http://dergipark.org.tr/trkjnat
Trakya University Journal of Natural Sciences, 24(2): 91-100, 2023
ISSN 2528-9691
DOI: 10.23902/trkjnat.1324202

Research Article

IDENTIFICATION OF LENS CULTIVARS IN MARKET BY MOLECULAR TOOLS: DNA BARCODING AND SSRs

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Cite this article as:

Tatlıses M.B. & Hasançebi S. 2023. Identification of lens cultivars in market by molecular tools: DNA barcoding and SSRs. *Trakya Univ J Nat Sci*, 24(2): 91-100, DOI: 10.23902/trkjnat.1324202

Received: 07 July 2023, Accepted: 10 October 2023, Published: 15 October 2023

Abstract: Substitution of plant cultivars of high commercial value with a cheaper, lower quality one is a common fraud committed against consumers and producers. Since it is one of the most widely grown legumes, lentil (*Lens culinaris* Medik.) is suitable for such frauds. This study aimed to identify lentil cultivars which are registered and authorized in the market in Türkiye by using current molecular methods. For this purpose, 26 lentil cultivars were analyzed for 15 SSR markers and two DNA barcode regions (*trnH-psbA* and *matK*). A high allele diversity was observed by 12 scorable SSR markers, and the average number of alleles was determined to be 16. One of the important findings was the presence of "cultivar-specific alleles" that can be used to identify each cultivar in the lentil market in Türkiye. At least one "cultivar-specific allele" was obtained for each cultivar. The lentil cultivars were also analyzed by two DNA barcode regions as *trnH-psbA* and *matK*. While it was observed that the rate of the intra-species variation for the *trnH-psbA* region was low and 26 varieties were divided into 7 groups, higher rate was found for *matK* and samples were distributed into 14 groups. Nevertheless, it was observed that intra-species discrimination can be made more effective when both loci are used together and 26 species were distributed into 18 different groups. We expect that the results of this study, especially the cultivar-specific SSR alleles and DNA barcoding sequence data may be used routinely to identify production and packaged products that are commercially available in markets.

Özet: Ticari değeri yüksek bitki çeşitlerinin daha ucuz ve düşük kaliteli olanlarla değiştirilmesi, tüketicilere ve üreticilere karşı yaygın bir hiledir. Mercimek (*Lens culinaris* Medik.) en yaygın yetiştirilen baklagillerden biri olduğu için bu tür hileler için uygun bir üründür. Bu çalışmada, güncel moleküler yöntemler kullanılarak Türkiye'de tescilli ve piyasada izinli mercimek çeşitlerinin tanımlanması amaçlanmıştır. Bu amaçla, 26 mercimek çeşidi 15 SSR markırı ve 2 DNA barkod lokusu (*trnH-psbA* ve *matK*) ile analiz edilmiştir. Değerlendirilen 12 SSR markırı ile yüksek bir allel çeşitliliği gözlenmiş ve ortalama allel sayısı 16 olarak belirlenmiştir. Türkiye'deki mercimek pazarında her bir çeşidi tanımlamak için kullanılabilecek "çeşide özgü allellerin" varlığı önemli bulgulardan biridir. Her bir çeşit için en az bir "çeşide özgü allel" elde edilmiştir. Mercimek çeşitleri ayrıca *trnH-psbA* ve *matK* olmak üzere iki DNA barkod bölgesi açısından da analiz edilmiştir. *trnH-psbA* bölgesi için tür içi varyasyon oranının düşük olduğu ve 26 çeşidin sadece 7 gruba ayrıldığı gözlenirken, *matK* için bu oran daha yüksek bulunmuş ve örnekler 14 grupta dağılım göstermiştir. Bununla birlikte, her iki lokus birlikte kullanıldığında tür içi ayrımın daha etkili hale getirilebileceği görülmüş ve 26 çeşit 18 farklı gruba dağılmıştır. Bu çalışmanın sonuçlarının, özellikle de çeşitlere özgü SSR allelleri ve DNA barkod dizisi verilerinin, piyasada ticari olarak bulunan üretim ve ambalajlı ürünlerin tanımlanmasında rutin olarak kullanılabileceğini düşünüyoruz.

