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Yetiştiriciliği Yapılan Bazı Türk Kişniş (*Coriandrum Sativum* L.) Çeşitlerinde Genetik Çeşitliliğin ISSR ve SRAP Markörleri Yardımıyla Değerlendirilmesi

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Özet: *Coriandrum sativum* L., *Umbelliferae / Apiaceae* ailesine ait uçucu yağ dahil olmak üzere tıbbi bitki olarak kullanılan bir baharat bitkisidir. Farklı kısımlarındaki çeşitli kimyasal bileşenleri, antibakteriyel, antifungal ve antioksidatif olarak kullanışlıdır. Dolayısıyla gıdaların bozulmasını önleyerek raf ömrünün uzatılmasında önemli bir rol oynamaktadır. Kişnişin yaprak ve tohumları çoğunlukla geleneksel tedavide kullanılır ve uçucu yağları insanlar için toksik olmaması nedeniyle farmasötik ürünler ve parfüm bileşenlerinde yer alır. Kişniş, moleküler olarak yeterince tanımlanmamıştır. Kişniş ıslah çalışmalarıyla genetik olarak tek düze *Coriandrum sativum* L çeşitlerine ulaşılabilecektir. Kabul edilebilir Genotipler, germplazmdaki genetik değişkenlik miktarıyla belirlenir ve kişniş için kullanılabilir bilgiler sınırlıdır. Genetik farklılığının belirlenmesinin çalışılması ve ıslah programlarında uygun genotip kullanımının öneminin vurgulanması, kişnişteki genetik tabanı genişletmek için gereklidir. Bu çalışmada; bazı Türk kişniş çeşitleri arasındaki genetik varyasyonlar iki farklı markör tekniği kullanılarak (ISSR ve SRAP) DNA düzeyinde araştırılmış ve ticareti yapılan bu çeşitler arasındaki genetik ilişkinin belirlenmesi amaçlanmıştır. Her iki primer toplam 473 skorlanabilir bant vermiş ve her markörden elde edilen sonuçlar genotipler arasındaki filogenetik ilişkileri göstermiştir.

Anahtar kelimeler: *Coriandrum sativum* L., ISSR, Moleküler markör, SRAP

Assessment of Genetic Variation on Some Cultivated Turkish Coriander (*Coriandrum Sativum L.)* Varieties Based on ISSR and SRAP Markers

Abstract: *Coriandrum sativum* L. is the spices plant that used as medicinal plant including essential oil, belonging to the family *Umbelliferae/Apiaceae.* Its various chemical components in different parts are useful as antibacterial, antifungal and antioxidative. Therefore plays a major role in preserving the shelf life of foods by preventing their spoilage. Leaves and seeds of coriander mostly used in folk medicine and its essential oil used for pharmaceutical products and as an ingredient in perfumes cause of non-toxic to humans. Coriander is not much defined as molecularly. Coriander breeding studies will probably result in more or less genetically identical varieties of *Coriandrum sativum L.* The acceptability of the genotypes is determined by the amount of genetic variability in the germplasm and the utilizable information is limited in coriander. Studying to determine the genetic diversity of identified characters and emphasis of importance to use convenient genotype in a breeding program is needed in order to enlarge the genetic base in coriander. In this study; Genetic variations among some Turkish coriander varieties were investigated at the DNA level using two different marker techniques (ISSR and SRAP) and the aim was to determine the genetic relationship between these traded varieties. Both primers gave totally 473 scorable bands and all results from each marker have shown the phylogenetic relationships between genotypes.

Keywords: *Coriandrum sativum* L., ISSR, Molecular marker, SRAP

Introduction

The aromatic plants and spices have been used in many different areas like food preservation, pharmaceuticals, folk medicine and natural therapies all over the world. *Coriandrum sativum L.* (*C. sativum*) belonging to the family Umbelliferae/Apiaceae is mostly used for medicinal properties due to its essential oil. Especially the leaves and seeds of the plant are commonly used in folk medicine and spices in

