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Determination of Genetic Diversity in Banana and Bell Pepper Lines Using Molecular Markers*

Moleküler Markerlar Kullanarak Çarliston ve Dolmalık Biber Hatlarında Genetik Çeşitliliğin Belirlenmesi

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Abstract: Successful hybrid cultivar breeding is depend on the high genetic diversity of the plant sources, as well as the homozygous and genetically distant lines requiring hybridization. The aim of this study is to determine the genetic distance between the inbred lines of pepper (*Capsicum annuum* L.) in order to increase efficacy of the breeding program. In this study, the genetic distances between the inbred lines of 44 bell peppers and 35 banana peppers were investigated using the SRAP (Sequence Related Amplified Polymorphism) markers. Based on pattern scores, dendrograms were produced by the UPGMA (unweighted pair-group method of mathematical averages method). Out of the 71 primer combinations tested, 50 combinations revealed polymorphisms among the banana pepper lines, and a total of 123 polymorphic bands were obtained. In the bell pepper lines, 24 SRAP primer combinations were tested and 15 combinations had 25 polymorphic bands. Based on the UPGMA cluster analysis, the pepper lines divided into groups as bell peppers and banana peppers. While the genetic similarity among the banana pepper lines varied between 0.62 and 0.98, the genetic similarity among the bell pepper lines varied between 0.54 and 1.00. As a result, it can be stated that the SRAP markers can be used successfully for determining the genetic distances of the pepper inbred lines thus will help the breeding programme.

Keywords: Pepper, genetic relationship, SRAP, polymorphism

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Öz: Hibrit çeşit ıslahında başarının sağlanması; genetik çeşitliliği yüksek bitki kaynağına, melezlenecek hatların homozigot olmasına ve melezlenecek hatların genetik olarak birbirinden uzak olmasına bağlıdır. Bu çalışmada, biber (*Capsicum annum* L.) saf hatları arasındaki genetik uzaklığın belirlenmesi ve ıslah programının etkinliğinin arttırılması amaçlanmıştır. Çalışmada 44 adet dolmalık ve 35 adet çarliston biber saf hatları arasındaki genetik uzaklık, SRAP (Sequence Related Amplified Polymorphism) markerı ile araştırılmıştır. Dendrogramlar UPGMA (unweighted pair-group method of mathematical averages method) yöntemine göre oluşturulmuştur. Test edilen 71 primer kombinasyonundan, çarliston biber hatlarında 50 kombinasyon polimorfizm göstermiş ve toplamda 123 adet polimorfik bant elde edilmiştir. Dolmalık biber hatlarında 24 SRAP primer kombinasyonu test edilmiş ve 15 kombinasyondan 25 polimorfik bant elde edilmiştir. UPGMA küme analizine göre biber hatları dolmalık biber ve çarliston biber olarak gruplara ayrılmıştır. Çarliston biber hatları arasındaki genetik benzerlik 0.62 ile 0.98 arasında değişirken, dolmalık biber hatları arasındaki genetik benzerlik 0.54 ile 1.00 arasında değişmiştir. Sonuç olarak, SRAP markerlarının biber saf hatlarının genetik uzaklıklarının belirlenmesinde başarılı bir şekilde kullanılarak ıslah programına yardımcı olacağı ifade edilebilir.

Anahtar kelimeler: Biber, genetik akrabalık, SRAP, polimorfizm

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*This study represents first author's master thesis.

INTRODUCTION

Pepper (*Capsicum annum* L.), a member of the *Solanaceae* family is one of the most globally important vegetable crops because of its consumption preferences and high nutritional value. It is becoming increasingly popular among consumers, with industrial applications also rising worldwide. In 2020, the global production of pepper was 36 million tons (FAOSTAT, 2022).

The Conservation of Plant Genetic Resources is vital both for plant breeding and society (Moreira et al., 2018). While the ability of breeders to achieve their goals depends on the plant genetic resources (Alvares Bianchi et al., 2020) and its genetic diversity. The genetic diversity of breeding lines has become smaller due to the breeding activities (Lee et al., 2016).