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Key words:
Food traceability
DNA barcoding
SSR
Lens culinaris

Introduction

In Türkiye and in the world in general, the seed sector is developing rapidly. The ever-increasing nutritional demand of the world's human population makes it inevitable to develop new cultivars with high quality and yield. This has led to a rapid increase in the number of cultivars in plant species that have economic value. Lentil (*Lens culinaris* Medik.) contains high levels of vegetable protein and is commonly found on tables of people. It is therefore widely consumed and has extensive breeding programs. Due to the fact that Türkiye is the gene center of lentils and has important cultivation areas (Ford *et al.* 2007), there are many different cultivars of lentils in the market. The yield and quality potentials of all lentil cultivars differ from each other. Therefore, it is extremely

important to identify the cultivars to minimize cheating/confusions in both the use of cultivars suitable for soil/climatic conditions that are demanded in production and in trade while choosing parents in breeding activities.

All food products must comply with the description provided by the manufacturers or processors with reference to the origin of the ingredients, as well as the identity of the species, breeds or cultivars used. Substitution of plant cultivars of high commercial value with a cheaper, lower quality one is a common fraud committed against consumers and producers. Such fraud causes confusion in the market, disaffection towards genuine products, deception of consumers and unfair competition. Therefore, a wide variety of analytical methods are used to detect these type adulterations. In the last decade, DNA-based molecular methods offer testing tools to ensure food safety, origin and authenticity of primary products entering food chains in terms of both fresh and processed food (Böhme *et al.* 2019, Dawan & Ahn 2022). Molecular methods are widely preferred because they provide more reliable, sensitive and faster results from the field to the market and can also reveal the origin of the products (Fanelli *et al.* 2022). Among them, molecular markers such as SSRs are well established methods for food tests, while some new approaches based on sequencing such as DNA barcoding have recently been applied with their great potential.

SSR markers are highly polymorphic species specific markers that have been useful for identifying origins of raw materials and ingredients of processed food. The high level of polymorphism is due to different numbers of repeats in the microsatellite loci that are distributed to the entire genome. Therefore, SSRs are amenable to high throughput genotyping and also a useful testing tool for paternity analysis, construction of high-density genome maps, marker-assisted selection and for establishing genetic, evolutionary relationships and food safety (Kalia *et al.* 2011). For instance, Beser & Mutafcilar (2020) detected and used some variety specific SSR markers that can be used to differentiate and identify varieties for adulteration of Turkish rice markets. In addition, Ganopoulos *et al.* (2011) genotyped Basmati and non-Basmati varieties by integrating five SSRs into High Resolution Melting analysis for detecting of adulteration. DNA barcoding is another method that has been successfully used for the authenticity of different kinds of food (Dawan & Ahn 2022). There are many good examples especially for meat and sea food. In addition top bananas (Dhivya *et al.* 2020), mushrooms (Zhang *et al.* 2021), vegetables (Thongkhao *et al.* 2020), cherries (Feng *et al.* 2018) and saffron species (Khilare *et al.* 2019) are identified with a single barcode locus, while two barcode loci are used in combination when identifying herbal medicinal products (Vassou *et al.* 2015, Intharuksa *et al.* 2020) and citrus species (Mahadani & Ghosh 2014). For lentil; Bosmali *et al.* (2012) identified a special lentil species by integrating five SSRs and *rpoC1* barcode loci into High Resolution Melting analysis.