food preparation (Mandal and Mandal 2015). The most important constituents of coriander are the essential and the fatty oil. The essential oil content of coriander varies between 0.03 and 2.6% and the content of fatty oil between 9.9 and 27.7% (Diederichsen 1996). Coriander for medical purposes was used in ancient Egypt for the first time. Coriander fruits are still used in medicine and placed in the German and Austrian official pharmaceutical plant drug lists (Ebert 1982). Coriander is mostly consumed as curry powder and fresh green herbs because of its flavor in India, China, Thailand, Malaysia, Indonesia, and the American Midwest and in the Near East (Prakash 1990; Purseglove et al. 1981). From the eastern Mediterranean to India, China and rest of the world, it exists as a native plant (Coskuner and Karababa 2007). Coriander can also be invoked as medicine, beverage and pharmaceutical industries besides uses as the salad (Kalemba and Kunicka 2003). Its cultivation mostly occurs on a small scale. Studying the genetic diversity of identified characters and establishing a breeding program require a large coriander genetic base (Singh et al. 2005). Molecular marker techniques to preserve the plant genetic resources and the assessment of genetic variability can be more efficient. PCR-based dominant marker systems (ISSR; Zietkiewicz et al 1994), and amplified fragment length polymorphism (AFLP; Vos et al. 1995) have been used to estimate genetic variation in plants (Agarwal et al 2008). Amplifying the sequence between two SSRs from PCR reaction, yielding a multi-locus marker system make possible to use this method for fingerprinting, diversity analysis and genome mapping (Godwin et al. 1997). On observing genetic relatedness among individuals that consisted of a population frequently depend on the use of molecular methods in many systematic studies such as phylogenetic relationships and tracking quantitative traits (Robarts and Wolf 2014). SRAP (sequence-related amplified polymorphism) markers are simple, reliable, acceptable throughput ratio and easy sequencing of selected bands. Also, PCR is simple, inexpensive, and effective for producing genome-wide fragments with high reproducibility and versatility. The aim of the SRAP technique is based on two-primer amplification that is the amplification open reading frames (ORFs) and the method uses primers of arbitrary sequence, which is 17–21 nucleotides in length (Aggarwal et al 2008). It is targeted coding sequences in the genome and shows a moderate number of co-dominant markers (Li and Quiros 2001) but SRAP markers are dominant and Compared with RAPD, SRAP used a pair of primers with 16 to 22 nucleotides instead of 10-mer short primers in RAPD, which gives SRAP a big advantage over RAPD thus one SRAP primer can combine with unlimited number of other primers (Li et al. 2013). Because of relatively new and still in early stages SRAP markers boundaries have not been exactly defined yet. Also Last decade, application of SRAP markers has accelerated, especially in the applied plant sciences and some researches show that SRAP primers amplify some non-nuclear material and a small proportion of the markers scored were hybrid specific, implies some recombination's (Robarts et al. 2014). And sometimes its dominance is regarded as moderately. There is no single technique is universally applicable (for reviews, see Doveri et al. 2008; Arif et al. 2010), and all molecular markers have strengths and weaknesses, method selection depends on the aimed to research and its requirements. In the present study, we have used five corianders representing different provinces of Turkey and The variety of Iraq, which is regarded as Kerkük in Turkey as a control in order to determine genetic profiles by using ISSR and SRAP markers. The study was aimed to determine phylogenetic relationships among coriander varieties using one by one two different marker system. In this way, two different marker systems examined and evaluated together to study of genetic divergence for six coriander genotypes. Consequently, we intended to encourage the farmers in Turkey to initiate the local coriander breeding programs and trying to introduce a coriander as an alternative crop. The study was conducted at the Yuzuncu Yil University Agriculture Faculty, Plant Biotechnology Department Van in Turkey.

Materials and Methods

Plant materials

Plant materials of this study consisted of Six coriander genotypes (Pelmus, Gürbüz, Erba, Arslan, Kerkük (Kirkuk) and Kudret-K) that collected from different areas of Turkey. These genotypes genetically assessed using two different markers.

DNA extraction

All fresh leaves samples were germinated in petri dishes and collected samples of genotypes were stored in the freezer using 2ml eppendorf tubes and kept at -80 °C for a night. Then ground to samples were used the polypropylene sample pestle for each tube to a fine powder. The genomic DNA was extracted from approximately 2 gr of leaf tissue of each variety of coriander. The extraction procedure was the CTAB as

reported by Doyle and Doyle (1987). DNA was checked for quantification using NanoDrop 2000 Versions (Thermo Scientific) by measuring A260/A280.

ISSR analysis

Selected eleven ISSR primers were used in the amplification procedure according to Katzir et al. (2000). Prepared PCR reaction mixture consisted of 10 ng genomic DNA, 5X buffer, 100µM of dNTPs and 0.3 µM were used for each primer with 1 unit Taq polymerase in 25 µl volume. The amplification were proceeded under thermal conditions as 7 min at 94 °C for denatured as three steps at 35 cycles to be 1 min at 94 °C, 1 min at 54 °C, 7 min at 72 °C before the program was terminated by holding at 4 °C. The amplified ISSR products were analyzed by electrophoresis on 1.8 % (w/v) of agarose gel containing 6 µl ethidium bromide, at 110 volts for 3 hours and screened using Vilber Lourmart Quantum ST4 visualization system.