The pepper genotypes generally are evaluated based on their agro-morphological characteristics and disease-pest resistance in the breeding programs, resulting in genetic bottleneck. Species identification based on morphological characteristics is often difficult. The most of these characteristics are under the influence of environmental factors and might not be distinguish genotypes (Rodriguez et al., 1999). Furthermore, the limited number of morphological characteristics allow the breeders to survey only a small portion of the genetic diversity of the entire germplasm, and the resulting data cannot be used for breeding programs based genome-wide variation. The germplasm with a minimum number of accessions and maximum genetic diversity in pepper breeding programme will facilitate easy access to genetic material as well as the use of hidden genetic diversity. Therefore, molecular markers have been used efficiently to characterize the genetic diversity of germplasm in *Capsicum* sp. (Lefebvre et al., 2001; Geleta et al., 2005; Tam et al., 2005; Ibiza et al., 2012; Pacheco-Olvera et al., 2012; Zhang et al., 2016). In fact, AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeats), SSR (Simple Sequence Repeat), RAPD (Random Amplification Polymorphism DNA) and SRAP (Sequence Related Amplified Polymorphism) are widely employed in the identification of genetic diversity (Finger et al., 2010; Thul et al., 2012; Wahyuni et al., 2013; Moses et al., 2014; Carvalho et al., 2014; Carvalho et al., 2015; Grover et al., 2016).

SRAP (Sequence Related Amplified Polymorphism), one of the PCR techniques is based on the reproduction of open reading regions (ORFs, Open Reading Frames) in DNA. The SRAP markers are used to determine the genetic relationships of species. It is also preferred for its practical use in gene tagging, genomics, and cDNA fingerprinting as well as map-based cloning, and for providing reliable results (Li and Quiros, 2001).

The genetic diversity of inbred pepper lines and heterotic groups studies has not yet been extensively analyzed. Some studies using AFLP (Aktas et al., 2009), AFLP, RAPD (Lefebvre et al., 2001) and RAPD (Kumar et al., 2007) markers have been reported but these surveys only considered a few accessions. The aim of this research was to determine the genetic relationships among the 79 inbred pepper lines (*Capsicum annum* L.) using SRAP molecular markers and thus to establish the basis for the selection of the lines to be used as parents in the breeding programs.

MATERIAL AND METHOD

Plant Material

Forty-four bell pepper pure lines and 35 banana pepper pure lines from the AD-Rossen Seed Company were used in the present research.

DNA Extraction

The DNA isolation of the pepper lines was carried out by using CTAB protocol according to Doyle and Doyle (1990). After the quantities of the DNA samples obtained were determined using the spectrophotometer, the samples were diluted to 10 µl/ng.

SRAP Markers

A total of 95 combinations were tested in the SRAP analysis, including 71 combinations for the banana genotypes, and 24 combinations for the bell peppers (Table 1).

Table 1. SRAP primer combinations used in the Banana and bell pepper lines.
Çizelge 1. Çarliston ve dolmalık biber hatlarında kullanılan SRAP primer kombinasyonları.