DNA barcoding is in recently used techniques that analyzes one or few standardized loci for identifying all species. The mitochondrial gene Cytochrome c oxidase subunit 1 (*cox1* or COI) was proposed as a DNA barcode locus for identification of animal species by Hebert *et al.* (2003). However, mitochondrial DNA barcode candidates are useless for plants because plant mitochondrial sequences evolve slowly (Mower *et al.* 2007). Therefore, the attention of researchers is focused on plastid genomes. Plastid genes (*rpoC1*, *rpoB*, *matK*, *rbcL*), plastid intergenic spacers (*atpF–atpH*, *trnH–psbA and psbK–psbI*) and the internal transcribed spacer region (*ITS)* have been initially proposed as candidate barcoding loci (Kress *et al.* 2005, Chase *et al.* 2007, Fazekas *et al.* 2008). The CBOL Plant Working Group recommended a two-locus combination of *rbcL* + *matK* as the core barcode for land plants (Hollingsworth *et al.* 2009). Accordingly, it has been reported that the *rbcL*, *matK* and *trnH - psbA* regions have a high level of distinctive characteristics between species when used together (Kress & Erickson 2007, Hollingsworth *et al.* 2009, 2011). In comparison to other plant barcode loci, *rbcL* has a low mutation frequency. However, it is informative for the intra-species level. *matK* is one of the most rapidly evolving plastid regions and shows high levels of discrimination among angiosperm species (Hilu & Liang 1997, Fazekas *et al.* 2008). The presence of the highly conserved coding sequences of *trnH-psbA* makes the design of universal primers feasible with a single primer pair likely to amplify nearly all angiosperms (Shaw *et al.* 2005, 2007). *trnH-psbA* exhibits the most sequence divergence and has high rates of insertion/deletion (Kress & Erickson 2007).

In this study, we identified registered lentil cultivars in Türkiye with two commonly recommended plant DNA barcoding loci (*matK* and *trnH-psbA*) and 15 SSR markers, for ensuring the traceability of lentil cultivars in the market.

Materials and Methods

Plant material and DNA isolation

26 lentil cultivars that have a production permit in Türkiye and one Canadian cultivar which is one of most frequently imported cultivars by the country were used (Table 1). Total genomic DNAs (gDNA) were isolated from a single lentil seed by a Plant/Fungi DNA Isolation Kit (Norgen).

SSR analysis

For genotypic analysis, 15 SSR markers with high PIC values were selected among 149 SSRs which were developed by Andeden *et al.* (2015) (Table 2). Amplification of each SSR marker locus was carried out by PCR, which was performed in a 20 µL reaction mixture containing 60 ng DNA, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM primers and 1 U Taq polymerase (Invitrogen). Amplification was performed in a T100 Bio-Rad thermal cycler (CA, USA) by following the cycles of

5 min at 94°C for initial denaturation, and 35 times 45 sec at 94°C, 45 sec at 50-65°C (depending on the primer Tm), 1 min at 72°C and 10 min at 72°C for final extension. The amplified PCR products were separated and analyzed by using an AATI Fragment Analyzer System (Advance Analytic, IA, USA). The PCR products were prepared for the capillary system according to the manufacturer's instructions (DNF-905 dsDNA Reagent, IA, USA). Each reaction mixture was diluted at a 1:5 ratio by a dilution buffer, and 24 µL of the mixture was transferred to a 96-well plate. Each well was covered by mineral oil, and electrophoresis was performed by applying 9.0 kV for 80 min. For sizing of the SSR alleles, 1-500 bp DNA ladders were used in each run, and DNA fragment sizes were calculated by the system software ProSize 3.0. Each allelic DNA fragment produced from the SSR loci was scored for statistical analysis.

Table 1. List of lentil cultivars used in the study.

Material No.	Material Name	Type	Material No.	Material Name	Type
1	Çiftçi	R	**14**	Sazak	R
2	Özbek	R	**15**	Kayı	G
3	Kafkas	R	**16**	İpek	R
4	Fırat-87	R	**17**	Orhas-2019	G
5	Altıntoprak	R	**18**	Şanlıbey	R
6	Meyveci-2001	G	**19**	Atacan	R
7	Sultan-1	G	**20**	Yazlık Yeşil	G
8	Ankara Yeşili	G	**21**	Emre-20	R
9	Ceren	G	**22**	Mansur	R
10	Bozok	G	**23**	Canadian Cultivar (Sultani)	G
11	Gümrah	G	**24**	Şahan	G
12	Karagül	G	**25**	Eva-2017	R
13	Yerli Kırmızı	R	**26**	Yürekli	R

(R: red type, G: green type)

Table 2. List of high polymorphic SSR markers (Andeden *et al.* 2015).

