.7	~143	
UDP96-005	GTAACGCTCGCTACCACAAA CCTGCATATCACCACCCAG	57°C	76.92	82.7	~155	
UDP96-010	CCCATGTGTGTCCACATCTC TTGATGATTCCATGCGTCTC	57°C	78.53	82.13	~131	
UDP97-402	TCCCATAACCAAAAAAAACACC TGGAGAAGGGTGGGTACTTG	57°C	71.17	75.49	~136	
UDP98-406	TCGGAAACTGGTAGTATGAACAGA ATGGGTCGTATGCACAGTCA	57°C	72.41	79.69	~101	
UDP98-409	GCTGATGGGTTTTATGGTTTTC CGGACTCTTATCCTCTATCAACA	57°C	74.51	79.51	~129	

Primer	Sequence (Forward and Reverse, 5′→3′)	Melting Temperature	Temperature Optimization for Principal Component Analysis Lower Limit	Upper Limit	Expected Size (bp)	Reference
UDP98-021	AAGCAGCAATTGGCAGAATC GAATATGAGACGGTCCAGAAGC	57°C	72.0	82.04	~145	Testolin et al., 2000
PS12A02	GCCACCAATGGTTCTTCC AGCACCAGATGCACCTGA	60°C	76.86	84.56	~200	Downey & Iezzoni, 2000

2.3. HRM-PCR amplification and Data Analysis

Firstly, normalization the concentration of all the DNA samples was adjusted to 10ng/µl before HRM analysis. HRM amplifications were performed on Rotor-Gene-Q 5plex thermal cycler (Qiagen, Germany) with a 72-well carousel. The HRM mix was prepared as 10 µl total volume consisting of 5µl Luminaris Colour HRM Master Mix (Thermo Scientific, USA), 0.5 µl of 10 mM each primer (Sentebiolab, Turkey), 10 ng DNA, and nuclease-free water to 10 µl. We used a three-step cycling protocol as 95°C 10 min initial denaturation followed by 45 cycles of 95°C 10 s denaturation, 60°C 30 s annealing, and 72°C 30 s extension. Data acquiesced following each extension step. We added 95°C 30 s and 50°C 30 s steps for heteroduplex formation to the end of the cycle. We performed HRM immediately after the amplification in increments of 0.1°C s^{-1} hold time from 65°C to 95°C and data acquiesced continuously. All the reactions were performed as three replicates and no template control (NTC) was included in the reactions.

We analyzed the HRM data using both Rotor-Gene-Q Software (2.3.5). We first normalized the HRM curves by removing the background fluorescence; then, drew difference plots of AS against the other cultivars for each SSR primer. Next, the software calculated Genotype Confidence Percentages (GCPs) for each cultivar against AS by setting each cultivar as "genotype". We set the confidence threshold to 90% for more reliable results.

2.4. Developing R Script for Principal Component Analysis of HRM data

Although there is a well-developed R Script available for HRM-PCA analysis (Chou et al., 2020), we needed to improve it with different libraries to improve visualization performance. The improved script uses the new R Script to visualize the data by Principal Component Analysis (PCA) to be used to help distinguish the cultivars, which HRM failed. Following the HRM reaction, raw HRM temperature and normalized fluorescence data were exported with the Rotor-Gene-Q (2.3.5) software as comma-separated values (csv) file. Clustering and PCA were performed in RStudio (RStudio Team, 2020). The samples were clustered with 'mclust' (Scrucca et al., 2016) and visualized with 'ggplot2' (Wickham, 2016) packages. The script was improved by means of compatibility and better visualization by commonly used and recent packages. The PCA results can be exported as high-quality image formats as well as PDF for better publishing. We also added self-explanatory comments to the script in both English and Turkish to increase usability. The RStudio script is publicly available on GitHub (https://github.com/biologisthurkan/hrm_pca) and in Supplementary Material 1. The script can be used with all the HRM supported devices. A sample data file is also available on both GitHub and in Supplementary Material 2.