Banana Pepper					
No	**Primer Combination**	**No**	**Primer Combination**	**No**	**Primer Combination**
1	me1 x em1	26	me3 x em2	51	me6 x em6
2	me1 x em2	27	me3 x em3	52	me6 x em7
3	me1 x em3	28	me3 x em4	53	me6 x em9
4	me1 x em4	29	me3 x em6	54	me6 x em10
5	me1 x em6	30	me3 x em7	55	me6 x em11
6	me1 x em7	31	me3 x em8	56	me6 x em12
7	me1 x em8	32	me3 x em9	57	me6 x em13
8	me1 x em9	33	me3 x em10	58	me6 x em14
9	me1 x em10	34	me3 x em11	59	me6 x em15
10	me1 x em11	35	me3 x em12	60	me6 x em16
11	me1 x em12	36	me3 x em13	61	me7 x em6
12	me1 x em13	37	me3 x em14	62	me8 x em2
13	me1 x em14	38	me3 x em15	63	me8 x em6
14	me1 x em15	39	me3 x em16	64	me9 x em6
15	me1 x em16	40	me3 x em17	65	me10 x em5
16	me2 x em1	41	me4 x em1	66	me10 x em6
17	me2 x em2	42	me4 x em2	67	me10 x em9
18	me2 x em3	43	me5 x em1	68	me11 x em6
19	me2 x em4	44	me5 x em2	69	me11 x em7
20	me2 x em6	45	me5 x em4	70	me12 x em5
21	me2 x em7	46	me5 x em6	71	me12 x em6
22	me2 x em8	47	me6 x em1		
23	me2 x em9	48	me6 x em2		
24	me2 x em10	49	me6 x em3		
25	me3 x em1	50	me6 x em4		
Bell Pepper					
No	**Primer Combination**	**No**	**Primer Combination**	**No**	**Primer Combination**
1	me1 x em11	9	me3 x em3	17	me6 x em15
2	me1 x em14	10	me3 x em9	18	me8 x em2
3	me1 x em19	11	me3 x em17	19	me8 x em7
4	me2 x em1	12	me4 x em1	20	me9 x em2
5	me2 x em2	13	me4 x em13	21	me9 x em7
6	me2 x em3	14	me5 x em2	22	me10 x em15
7	me2 x em6	15	me5 x em4	23	me11 x em9
8	me2 x em2	16	me6 x em6	24	me11 x em10

PCR Protocol

PCR amplifications were performed in 15 µL reaction volumes containing 25 ng μL^{-1} genomic DNA, 1 unit Taq polymerase, 1X PCR buffer, 2.5 mM of forward and reverse primers, 2mM $MgCl_2$, 250 µM of dNTP mix.

Thermal cycling conditions using a Thermocycler Mastercycler (Eppendorf-Germany) were at 94°C for 5 min, followed by 5 cyles of 94°C 1 min, 35°C 1 min, 72°C 1 min; followed by 29 cycles of at 50°C for annealing temperature. Finally, extension was performed at 72°C for 10 min. The PCR products were separated in 2.5% of agarose gel.

Data Analysis

The presence or absence of a SRAP band was scored as one (1) or zero (0), respectively. The data was evaluated in the NTSYS-pc version 2.2 (Numerical Taxonomy and Multivariate Analysis System) (Rohlf, 1992). The distance among the genotypes was calculated by applying the Jaccard similarity coefficient. The clustering was performed using UPGMA (Unweighted Pair-Group Method Algorithm) method by SHAN

clustering program. A correlation matrix was obtained by the SIMINT and a PCA (Principal Component Analysis) was performed.

RESULTS AND DISCUSSION

The Genetic Distance in Banana Pepper Lines

Out of the 71 SRAP primer combinations tested to determine the genetic relationship among the 35 banana pepper lines, 50 combinations yielded 123 polymorphic bands (Table 2). The SRAP combination that provided the most polymorphic bands among these lines was Me3 x Em16.The genetic similarity of 35 banana pepper lines varied from 0.62 to 0.98, revealing two main groups (Figure1).