No	SSR	Repeat Motifs
1	CULA105	(TA)7(TG)22
2	CULA107	(CA)7(CG)2(CA)7
3	CULA109	(TG)15A(GA)29
4	CULA211	(GT)23(GA)18
5	CULA308	(TC)20A(CA)6
6	CULA408	(CA)11
7	CULA413B	(AC)14
8	CULB7	(CT)7
9	CULB9	(CT)24
10	CULB206	(CA)17(CA)6
11	CULB217	(CT)31
12	CULB222	(GA)28
13	CULB310	(TC)18
14	CULB418	(GA)28
15	CULB423	(TC)6

SSR data analysis

The DNA fragment information observed for each SSR locus in each cultivar by capillary electrophoresis was statistically analyzed using the GenAlEx 6.5 program (Peakall & Smouse 2006, 2012). Genetic distance and similarity matrices were created using the DARwin 6.0 program (Perrier & Jacquemoud-Collet 2006), and the genetic relationship between the cultivars was revealed by drawing a phylogenetic tree using the UPGMA method (Sneath & Sokal 1973).

DNA barcoding

matK and *trnH-psbA* barcode loci in the chloroplast genome were used as the barcode regions. The different universal primers used in the amplification of the barcode regions are given in Table 3. Amplification of the barcode loci was carried out by PCR, which was performed in a 40 µL reaction mixture containing 60 ng gDNA, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM primers and 1 U Taq polymerase (Invitrogen). Amplification was performed following the cycles; 5 min at 94°C for initial denaturation, and 35 times 45 sec at 94°C, 45 sec at 50°C for *matK*, 55°C for *trnH-psbA*, 1 min at 72°C and 10 min at 72°C for final extension. The PCR products were analyzed by gel electrophoresis on 1.5% agarose gels in 0.5× TBE buffer stained with ethidium bromide and checked under ultraviolet light. Amplified DNA fragments of *matK* were cut from the gel and cleaned using the GeneJET Gel Extraction kit (Thermo Scientific) and the purified PCR products were used as a template for sequence analysis. For 14 samples, purified matK fragments were cloned to pJET1/2 vector and transformed to *Escherichia coli* (Migula) according to Chung *et al.* (1989) to overcome the sequencing problems.

Table 3. List of DNA barcoding loci and universal primers (* HPLC grade purification).

Locus	Primer	Sequence 5' - 3'	Reference
trnH-psbA	psbA3_f	GTT ATG CAT GAA CGT AAT GCT C	Sang *et al.* 1997
	trnHf_05r	CGC GCA TGG TGG ATT CAC AAT CC	Tate & Simpson 2003
trnH-psbA	*trnH-psbA.F	ACT GCC TTG ATC CAC TTG GC	Hamilton 1999
	*trnH-psbA.R	CGA AGC TCC ATC TAC AAA TGG	
matK	matK2.1F	CCT ATC CAT CTG GAA ATC TTA G	Kress & Erickson 2007
	matK_5R	GTT CTA GCA CAA GAA AGT CG	
matK	*matK472F	CCC RTY CAT CTG GAA ATC TTG GTT C	Fazekas *et al.* 2008
	*matK1248R	GCT RTR ATA ATG AGA AAG ATT TCT GC	Yu *et al.* 2011
	*matK-1FKIM.R	AAT ATC CAA ATA CCA AAT CC	Ki-Joong Kim, unpublished

Barcoding data analysis

The sequences were manually edited and aligned by MEGA X (Kumar *et al.* 2018). The alignment of each region was performed by ClustalW (Thompson *et al.* 1994). A phylogenetic tree was created according to the UPGMA method (Sneath & Sokal 1973) to determine the phylogenetic relationships between the cultivars by MEGA X (Kumar *et al.* 2018). Numbers of groups, as well as group and nucleotide diversity, were determined by using the DnaSP 6 software (Rozas *et al.* 2017).

Results

Results of SSRs

26 lentil cultivars were analyzed by 15 SSRs but were genotyped by 12 SSR markers. Three SSR markers (CULA413B, CULA107 and CULA109) did not produce scorable PCR products in all cultivars. Therefore, they were not used for genotyping analysis. The alleles of the SSRs were amplified by PCR and analyzed with an AATI Fragment Analyzer. The 12 successful SSR markers were found highly polymorphic, and 172 alleles in total were obtained from 26 cultivars. In the total assessment, the average number of alleles per SSR locus was calculated as 14. The most frequently observed alleles and their frequencies are presented in Table 4. The most polymorphic markers with 19 alleles among 172 were seen in the CULB310 marker. The distribution of the alleles belonging to each SSR marker, the frequencies of the most frequently observed alleles, their loci among the 26 cultivars that were scanned and their PIC values are given in Table 4.

