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Characterization of local Besni pepper (*Capsicum annuum* L.) genotypes with SRAP and ISSR markers

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Abstract

Türkiye has great plant diversity due to its geographical location and suitable climatic and soil conditions. This genetic richness will be meaningful with the studies to be done on it. Characterization studies carried out on local varieties are important in determining the characteristics of the variety and thus including those with superior characteristics in breeding studies. This study aimed to characterize the Besni Pepper genotypes a local variety with molecular (DNA) markers. In the study, 29 pepper genotypes were screened with 10 ISSR and four SRAP primers, and 62 scorable bands were produced. While the polymorphism rate was determined as 76% (47/62), the average number of polymorphic fragments for each primer was calculated as 3.3. According to the created dendrogram, 29 pepper genotypes formed 2 main groups, and these groups were divided into subgroups. The genetic similarity rate was found to be between 0.68-0.95. While the B1 and C3 genotypes were the most distant from each other, the G2 and G3 genotypes were the closest to each other. The study shows that even pepper genotypes from a narrow area have about 32% variation that can be used in future breeding studies. This variation is due to both the fertilization biology (%3-30 cross-pollination) of pepper and seed mobility between farmers. After selection studies for yield, quality, and stress conditions, genotypes can be included in breeding programs.

Keywords: *Capsicum annum*, Molecular characterization, SRAP, ISSR, Polymorphism

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INTRODUCTION

The genus *Capsicum* is native to South America and it is accepted that both Central America and Mexico are considered as a diversity region for the taxa and it is classified as an important genetic resource for agriculture and food. *Capsicum annuum* L., belonging to the genus *Capsicum* of the *Solanaceae* (nightshade) family, is commonly known by names such as sweet pepper, cayenne pepper, hot pepper, paprika, red pepper, Christmas pepper, and bell pepper (Zhigila et al., 2014). Pepper is an important species that is grown as an annual in temperate climates and as a perennial in tropical climates and can be grown both in open fields and in protective cultivation (Vural et al., 2000: Singh et al., 2011). Pepper is a fruit-bearing vegetable species and both its green unripe fruits and its ripe fruits of different colors are consumed in different ways, either fresh or processed (Swamy, 2023). One of the oldest cultivated crops, peppers were domesticated in parts of Mexico about 6,000 years ago (García-Gaytán et al., 2017). Pepper, which are native to South America and domesticated in Mexico, is of great commercial interest today and is consumed in different ways by a significant portion of the world's population (Barboza et al., 2022). Peppers have attracted attention not only for their importance in human nutrition but also for their potential as medicinal plants. This is due to the high phytochemical content of both ripe and unripe fruits that have been shown to be beneficial to human health. Beneficial phytochemicals contained in peppers include carotenoids, flavonoids, ascorbic acid and phenolic compounds with antioxidant properties, and capsaicinoids, which give peppers their spicy flavor (Bosland, 2000; Crosby et al., 2005). Studies have shown that capsaicin can reduce pain, protect cells from damage, and reduce inflammation (Fattori et al., 2016). Peppers are also an important source of vitamin A,

vitamin C, vitamin B, vitamin E, and folic acid (Wahyuni et al., 2011). Recent studies have added 5 new species to the 38 species in the *Capsicum* genus, bringing the total number to 43 (Barboza et al., 2019). Heiser and Smith (1953) reported that there are five economically important pepper species: *C. annuum*, *C. frustescens*, *C. pendulum*, *C. baccaum,* and *C. pubescens* (Samos and Kundt, 1984; Roy, 2016). A diploid and self-fertile species with 2n=24 chromosomes within the genus *Capsicum*, *C. annuum* is one of the major vegetable species grown in large quantities in Türkiye and the world (FAO, 2023). *Capsicum,* originating from the New World tropics and subtropics, has a genetic diversity center in and around Mexico. Peppers were introduced to Spain from the Americas by Columbus, who returned from one of his journeys to the Caribbean in the late 15th century. Pepper cultivation spread from the Mediterranean region to England in the mid-16th century and to Central Europe towards the end of the 16th century. The Portuguese brought it to India from Brazil in the last quarter of the 19th century. Cultivation began in China in the late 1700s (Vidhi, 2023). According to FAO (2023) statistics, approximately 38 million tons of fresh (green and red) and 5.8 million (M) tons of dried pepper were produced in the world, and the world's major pepper-producing countries are China (17 M tons), Mexico (3.7 M tons), Indonesia (3.1 M tons) and Turkiye (3.1 M tons), respectively. It was brought to Istanbul in the 16th century with the improvement of relations between the Ottoman Empire and European countries (Vural et al., 2000). There are also sources reporting that pepper was brought to Turkiye via the Middle East (Andrews, 1999)