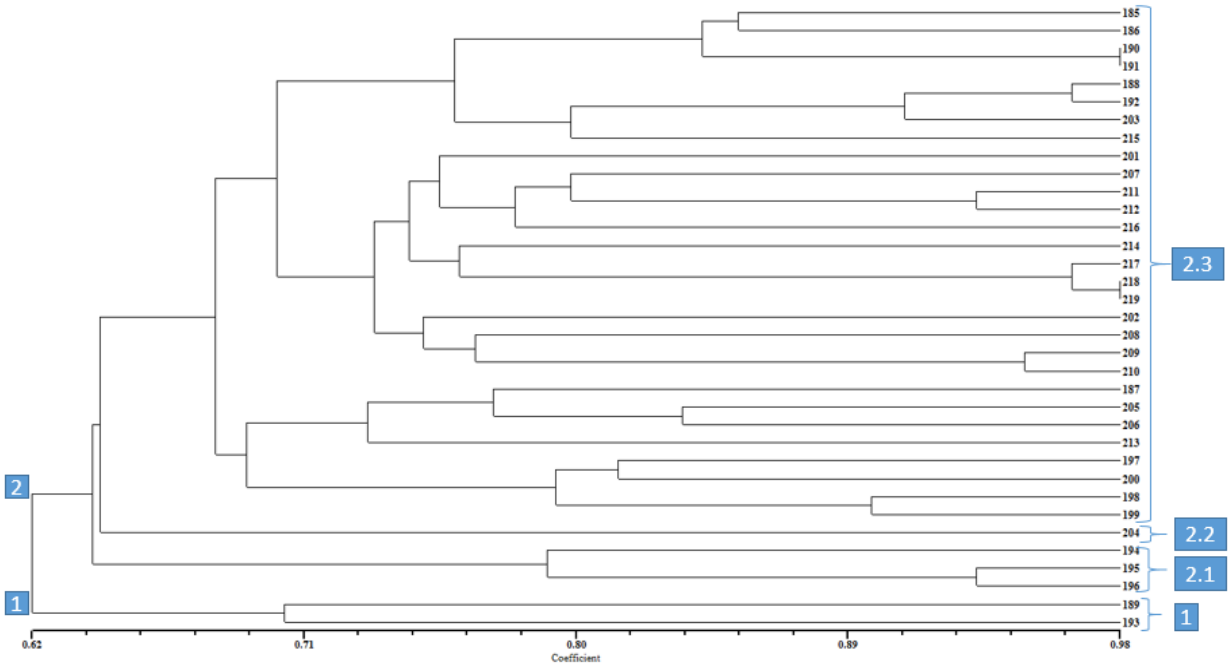

Figure 1. The genetic similarity among the Banana pepper lines created by the UPGMA method.
Şekil 1. UPGMA yöntemi ile oluşturulmuş çarliston biber hatları arasındaki benzerlik dengogramı.

The UPGMA dendrogram as defined by SRAP markers revealed two major groups. The first group included lines 189 and 193, which had genetic similarity of 69% (Figure 1).

The second group included remaining lines of the banana pepper. The genetic similarity of these genotype was 73%. Subsequently, second group was further divided in to 3 subgroups. The first subgroup consisted of lines 194, 195, 196; the second subgroup included only line 204, and the third subgroup consisted of remaining lines (Figure 1).

The genetic similarity between the lines 190 and 191 in third subgroup and between the lines 218 and 219 in second subgroup was 98%. The lines 185 and 193 had the farthest genetically distance (Figure 1).

Bozokalfa et al. (2017) found also that the genetic distance among the local pepper genotypes by SRAP markers ranged between 62 and 94% which was similar to our findings. However, the genetic similarity of the 22 accession of *C. annuum, C. baccatum, C. chinense, C. eximium, C. frutescens*, and *C. luteum* species, was reported to be between 23 and 88% for the RAPD markers and 11 and 96% for the ISSR marker (Thul et al., 2012). Zhang et al. (2016) also indicated that high polymorphism among the 372 pepper accessions was achieved by the use of the SSR marker. These results showed us that the genetic distance can be varied according to the genotype and molecular markers.

Table 2. SRAP primer combinations showing polymorphism in the Banana pepper lines.
Çizelge 2. Çarliston biber hatlarında polimorfizm gösteren SRAP primer kombinasyonları.

No	Primer Combination	Number of Polymorphic Bands	No	Primer Combination	Number of Polymorphic Bands
1	em1 x me3	2	12	me1 x em2	2
2	em1 x me5	1	13	me1 x em3	3
3	em1 x me6	4	14	me1 x em4	5
4	em6 x me2	1	15	me1 x em7	3
5	em6 x me3	3	16	me1 x em8	2
6	em6 x me5	3	17	me1 x em9	2
7	em6 x me6	2	18	me1 x em11	1
8	em6 x me7	3	19	me1 x em12	4
9	em6 x me9	2	20	me1 x em13	2
10	em6 x me10	1	21	me1 x em14	1
11	em6 x me11	3	22	me1 x em15	4
23	em6 x me12	1	37	me6 x em11	1
24	em9 x me6	1	38	me6 x em12	3
25	em9 x me10	2	39	me6 x em13	1
26	em3 x me3	2	40	me6 x em14	2
27	me3 x em4	1	41	me6 x em15	3
28	me3 x em7	4	42	me1 x em16	3
29	me3 x em9	3	43	me2 x em4	3
30	me3 x em10	3	44	me2 x em7	1
31	me3 x em11	3	45	me2 x em8	2
32	me3 x em12	3	46	me3 x em13	4
33	me6 x em2	3	47	me3 x em14	3
34	me6 x em3	1	48	me3 x em15	2
35	me6 x em4	3	49	me3 x em16	6
36	me6 x em7	2	50	me3 x em17	3