Detection of alleles that are unique for each cultivar is one of the significant findings of SSR analysis. With the 12 SSRs used in the scope of this study, unique cultivar-specific alleles were obtained. The Özbek cultivar had 8 unique, cultivar-specific alleles. These alleles, which are especially important for cultivar recognition/determination, can create an alternative solution for problems encountered in both lentil production and lentil seed trade. The obtained cultivar-specific alleles are presented in Table 5. Additionally, a dendrogram (Fig. 1) was created using the DARwin 6.0 program to reveal the phylogenetic relationships of the cultivars using the UPGMA method, which was performed based on the genetic similarity and distance between the cultivars according to the report by Sneath & Sokal (1973).

Results of DNA barcoding

In addition to SSRs, 2 barcode regions were used for genotyping the lentil cultivars. Both 2 barcode loci were successfully reproduced by PCR in all cultivars with all primer pairs (Table 3).

In the study, 2 different universal primers were used for each locus. For *trnH-psbA* barcode locus, trnH-psbA_F/trnH-psbA_R was the most successful primer pair for both amplification and sequencing. The amplified *trnH-psbA* barcode locus was ~350 bp, and it was directly sequenced and compared among the 26 cultivars. Successful primer pair for *matK* locus is the matK472.F/matK-1FKIM.R and produced 1000 bp DNA fragments but sequencing success was lower (53.8%). Therefore, cloning was performed for 14 samples that could not be sequenced, and then a successful result was achieved. *matK* sequences were compared among the 26 cultivars by the MEGA software. The number of groups, group diversity and nucleotide diversity results of the sequences belonging to the *matK* and *trnH-psbA* loci are given in Table 6.

Table 4. SSRs allelic diversity information.

No	SSR	Number of Allels	Min. Allel (bp)	Max. Allel (bp)	Max. Observed		PIC
					Allel	Freq.	
1	CULA105	11	142	178	158,178	0.173	0.868
2	CULA211	17	180	274	258	0.134	0.915
3	CULA308	13	230	294	294	0.211	0.863
4	CULA408	15	144	356	154	0.250	0.837
5	CULB7	16	208	260	216	0.153	0.911
6	CULB9	13	180	220	188	0.115	0.904
7	CULB206	18	202	264	244,264	0.115	0.928
8	CULB217	9	146	170	146	0.230	0.857
9	CULB222	17	126	184	136	0.192	0.909
10	CULB310	19	266	326	272	0.346	0.836
11	CULB418	9	220	274	224	0.346	0.770
12	CULB423	15	218	266	250,260	0.115	0.914

Table 5. List of variety-specific alleles.

Variety Name	SSR Loci	Allel	Variety Name	SSR Loci	Allel
Çiftçi	CULA308	260	Sultan-1	CULB222	160
Çiftçi	CULB7	240	Sultan-1	CULB310	268
Özbek	CULA211	268	Sultan-1	CULB418	236
Özbek	CULA408	144	Ceren	CULA211	274
Özbek	CULB7	212	Ceren	CULA408	178
Özbek	CULB206	248	Ceren	CULB9	190
Özbek	CULB222	164	Ceren	CULB206	260
Özbek	CULB310	266	Bozok	CULB7	217
Özbek	CULB310	286	Bozok	CULB222	178
Özbek	CULB423	238	Bozok	CULB423	230
Kafkas	CULA105	166	Gümrah	CULB7	260
Kafkas	CULA211	256	Gümrah	CULB222	170
Kafkas	CULA308	282	Gümrah	CULB310	326
Kafkas	CULB9	206	Karagül	CULB222	184
Kafkas	CULB206	216	Yerli Kırmızı	CULB222	130
Kafkas	CULB222	145	Yerli Kırmızı	CULB418	266
Kafkas	CULB418	270	Sazak	CULA105	154
Fırat	CULB7	224	Sazak	CULA308	240
Fırat	CULB206	214	Sazak	CULB222	168
Altıntoprak	CULB7	248	Kayı	CULB7	258
Altıntoprak	CULB206	258	Kayı	CULB206	240
Meyveci-2001	CULA105	142	Kayı	CULB222	180
Meyveci-2001	CULA211	240	Kayı	CULB310	320
Meyveci-2001	CULB222	134	İpek	CULA211	202
Meyveci-2001	CULB310	274	İpek	CULB222	154
Meyveci-2001	CULB310	288	İpek	CULB310	296
Ankara Yeşili	CULB222	162	İpek	CULB423	222
Orhas 2019	CULA308	258	Mansur	CULA211	264
Orhas 2019	CULA408	168	Mansur	CULB7	250
Orhas 2019	CULA408	324	Canadian V.	CULA308	252
Orhas 2019	CULB9	220	Canadian V.	CULA408	158
Orhas 2019	CULB206	250	Canadian V.	CULB206	204
Orhas 2019	CULB423	252	Şahan	CULA308	232
Şanlıbey	CULA308	230	Şahan	CULA408	150
Şanlıbey	CULB310	306	Şahan	CULB206	202
Atacan	CULA308	234	Şahan	CULB418	268
Atacan	CULB7	254	Şahan	CULB423	266
Atacan	CULB9	198	Eva 2017	CULB7	246
Atacan	CULB310	300	Eva 2017	CULB206	238
Yazlıkyeşil	CULA211	200	Yürekli	CULB206	206
Emre-20	CULB206	234	Yürekli	CULB423	258
Emre-20	CULB423	242			