3. Results

3.1. DNA extraction and PCR validation of SSR markers by Capillary Electrophoresis (CE)

DNA extraction from each cultivar was successfully performed with the used protocol. The DNA concentration that was obtained varied between 39.32 to 95.06 ng μl^{-1} and A260/230 ratio ranged from 1.234 to 1.766, which is sufficient for HRM analysis. Each SSR marker successfully amplified for five studied apricot cultivars. The CE validation of the markers showed that each marker had different patterns among Turkish apricot cultivars. The PCR fragments sizes and counts generally varied among the cultivars (Table 2). The SSR markers pchgms4 and UDP96-001 amplified only one fragment for all the cultivars while the remaining markers amplified more than one fragment. The longest fragment (347 bp) was amplified by the UDP96-005 marker on KB and the shortest one (96 bp) was amplified by UDP96-010 on HB. According to the fragment sizes, the UDP96-001 marker could distinguish AS, HB, and KB but not HH and SK. We present an example CE comparison electropherogram for the pchgms4 marker which compares the fragment peaks (Fig. 2). According to the peaks in Figure 2 and fragment data in Table 2, cultivars HB and HH have identical fragments (169 bp) and AS and SK also have identical fragments (194 bp). Therefore, based on CE results, the pchgms4 marker could not distinguish the cultivars HB and HH, and AS and SK.

Table 2. SSR fragment analysis by capillary electrophoresis. Amplicon size of each primer per cultivars showed in the table. Cultivar abbreviations AS: Aprikoz Şalak, HB: Hasanbey, HH: Hacıhaliloğlu, KB: Kabaaşı, SK: Şekerpare.

	Amplicon sizes for each cultivar (bp)				
Primer	AS	HB	HH	KB	SK
pchgms1	185	173, 180, 184, 190	180, 189, 193	173, 179, 184, 190	180
pchgms2	159, 170, 172	149, 163, 202	191, 196	165, 170, 181	158, 169, 172, 176
pchgms4	194	196	169	169	194
UDP96-001	137	133	135	198	135
UDP96-003	136	106	102, 106, 108, 116, 126	103, 136, 147	133
UDP96-005	139, 144, 150, 156, 158, 162, 167	125, 138, 144, 176	125, 138, 143, 149, 178	257, 276, 318, 347	126, 159, 164, 177, 179, 182

	Amplicon sizes for each cultivar (bp)				
Primer	AS	HB	HH	KB	SK
UDP96-010	105, 113, 117, 121	96, 105	98, 105	107, 114	98, 105
UDP97-402	146, 152	168, 173	164	165, 170	165
UDP98-406	107	100, 105, 117	105, 108, 119	104, 108	107, 119, 123
UDP98-409	153, 158, 173, 185, 188	168, 173, 182, 187, 190	176, 179, 185, 198, 206	223, 227	150, 155, 159
UDP98-021	141, 143	139, 141	139, 141	117, 138, 143, 147	118
PS12A02	173, 184, 187, 193	169, 172, 176, 178	168, 179, 182, 188	169, 179, 181, 186	176, 181, 187, 193, 196, 199, 201