SRAP analysis

Randomly selected eleven SRAP primers were experienced on six coriander genotypes to determine the genetic relationship among them. The reaction mixture and protocols were considered as described by Ferriol et al. (2003). Amplification was proceeding in total 25 µl reaction mixtures containing 25 ng DNA, 1.5 mM MgCI2, 0.5 µM primer, 0.2 mM, 1x Taq buffer and 1 unit Taq DNA polymerase. Thermocycler were programmed to be 5 min at 94 °C to denatured and followed three steps as repeated as five cycles 1 min at 94 °C, 1 min at 35 °C and 2 min for elongation at 72 °C before 30 cycles at 50 °C with a final extension step of 5 min at 72 °C. The PCR products were electrophoresis on 2 % (w/v) of agarose gel containing 6 µl ethidium bromide, at 110 volts for 3 hours and screened using Vilber Lourmart Quantum ST4 system.

Data scoring and analysis

DNA bands were screened by Quantum ST4 software and two or three times checked with eye exam by the different researcher; just clear bands were taken into account and not fully distinguishable bands were ignored. The presence of a band was calculated '1' and '0' for the absence of the band. Binary data have entered the computer as an Excel sheet using for further analysis SPSS Statistics (SPSS Inc. 2001) to occur with distance matrices and construction of dendrogram. The phylogenetic tree was constructed on a Dice genetic similarity coefficient (Nei and Li 1979) using the Unweighted Pair Group Method of Arithmetic means (UPGMA) and the binary data were imported into SPSS statistical software to build a similarity matrix. Genetic distance built on the Jaccard coefficient (Jacquard 1908) was calculated in accordance with making hierarchical clustering employing agglomerative, proximity matrix. The goodness of fit of the clustering compared with the basic data matrix was verified. Polymorphic Information Content (PIC) for these markers was determined as PIC = 1-[f2+ (1-f)2], PIC value is calculated as where "f" is the frequency of the marker in the data set (Riek et al. 2001).

Results and Discussion

Selected eleven primers for polymorphism across the six coriander genotypes have been characterized for molecularly after PCR amplifications. Mean PIC values of these 11 ISSR and 11 SRAP primers are presented in Table 1 and Table 2.

Polymorphic Information Content (PIC) for dominant markers was determined as: PIC = 1-[f2+ (1-f) 2], meantime for the co-dominant markers as: PIC = 1 - Σpi2. PIC value is calculated as where "f" is the frequency of the marker in the data set. Maximum PIC values were determined for the ISSR marker is 0.44 and SRAP marker is 0.47. For each primer, the PIC value is the mean of calculated PIC of all loci. We used 11 primers for each marker and amplified countable bands total of 271 for ISSR and 207 for SRAP marker to indicate the rank of genetic variability. Knowledge of these primers for each marker with the inclusion of mean polymorphism information content (PIC) values and band polymorphism is shown in Table 1 and Table 2. The highest number of bands was obtained from ISSR LOL10 primer (28) and SRAP Me3 and Me7 primers (29) whereas obtained the lowest PIC value (0.1975) from the ISSR primer 7 and the SRAP Primer Me11 (0.2778). These results stated that this study displayed an extensive range of genomic DNA diversity between six coriander genotypes. The dendrogram was generated from binary data screening eleven ISSR and SRAP primers for six coriander (Pelmus, Gürbüz, Erba, Arslan, Kerkük and Kudret-K)

genotypes were imported into the SPSS statistics (SPSS Inc. 2001) to identify genetic relationships between the genotypes to be held with distance matrices and construction of dendrogram in Figure 1 and 2.

Table 1. Eleven ISSR primers to detection of polymorphism among 6 Coriander (*Coriandrum sativum L.*) genotypes

ISSR primers	Sequence (5'–3')	Tm(^{0}C)	Number of bands[a]	Number of polymorphic bands	Percentage of polymorphism(%)	Mean PIC value
12	$(AG)_8$YG	55	20	8	40	0.2778
7F	$(AG)_8$YC	55	30	12	40	0.2778
4F	$(GA)_8$YC	55	23	12	52.1	0.3578
LOL10	$(GAG)_3$GC	38	28	28	100	0.3457
PHV7	GTG $(GT)_8$	57	12	12	100	0.4444
ISSR6	VDVGTGTGTGTGTGTGTGT	56	30	0	0	0
2F	$(CA)_8$RY	52	12	12	100	0.4444
SOLA6	BDBCACCACCACCACCAC	60	30	0	0	0
13F	$(AC)_8$YT	52	29	18	62	0.3133
889	AGTCGTAGTACACACACACACAC	61	23	18	78.2	0.3578

[a] Total accountable bands appearing in two or three times repeated experiments.