Türkiye, located at the intersection of the Mediterranean, European, and Near Eastern gene centers, exhibits significant regional diversity in climate and soil characteristics due to both its location and topographic structure. In addition, as one of the oldest regions in the world where agriculture was first practiced, it has caused many plant species to show diversity and to be a micro gene center (Tan and Inal, 2003). As mentioned above, pepper production is carried out in almost all regions of Türkiye, one of the important pepper-producing countries (3.4 M tons) (TUIK, 2024). Over time, significant genetic diversity has occurred due to new introductions and cross-pollination, and many local pepper varieties have emerged in different regions of Turkiye. Besni pepper, the subject of this study, is one of them. Genetic diversity depends on the genetic characteristics and fertilization biology of the species (Figdore et al., 1988). Pepper tends to cross-pollinate up to 30% depending on the flower structure (heterostyle) and pollinator activity (Rego et al., 2012).

Plant germplasm is an indispensable resource for the development of high-yielding/high-quality cultivars that are tolerant or resistant to environmental (biotic, abiotic) stress factors to secure the long-term sustainability of agricultural production. Local populations consisting of landraces that have adapted to their environmental conditions for long periods of time and have survived against some abiotic and biotic stress factors are important resources for plant breeding. Türkiye, one of the world's major vegetable-producing countries, has unique resources in terms of locally cultivated plant varieties developed by the selection of local farmers and still exhibiting significant diversity. Climate change, replacement of local varieties with modern varieties, forest fires, increased land clearance, erosion, urbanization, road and dam construction, alternations in plant production and plant protection methods (intensive use of agricultural chemicals), and the continuous collection of bulbous plants from nature cause the diversity of plant genetic resources to gradually decrease. Countries and their governments that are aware of this important problem in plant germplasm have long begun to work on the collection, characterization, evaluation, and protection (*in-situ* and *ex-situ*) of resources (Tan, 1992; Sahin et al., 2022). Characterization of plant resources can be done with both classical and molecular/DNA markers. Genetic markers used in the characterization of organisms are generally classified into two categories: classical and DNA/molecular markers. While morphological, cytological, and biochemical markers are classified as classical markers, examples of molecular markers are PAPD (Randomly Amplified Polymorphic DNAs), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplification Fragment Length Polymorphism), SSRs (Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeats), SNP (Single Nucleotide Polymorphism) and DarT (Diversity Array Technology markers) (Jiang, 2013; Hulse-Kemp et al., 2016; Karataş et al., 2017). Molecular markers are preferred because they are not affected by environmental conditions, like morphological and biochemical markers, and can be repeated in different places and conditions.

With the development of biotechnology, genetic similarities and differences can be clearly revealed as a result of molecular characterization analyses performed on plants (Geleta et al., 2005). As with many other plant species, many molecular characterizations have been carried out in pepper. Lijun and Xuexiao (2012) screened 28 pepper lines from 5 pepper varieties with 13 ISSR primers to examine genetic diversity within and between species. Of the 135 bands produced, 105 were found to be polymorphic, and the polymorphism rate was calculated as 78%. They reported that pepper varieties were divided into 2 main groups in the dendrogram. They reported that Shannon's information index (I) was between 0.06 and 0.13, and Nei's genetic diversity index (H) was between 0.03 and 0.08. In another study where 16 genotypes belonging to Cırgalan pepper (*C. annuum*), one of the local varieties of Türkiye, were characterized with ISSR markers, 12 of the 16 ISSR primers gave results, and the polymorphism rate was 22% (23/104). The genotypes were divided into two main groups, 10 in the first cluster and 6 in the second cluster. Researchers reported that the genetic similarity rate among Cırgalan pepper genotypes varied from 0.95 to 0.99, and genetic diversity was low (Pınar et al., 2017). In a study in which 26 pepper genotypes were characterized with 26 SRAP primers, while no amplification was observed in 8 SRAP primers, 90