We determined line specific markers for lines 208 and 193 (Table 3). While the line 208 had a specific SRAP marker at 310 bp by the Em6 x Me11 primer combination. The line 193 had 3 specific SRAP markers at 300 bp by Me3 x Em12 and by Me1 x Em12 primer combinations, and at 200 bp by Me1 x Em4 combination (Figure 2). It can be stated that these markers can be useful for parental selection during multiplication of these lines, thus improving the hybrid breeding and seed production when these lines used as parental lines.

Table 3. Specific markers obtained for the Banana pepper lines.
Çizelge 3. Çarliston biber hatlarında elde edilen spesifik markörler.

Inbred lines	Primer combination	bp
208	em6 x me11	310
193	me3 x em12	300
193	me1 x em4	200
193	me1 x em12	300

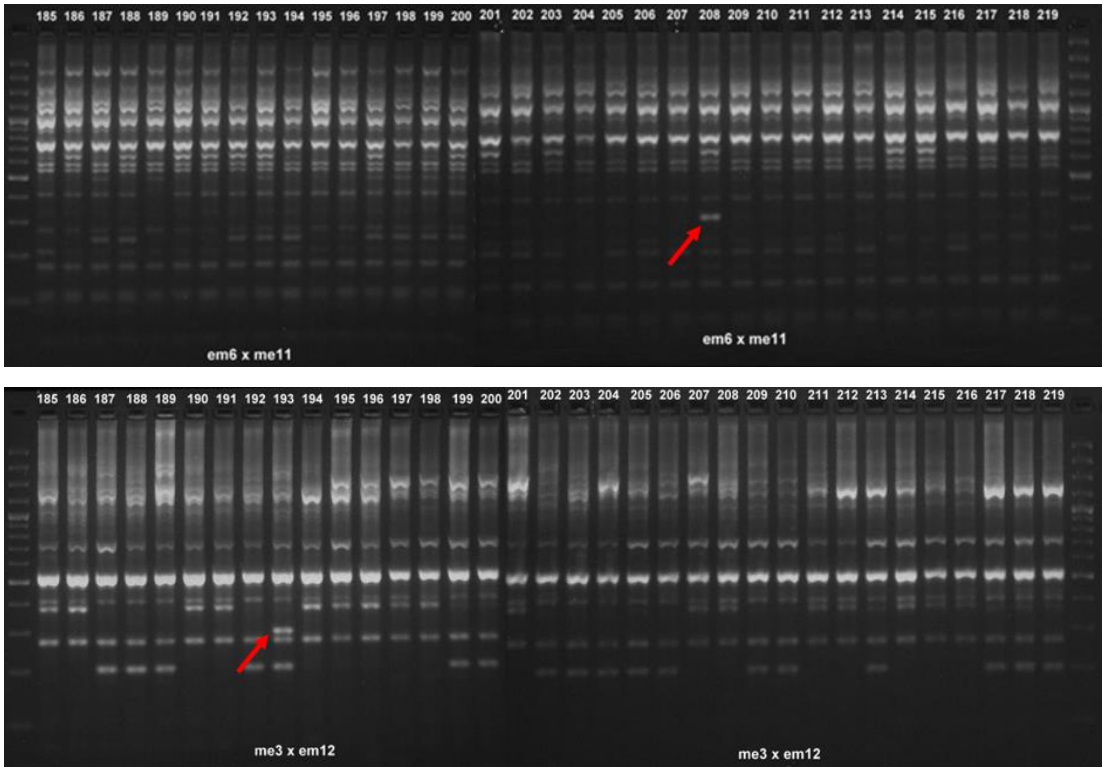

Figure. 2. Specific markers belonging to the Banana inbred lines 208 and 193.
Şekil 2. 208 ve 193 nolu çarliston saf hatlarına ait spesifik markörler.