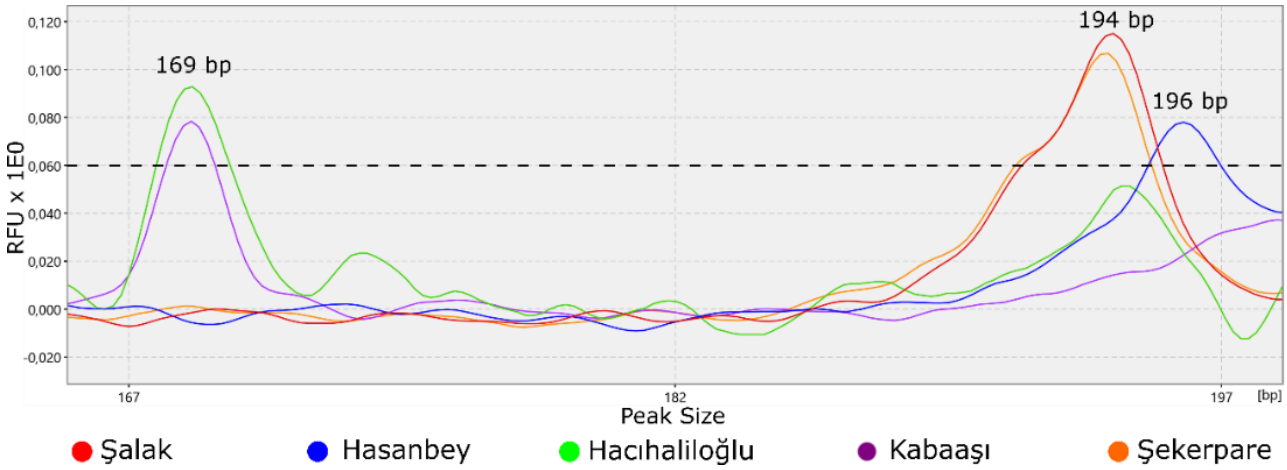

Figure 2. Capillary Electrophoresis comparison electropherogram view of pchgms4 marker for five studied cultivars. Each cultivar is color-coded and peak sizes marked on each peak. Black dashed line represents the threshold for RFU signal.

3.2. HRM Results

The HRM analysis results of five closely related apricot cultivars scanned by 12 SSR markers are shown as difference plot curves (Fig. 3) and as GCP table (Table 3). The plot curves showed that 11 of the 12 SSR markers generated unique HRM curves for AS and distinguished it from the other cultivars. In a detailed look on the pchgms4 marker, which could not distinguish AS and SK cultivars according to the CE results, HRM curves of AS and SK differs from each other. The marker UDP96-001 generated almost identical curves for AS and KB and could not distinguish the cultivars. Auto calling function of the Rotor-Gene Q software calculates an R-value to provide a percentage of confidence. This percentage is used to call other genotypes as the positive control (AS in this study). The GCP lower than 90% were accepted as different genotypes while higher ones were accepted as the same genotype. The GCPs support the distinction among the five cultivars for 11 markers, except UDP96-001. The pchgms4 marker could clearly distinguish AS and SK from each other with 24.34 GCP. For the UDP96-001 marker, the GCP for KB was calculated as 99.73%, which is quite higher than the 90% threshold and supports the plot curve result. Therefore, the UDP96-001 is indeed the only marker that could not distinguish AS from the others. The most distinguishing marker for AS was UDP98-409 based on GCPs.

Generally, HRM performed greater in the study. All the markers we tested except UDP96-001 generated unique melting shapes and this resulted in differentiation of the PDO cultivar. The marker pchgms4, which could not distinguish AS and SK by CE, worked much better on HRM analysis. HRM distinguished AS from SK with 24.34% GCP (Table 3) and drew different melting shape (Fig. 3) even they generated identically sized fragments in CE results.

Table 3. Genotype distinguishing performance of each marker for five studied apricot cultivars. The confidence threshold is 90%. Cultivar abbreviations AS: Aprikoz Şalak, HB: Hasanbey, HH: Hacıhaliloğlu, KB: Kabaaşı, SK: Şekerpare.

pchgms1			pchgms2			pchgms4			UDP96-001		
Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %
AS	AS	100.00	AS	AS	100.00	AS	AS	100.00	AS	AS	100.00
HB	Variation	32.72	HB	Variation	0.00	HB	Variation	15.25	HB	Variation	10.08
HH	Variation	10.58	HH	Variation	0.06	HH	Variation	62.59	HH	Variation	76.60
KB	Variation	72.04	KB	Variation	0.01	KB	Variation	0.05	KB	AS	99.73
SK	Variation	3.38	SK	Variation	74.04	SK	Variation	24.34	SK	Variation	35.95