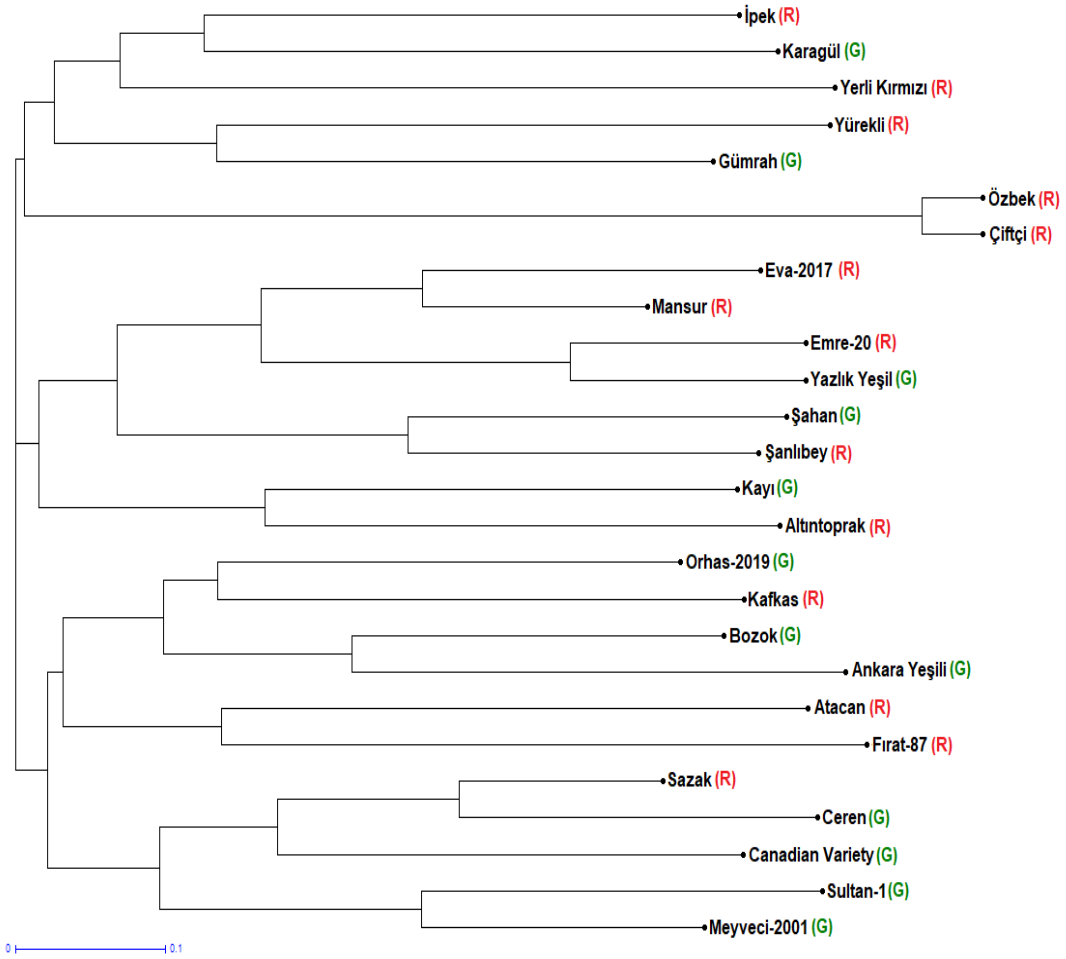

Fig. 1. UPGMA dendrogram based on SSR data obtained from DARwin 6.0 software.

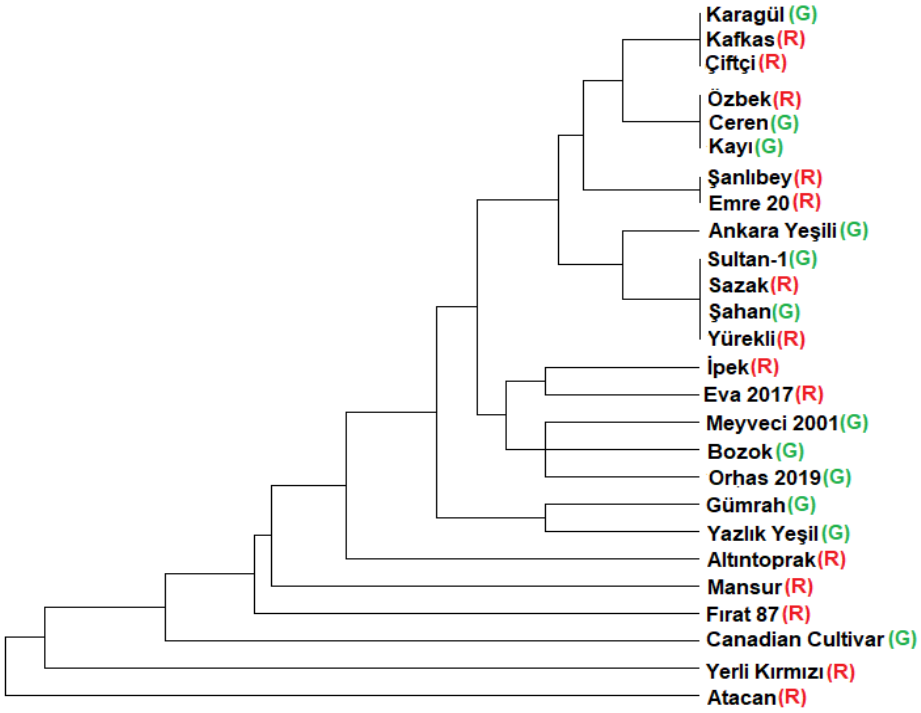

Fig. 2. UPGMA dendrogram based on sequencing data on combination of *trnH-psbA* + *matK* regions (R: red lentil cultivars, G: green lentil cultivars).

Table 6. Number of groups, group diversity and nucleotide diversity of *matK*, *trnH-psbA* and *matK* + *trnH-psbA* sequences.

	trnH-psbA	***matK***	***trnH-psbA+matK***
Number of Groups	7	14	18
Group Diversity	0.680	0.917	0.960
Nucleotide Diversity	0.00734	0.00292	0.00364

According to the results, the highest group number for the cultivars was obtained with the combination of the *trnH-psbA* + *matK* regions. A phylogenetic tree was constructed with the sequencing data of the *trnH-psbA* + *matK* regions for the analyzed lentil cultivars (Fig. 2). Each branch of the phylogenetic tree is associated with groups. As demonstrated in Fig. 2, the Çiftçi-Kafkas-Karagül, Şanlıbey-Emre 20, Sazak-Yürekli-Sultan 1-Şahan and Özbek-Ceren-Kayı cultivars were in the same branch as a result of a similar sequence. On the other hand, other red and green cultivars had unique sequences, and each cultivar fell into a different branch. The dendrogram based on sequence data (Fig. 2) proved that the combination of *trnH-psbA* + *matK* could discriminate 14 lentil cultivars in the market. All obtained sequences were recorded to BOLD system (http://boldsystems.org). The sequences and accession numbers were presented as Supplementary Materials S1, S2 and S3.