The genetic distance between banana genotypes were further examined by 2-dimensional and 3-dimensional scatter plots of different genotypes derived by the PCA (Principal Component Analysis). SRAP data showed that 77.21% of the total variation could be explained by the three principal components based on the first, second and third Eigen vectors which account for 69.91%, 3.90% and 3.40% variation respectively (Figure 3 and Table 4).

Table 4. Factor groups formed as a result of the SRAP marker analysis in the Banana pepper lines.
Çizelge 4. Çarliston biber hatlarında SRAP markör analizleri sonucunda oluşan faktör grupları.

PC axes	Eigenvalues	Variation (%)	Total variation (%)
1	24.46	69.91	69.91
2	1.36	3.90	73.81
3	1.19	3.40	77.21

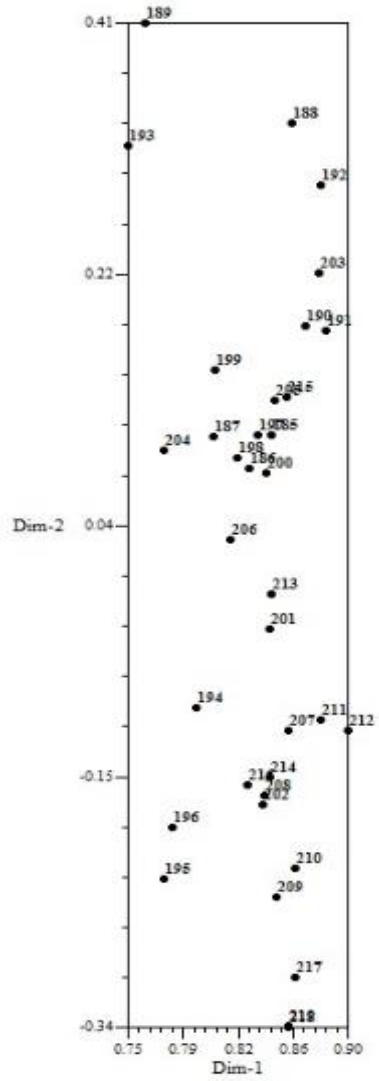

Figure 3. Two dimensional PCA scaling of 35 banana peppers genotypes using SRAP markers
Şelil 3. SRAP markerları kullanılarak 35 çarliston biber genotipinin iki boyutlu PCA ölçeklemesi

Genetic Distance in Bell Pepper Lines

Out of the 24 SRAP primer combinations tested to determine the genetic relationship among the 44 bell pepper lines, as well as in the Banana pepper lines (Table 1). 15 combinations yielded 25 polymorphic bands (Table 5).

Table 5. SRAP primer combinations showing polymorphism in the bell pepper lines.
Çizelge 5. Dolmalık biber hatlarında polimorfizm gösteren SRAP primer kombinasyonları.

No	Primer Combination	Number of Polymorphic Bands	No	Primer Combination	Number of Polymorphic Bands
1	em1 x me4	3	9	em13 x me4	4
2	em2 x me3	1	10	em15 x me6	1
3	em6 x me6	3	11	em15 x me10	1
4	em7 x me9	1	12	me1 x em19	3
5	em9 x me3	2	13	em6 x me2	1
6	em9 x me11	1	14	em2 x me2	1
7	em10 x me11	1	15	me3 x em17	1
8	em11 x me1	1			