UDP96-003			UDP96-005			UDP96-010			UDP97-402		
Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %
AS	AS	100.00	AS	AS	100.00	AS	AS	100.00	AS	AS	100.00
HB	Variation	51.62	HB	Variation	72.75	HB	Variation	31.64	HB	Variation	50.69
HH	Variation	27.10	HH	Variation	22.19	HH	Variation	15.44	HH	Variation	19.31
KB	Variation	8.06	KB	Variation	46.00	KB	Variation	50.32	KB	Variation	18.03
SK	Variation	6.43	SK	Variation	75.47	SK	Variation	38.35	SK	Variation	9.62
UDP98-406			UDP98-409			UDP98-021			PS12A02		
Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %
AS	AS	100.00	AS	AS	100.00	AS	AS	100.00	AS	AS	100.00
HB	Variation	11.38	HB	Variation	4.80	HB	Variation	71.30	HB	Variation	72.15
HH	Variation	64.33	HH	Variation	15.19	HH	Variation	22.45	HH	Variation	75.72
KB	Variation	73.80	KB	Variation	4.75	KB	Variation	21.84	KB	Variation	66.76
SK	Variation	68.16	SK	Variation	30.52	SK	Variation	0.63	SK	Variation	30.34

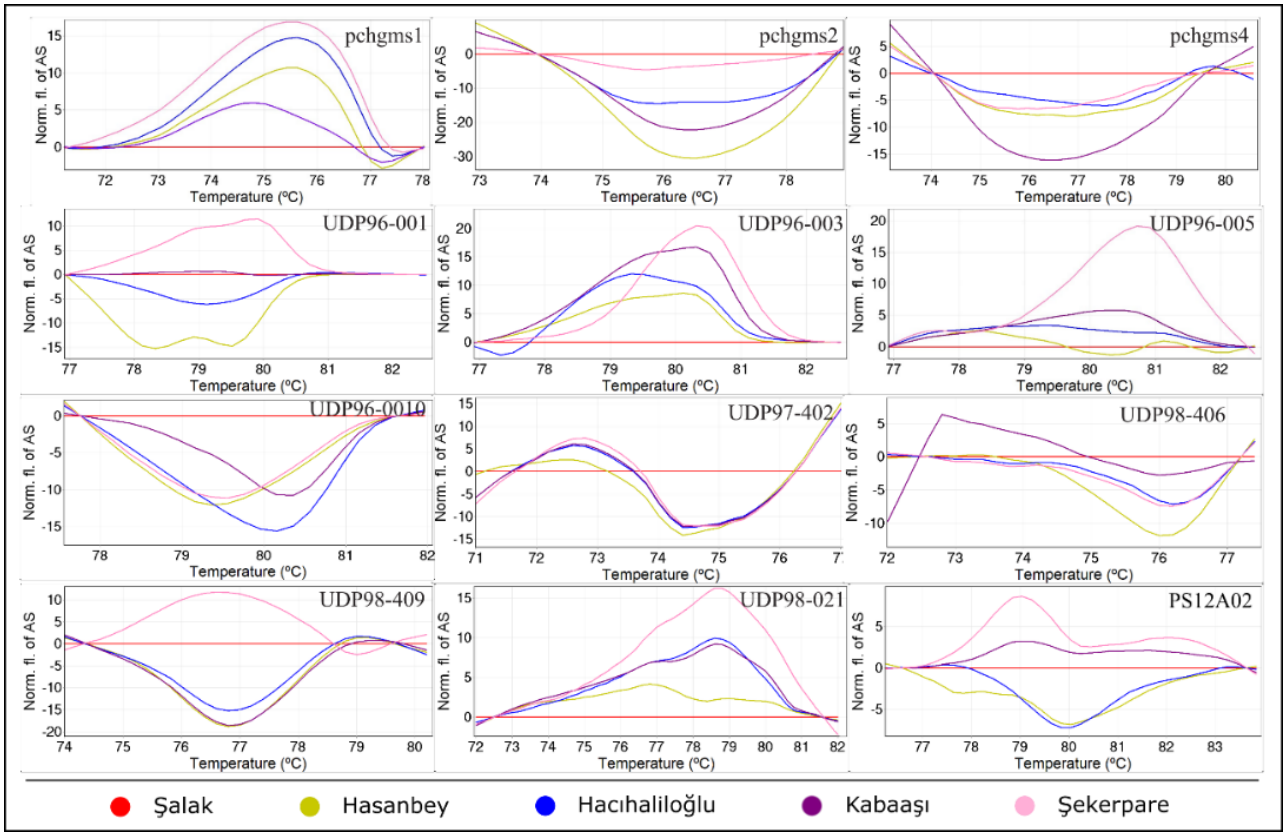

Figure 3. HRM analysis of five closely related apricot cultivars with 12 SSR marker. Difference plot curves were drawn based on AS cultivar.

To deeper analyze the HRM results, we used a third tool PCA clustering the data for failed marker UDP96-001. PCA could successfully cluster AS and KB and distinguished them (Fig. 4). According to the results, we were successfully able to distinguish the PDO AS apricot from closely related cultivars by HRM using 12 markers. Supporting HRM data with PCA resulted better resolution and distinguishing power.

4. Discussion