Discussion

There are problems in the global food trade that adversely affect consumers, such as the deliberate fraudulent substitution, falsification or mislabeling of food and its ingredients or food packaging for a variety of reasons, including economic interests (Robson *et al.* 2021). DNA-based tools offer a fast, accurate and cost-effective solution to tackle these negative issues. The main goal of this study was identification of lentil varieties in Turkish market via SSR markers and DNA barcoding methods.

SSR markers have been successfully used in food ingredient identification and the detection of adulteration due to their reproducible and reliable results, species specificity and high polymorphism. Some of the studies in this direction include the identification of grape varieties along the entire production chain for products such as grapes, must and wine (Di Rienzo *et al.* 2017, Zambianchi *et al.* 2021), determination of olive varieties in olive oil (Gomes *et al.* 2018, Chedid *et al.* 2020), assessments on the accuracy of the raspberry variety (Pinczinger *et al.* 2020), verification of the content of rice in packaged foods (Beşer & Mutafcılar 2020) and identifying varieties of zucchini (Verdone *et al.* 2018). In another study, common wheat contamination was detected in semolina and bread produced from durum wheat using wheat D-genome-specific SSRs (Silletti *et al.* 2019). No such study has been found for lentils in Turkish market, so it is expected that our results on "cultivar-specific" alleles can be used successfully for identification of the 26 lentil cultivars for adulteration.

As a result of the DNA barcoding process, sequencing data that could identify 26 lentil cultivars into 18 groups were found. It was observed that the *matK* locus was more successful in intra-species identification, but the combined usage of the barcoding loci would increase the success in the discrimination of cultivars as recommended by Kress *et al.* (2005). In other studies, barcode loci were used in combination to identify different kind of products with intraspecific or interspecific content (Mahadani & Ghosh 2014, Vassou *et al.* 2015, Intharuksa *et al.* 2020). However, for more superficial identifications, single barcode loci are being used (Feng *et al.* 2018, Dhivya *et al.* 2020, Thongkhao *et al.* 2020, Zhang *et al.* 2021). Identification with DNA barcoding is applied to foods such as rice (Genievskaya *et al.* 2017), olives (Kumar *et al.* 2011, Uncu *et al.* 2017), saffron (Khilare *et al.* 2019), cinnamon (Swetha *et al.* 2014), fruit mixtures (Bruno *et al.* 2019) and spices (Gismondi *et al.* 2013, Parvathy *et al.* 2014, Parveen *et al.* 2019) which are frequently adulterated. In addition, different studies have shown that the low sequencing success of the *matK* locus in our study is a feature specific to this locus. In particular, the CBOL Plant Working Group found that the sequencing success of 400 plants of different species tested using a single *matK* primer pair was low, down to 10% in some species (Hollingsworth *et al.* 2009). In our study, sequencing ratio was %100 by cloning of unsequenced 14 samples.

In conclusion, in this study, two DNA-based methods, DNA Barcoding and SSRs, were used to identify lentil species in Türkiye. DNA barcoding has the disadvantage of being a sequencing-based method compared to SSRs due to the low intraspecific discrimination of the *trnH-psbA* locus and the difficulty of sequencing the *matK* locus. SSR-based identification is faster and more practical when species-specific allelic data are available. Both methods can be successfully used to identify adulteration depending on the circumstances. The data on Turkish varieties reported in this study will help protect the producer, consumer and even the seller.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: Barcode sequence data has been uploaded to the Barcode of Life Data System database. The data is available for bublic uses (http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?searchMenu=taxonomy&query=lens+culinaris&taxon=lens+culinaris) and in the Supplementary Material of the article.

Author Contributions: Concept: M.B.T., S.H., Design: M.B.T., S.H., Execution: M.B.T., S.H., Material supplying: M.B.T., S.H., Data acquisition: M.B.T., S.H., Data analysis/interpretation: M.B.T., S.H., Writing: M.B.T., Critical review: S.H.

Conflict of Interest: The authors have no conflicts of interest to declare.

Funding: The study was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) with project number 218O172.

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