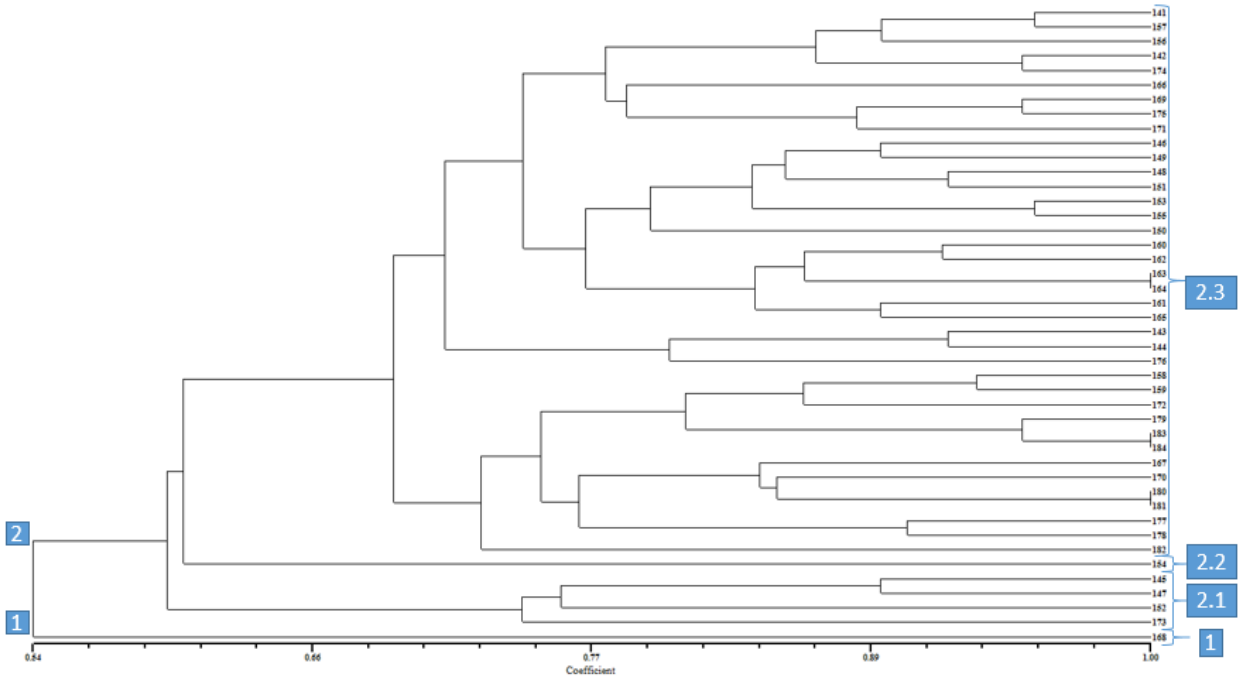

Figure 4. The genetic similarity among the bell pepper lines, created by the UPGMA method.
Şekil 4. UPGMA yöntemi ile oluşturulmuş dolmalık biber hatları arasındaki benzerlik dengogramı.

A dendrogram was created employing the UPGMA by using the similarity index in the bell pepper lines (Figure 4). The genetic similarity of the bell pepper lines varied from 0.54 and 1.00, and consist of two main groups (Figure 4).

The UPGMA dendrogram as defined by SRAP markers revealed two major groups, as well as banana pepper. The first group included line 168 while the second group included remaining lines of the bell pepper. The second main group is also divided into 3 subgroups which had genetic similarity of 61%. The first subgroup consisted of lines 145, 147, 152, 173; the second subgroup included only line 154, and the third subgroup consisted of remaining lines (Figure 4).

The most distant relationship in the bell pepper lines is seen between lines 141 and 168 (Figure 4). Also, the lines that are genetically closest to each other are 180-181; 183-184; 163-164.

Xu et al. (2011) on investigation of the genetic distance between 72 pepper accessions using 17 pairs of SRAP markers reported genetic similarity which varied among 0.56 and 0.91. However, Rai et al. (2013) examined the genetic diversity in 48 pepper genotypes, collected from 9 different countries, using the SSR and RAMPO (random amplified microsatellite polymorphism) markers. Genetic similarity found with the SSR markers ranged from 0.26 to 0.89, while with the RAMPO markers the similarity between the genotypes ranged from 0.37 to 0.98. These results were similar to the findings of the present study. However, Göçmen (2019), in which 16 pepper genotypes collected from different regions were investigated in terms of the phylogenetic relationship with SRAP markers, a total of 155 polymorphic bands were obtained from 31 combinations of the SRAP primers. These values were higher than the results obtained in this study. The high number of polymorphic bands is attributed to the peppers being collected from different locations.