Distinguishing the special food products from closely related varieties is quite important due to high adulteration potential. "Iğdır Kayısısı" (*Prunus armeniaca* cv. Şalak) is a PDO product special to Iğdır region, which is the fifth ranking apricot producing region in Turkey (TÜİK, 2020). DNA based authentication methods are reliable and provide traceability. DNA barcoding, which uses the nucleotide sequences of short DNA fragments, is a gold standard to distinguish closely related species and reconstructing the phylogeny for many organism groups (Lahaye et al., 2008; Hollingsworth et al., 2011) particularly for animals and most plants (Pentinsaari et al., 2016).

Nevertheless, it is not a convenient method for distinguishing at cultivar-level due to the slow mutation rate. Moreover, DNA barcoding requires experience in bioinformatics and specialization in computer software. SSR markers are more useful for screening genetic differences at the cultivar level since they are more stable and co-dominant transmission and conserved microsatellites are still widely used in taxonomy studies (Tuler et al., 2015). Due to those advantages, we combined the stability of SSR markers and distinguishing power of HRM on five closely related apricot cultivars including the PDO "Iğdır Kayısısı" with 12 potential SSR markers that were validated before for *Prunus* species and cultivars (Cipriani et al., 1999; Downey & Iezzoni 2000; Testolin et al., 2000; Sosinski et al., 2000). This study is the first to use SSR-HRM to detect a PDO apricot and distinguish closely related apricot cultivars.

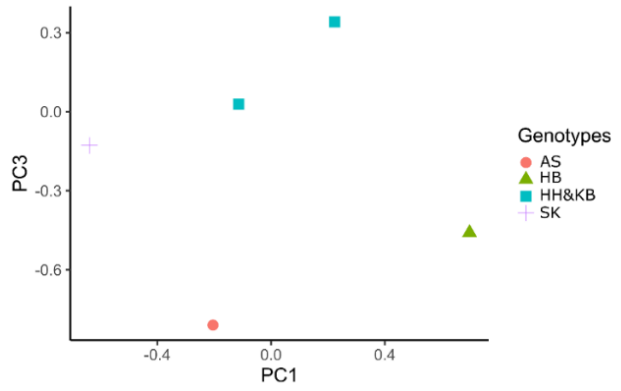

Figure 4. Principal Component Analysis, calculated by the R Script, for UDP96-001 marker distinguishes AS and KB cultivars.