Table 2. Eleven SRAP primers to detection of polymorphism among 6 Coriander (*Coriandrum sativum L.*) genotypes

Primer name	**Forward primer (5' to 3')**	**Primer name2**	**Reverse primer (5' to 3')**	**[a]Allels**	**Mean PIC value**
Me1	TGAGTCCAAACCGGAAA	Em1	GACTGCGTACGAATTAAT	16	0.4717
Me2	TGAGTCCAAACCGGAAT	Em2	GACTGCGTACGAATTAAC	16	0.4444
Me3	TGAGTCCAAACCGGAAC	Em3	GACTGCGTACGAATTATG	29	0.3133
Me4	TGAGTCCAAACCGGAAG	Em4	GACTGCGTACGAATTACG	24	0.3200
Me5	TGAGTCCAAACCGGATA	Em5	GACTGCGTACGAATTAGC	11	0.4753
Me6	TGAGTCCAAACCGGACA	Em6	GACTGCGTACGAATTTAG	24	0.3200
Me7	TGAGTCCAAACCGGACT	Em7	GACTGCGTACGAATTTGA	29	0.3133
Me8	TGAGTCCAAACCGGACC	Em8	GACTGCGTACGAATTTGC	13	0.4012
Me9	TGAGTCCAAACCGGACG	Em9	GACTGCGTACGAATTTCA	18	0.3750
Me10	TGAGTCCAAACCGGAGA	Em10	GACTGCGTACGAATTTCG	22	0.4753
Me11	TGAGTCCAAACCGGAGC	Em11	GACTGCGTACGAATTCAA	5	0.2778

[a]Number of bands from two or three times repeated experiments.

Figure 1 indicates that 6 genotypes distributed themselves into two major groups and one of these genotypes (Erba) is completely kept separate from the others. The other major group divided three subgroups contained 5 other coriander genotypes. Similarly, the Pelmus variety in the group was totally taken placed differently. Kudret and Kerkuk varieties have found the most similar varieties with 97% similarity ratios (Table 3). Binary data obtained from SRAP primers have shown that (Figure 2) similar results among the varieties.

Table 3. Genetic similarities based on the Dice coefficient obtained among the six coriander varieties using 11 ISSR markers

	PELMUS	GURBUZ	ERBA	ARSLAN	KERKUK
GURBUZ	0.91				
ERBA	0.83	0.85			
ARSLAN	0.81	0.85	0.79		
KERKUK	0.89	0.96	0.84	0.90	
KUDRET	0.88	0.92	0.82	0.93	0.97

Table 4. Genetic similarities based on the Dice coefficient obtained among the six coriander varieties using 11 SRAP markers

	PELMUS	GURBUZ	ERBA	ARSLAN	KERKUK
GURBUZ	0.52				
ERBA	0.61	0.56			
ARSLAN	0.50	0.89	0.55		
KERKUK	0.55	0.86	0.60	0.83	
KUDRET	0.54	0.87	0.62	0.85	0.99