The top five principal components were used to analyze population structure. The results showed that the five PCs had contribution rates of 31.01%, 2.57%, 2.10%, 1.51% and 1.08% respectively (Table 6). PCA separated the 44 genotypes into two major groups which were consistent with the UPGMA results (Figure 5).

Table 6. Factor groups formed as a result of the SRAP marker analysis in the bell pepper lines.
Çizelge 6. Dolmalık biber hatlarında SRAP markör analizleri sonucunda oluşan faktör grupları.

PC axes	Eigenvalues	Variation (%)	Total variation (%)
1	31.01	70.49	70.49
2	2.57	5.86	76.35
3	2.10	4.77	81.13
4	1.51	3.45	84.58
5	1.08	2.46	87.04

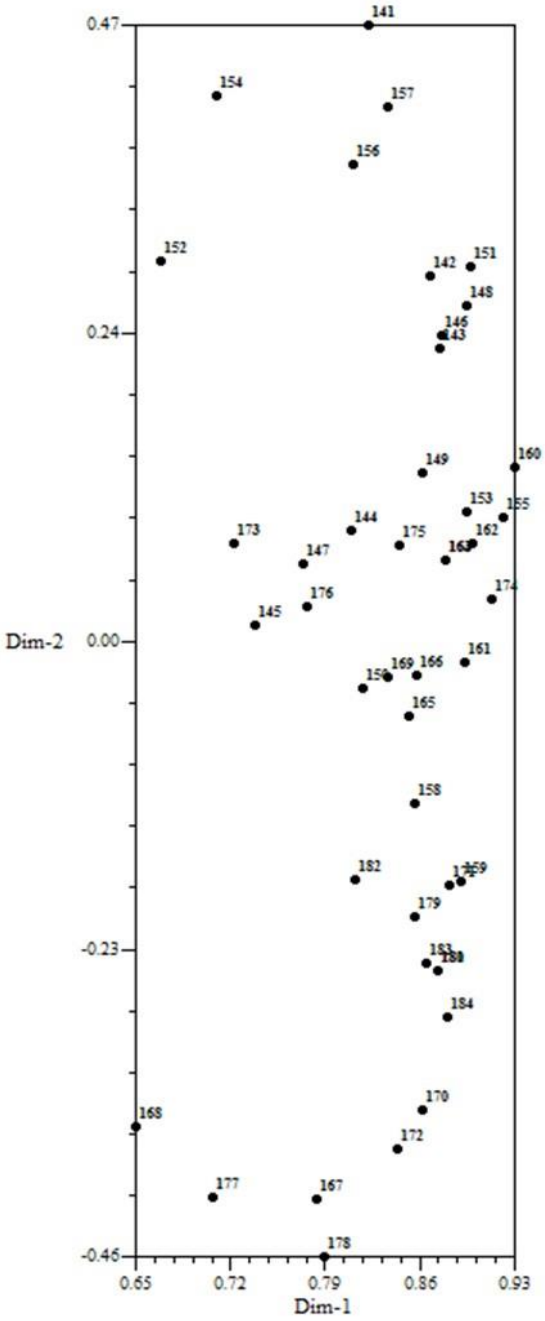

Figure 5. Two dimensional PCA scaling of 44 bell peppers genotypes using SRAP markers
Şekil 5. SRAP markerları kullanılarak 44 dolmalık biber genotipinin iki boyutlu PCA ölçeklemesi

CONCLUSION

Our study revealed that the SRAP marker system is useful for determining the genetic distances of the inbred lines. In this context, it is deduced that more primer combinations require testing, and morphological characterization should be made besides molecular characterization, thus enabling stronger results. Therefore, the findings obtained by this study demonstrated that the genetic variation between the inbred lines could easily be researched utilizing the SRAP marker.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest related to this article.

AUTHORS' CONTRIBUTIONS

Both authors read and approved the final manuscript.

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