polymorphic bands were produced with 18 SRAP primers and evaluated. The genetic variation between genotypes was between 24 and 72%, and the PIC (Polymorphic Information Content) value was between 0.09-0.99 (Adalı 2017). The genetic relationship of 30 pepper varieties, including capia, elongate, Charleston, and bell peppers, was studied with six SSR markers and it was found that genetic diversity was low in SSR loci (Şeker 2018). In the study conducted by Cesur et al. (2020) with the SSR maker on 10 pepper genotypes, it was determined that the genotypes were divided into two main groups; the total allele number was 162, the specific allele number was 60, and the band size varied between 164 and 294 bp. The PIC ranged from 0.04 to 0.89. In a study where *C. annuum* var. *acuminatum*, *C. annuum* var. *grossum*, *C. annuum* var. *abbreviatum*, *C. frutescens* var. *baccatum* species and varieties were used as plant material in West Africa, 75 loci were identified, and 14 of them were recorded as polymorphic. The PIC value was determined as 0.67, and heterozygosity (He) was 0.78. Based on the results obtained, the authors suggested that *C. frutescens* var. *baccatum* should be considered as a variety of *C. annuum* (Olatunji and Afolayan, 2019). Tatar (2022) characterized Gaziantep pepper genotypes using 31 SRAP and 13 ISSR primers. The polymorphism rates were reported as 83% (211/254) in the SRAP method and 85% (110/128) in the ISSR method, respectively. According to the SRAP and ISSR methods, the similarity rate was determined to be 0.40-1.00 and 0.48-0.80, respectively.

Local varieties preferred by the local people and grown in limited areas are part of the sustainable local economy and are indispensable for countries. Along with the changing world, the characteristics of the products demanded by the consumer are also changing. For this reason, it is important to determine the characteristics of local varieties and include the superior ones in breeding studies. Furthermore, determining the genetic relationship between the genotypes with determined characteristics is also extremely important for the breeders. This study aimed at the molecular characterization of Besni pepper genotypes grown in Besni district of Adıyaman province, whose morphological characterization was performed by Sahin et al. (2022).

MATERIAL AND METHODS

Experimental site and plant materials

This study was carried out in Erciyes University Faculty of Agriculture research areas, greenhouses, and laboratories located at latitude 38° 42’ 33’’ N and longitude 35° 32’ 33’’. In the study, 26 Besni pepper genotypes were obtained from farmers in the villages of Besni (20) and Gölbaşı district (6), and a total of 29 pepper genotypes, including Cırgalan, Yalova Çorbacı, and Sera Demre as control varieties, were used (Table 1). Pepper genotypes were collected by visiting villages and interviewing farmers. In the morphological characterization study of Sahin et al. (2022), pepper genotypes were divided into three groups according to fruit shape: conical, bell, and elongated.