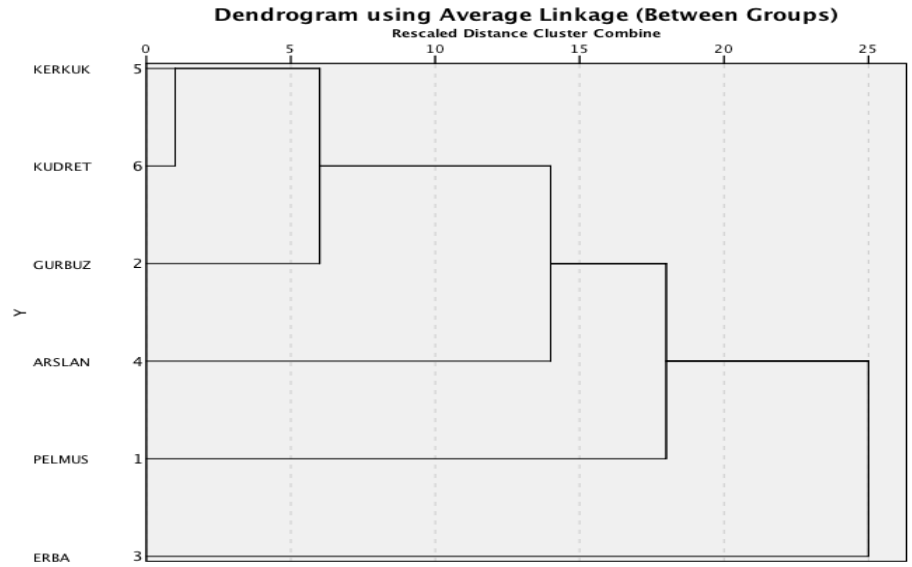

Figure 1. Dendrogram of 6 coriander genotypes generated with data from eleven ISSR primers.

While the varieties studied were mainly divided into 2 main groups, the Erba variety was clearly distinguished from the other varieties. Again Kerkuk and Kudret were the closest varieties of 99% similarity ratio (Table 4). Both markers results showed that Pelmus, Arslan and Erba varieties the most dissimilar varieties. These varieties found most distant varieties each other in a phylogenetic assessment for both marker system in the range of 50% and 79% dissimilarity ratios. These results showed that both two markers ISSR and SRAP could make desirable distinguishing power in determining genetic diversity and relationships among varieties.

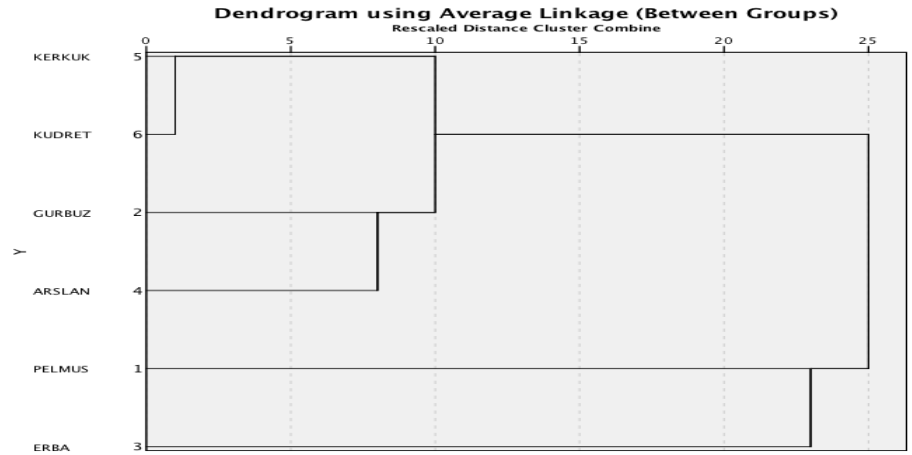

Figure 2. Dendrogram of 6 coriander genotypes generated with data from eleven SRAP primers.

This research is essential to show the importance of investigation genetically relationships of the coriander plant. There is a limited number of study has been performed for genetic relationships of the Turkish coriander plant at the DNA level. Using molecular markers is highly applicable for estimation of genetic diversity and genetic relationships in plant breeding. In this study, two different molecular markers were used to identifications of genetic similarities of mostly cultivated and traded Turkish coriander varieties. Inter-SSR (ISSR) fingerprinting has advantages such that no sequence knowledge is required. Amplifying the sequence between two SSRs from PCR reaction, yielding a multi-locus marker system make possible to use this method for fingerprinting, diversity analysis and genome mapping (Godwin et al. 1997). Preferred marker method should access a very large number of polymorphisms that are distributed within the genome. At the same time, preferred markers should be as cheap as effectively as possible to use, and the analytical method should be easy to perform (Flavel et al. 1992). Most of the researches on coriander varieties have been based on morphological traits and it's also known that environmental effects influence many of those traits. In this study has been presented an approach to investigate the genetic variability of the highly traded coriander varieties that collected from different areas of Turkey, using two different molecular markers as known ISSR and SRAP marker methods. The study also gave a chance to compare two distinct marker molecular analyses on coriander for the first time among the Turkish varieties.

Conclusion

The results derived from this research indicate that the marker-assisted characterization can be applied to develop the coriander breeding programs. It's obvious that genetic observations on coriander are limited, especially for the first time has been observed at the DNA level for most of the coriander's varieties. Applying molecular methods can offer opportunities to improve the desirable selection of proper coriander genotypes and it may help to make appropriate choices for market demands in the future.

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