We first validated the SSR markers whether they are successfully amplified on *Prunus armeniaca* cultivars by CE. The obtained fragment sizes are quite similar to the literature (Table 1 and Table 2) except for two markers. The primer pairs of the marker UDP97-402 amplified larger fragments than defined in the literature (expected: ~136 bp,) for HB (168 and 173 bp), HH (164 bp), KB (165 and 170 bp), and SK (165 bp) cultivars. The amplified fragments of the UDP98-409 marker ranging between 150 – 227 bp among cultivars were also larger than reported in the literature (expected fragment size ~129 bp). Due to the nature of microsatellites, it varies by means of length due to repeating regions (Li et al., 2018) thus, microsatellite markers could distinguish the species or cultivars. Although most of the markers generated different fragments for each cultivar, the pchgms4 marker amplified identical fragments for AS and SK (194 bp), and HH and KB (169 bp). Therefore, even scanning by high resolution CE, it is not possible to distinguish those cultivars. HRM generates melting curve shapes by continuously screening the level of fluorescence dye that intercalates with dsDNA during melting, the software can measure the distance between curves. The curve shapes depend on GC content, amplicon size, and the nucleotide sequence (Wittwer 2009). Thus, although the fragment sizes of AS and SK amplified by the primers of pchgms4 marker are identical, different GC content or/and different nucleotide sequence separated AS and SK easily by HRM. HRM behaves quite different for the UDP96-001 marker. According to the CE results, the sizes of the fragments for AS (137 bp) and KB (198 bp) were different but HRM failed to distinguish those cultivars. We investigated the melting curve shape and determined that the shape is almost identical although the signal level is different (Fig. 5). Therefore, the failure of HRM might be because of the identical melting shape.

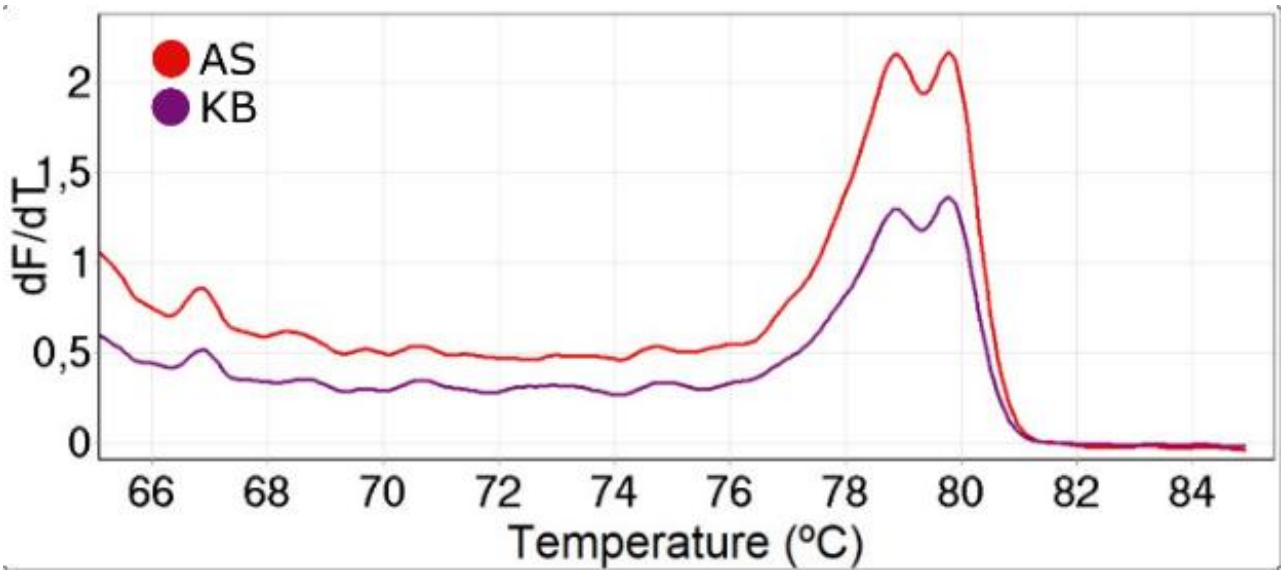

Figure 5. Melting Curve shapes of AS and KB cultivars.

New special food products, such as PDO products, bring new potential adulterants. Therefore, taking advantage of new molecular biology methods would help to manage food safety. In this study, we showed that HRM-SSR is a powerful approach for distinguishing closely related apricot cultivars. Moreover, when HRM failed, the raw HRM fluorescence data can be used on PCA for advanced analysis to distinguish the cultivars.

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Conflict of interest: The author declares that there is no conflict of interest.

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