Table 1. Pepper genotypes used in the study and locations where they are collected

Genotypes	Supplied location	Genotypes	Supplied location
B_1	Besni-Oyratlı	B_{16}	Besni-Oyratlı
B_2	Besni-Oyratlı	B_{17}	Besni-Oyratlı
B_3	Besni-Oyratlı	B_{18}	Besni-Oyratlı
B_4	Besni-Oyratlı	B_{19}	Besni-Oyratlı
B_5	Besni-Oyratlı	B_{20}	Besni-Toklu
B_6	Besni-Oyratlı	G_1	Gölbaşı-City Center
B7	Besni-Oyratlı	G_2	Gölbaşı-City Center
B_8	Besni-Oyratlı	G_3	Gölbaşı-City Center
B_9	Besni-Oyratlı	G_4	Gölbaşı-Maltepe
B_{10}	Besni-City Center	G_5	Gölbaşı-Maltepe
B_{11}	Besni-Oyratlı	G_6	Gölbaşı-Maltepe Village
B_{12}	Besni-Oyratlı	C_1 (Cırgalan)	ERU Faculty ofAgriculture
B_{13}	Besni-Çamurcu	C_2 (Yalova Çorbacı)	ERU Faculty of Agriculture
B_{14}	Besni-Çamurcu	C_3 (Sera Demre)	ERU Faculty of Agriculture
B_{15}	Besni-Oyratlı		

B: Besni; G: Gölbaşı; C: Control; ERU: Erciyes University

Cultivation of plants

Seeds of the genotypes were sown in a 3:1 peat-perlite mixture under unheated greenhouse conditions on 16.04.2021. The seedlings were fertilized twice with 15:15:15 (N:P:K) + microelement fertilizer until they reached planting size (3-4 true leaves). The electrical conductivity of the fertigation water was adjusted to 2 dS/m. When the seedlings reached the 3-4 true leaf stage, 3 plants from each genotype were planted in an unheated greenhouse at 80x30 cm distances on 25.05.2021. Drip irrigation was used as the irrigation system. Irrigation was done based on plant and soil observations. Fertilization was done depending on the plant development period by fertigation (Vural et al., 2000; Ifas, 2021). Black plastic mulch was used for weed control. Certified pesticides and fungicides for pepper were applied according to the disease and pest occurrence. Weeds between the rows were manually removed.

Molecular characterization

PCR-based ISSR and SRAP marker analysis methods were used for molecular characterization. Ten ISSR primers (Table 2) and four SRAP primer pairs (Table 4) were used in the study. Isolation of pepper DNA was performed using 50-60 mg of young leaf material using the CTAB extraction protocol reported by Doyle (1991). The PCR mixture was prepared using 30 ng of template DNA, 1U Taq DNA polymerase enzyme, 0.25 mM each dNTP, 1 µM primer, 1.5 µl 10X PCR buffer solution, 1.5 mM $MgCl_2$, and H_2O, with a total volume of 20 µl. The PCR cycles (Table 3) were performed according to Uzun (2009).

Table 2. ISSR primers used in molecular characterization

Pimer name	Primer sequence 5'-3'
AGC6G	AGCGGGGGG
AG7YC	AGAGAGAGAGAGAGYC
TCC5RY	TCCTCCTCCTCCTCCRY
CA6AC	CACACACACACAAC
CAC3GC	CACCACCACGC
GA8Y9	GAYGYGYGYGYGYGYGYG
GACA4	GACAGACAGACAGACA
AG8T	AGAGAGAGAGAGAGAGT
CA8R	CACACACACACACACAR
BDBCA7C	BDBCACACACACACACAC

Table 3. PCR conditions for ISSR primers used in the study

Step		Temperature	Time	Number of cycles
1	Initial denaturation	94 ˚C	3 min	1
	Denaturation	94 ˚C	1 min	35
2	Annealing	53 ˚C	50 s	35
	Extension	72 ˚C	2 min	35
3	Final extension	72 ˚C	7 min	1
	Storage	4 ˚C	∞	1

In the SRAP method, PCR mixture was prepared using a total volume of 20 µl, 30 ng Template DNA, 1U Taq DNA polymerase enzyme, 0.25 mM each dNTP, 1 µM primer, 1.5 µl 10X PCR buffer solution, 1.5 mM $MgCl_2$, and H_2O. The PCR cycles (Table 3) were performed according to Uzun (2009).

Table 4. SRAP primer pairs used in molecular characterization

Primer name	Primer sequence 5'-3'	
	Forward	Reverse
Em11-Me2	GACTGCGTACGAATTCTA –	TGAGTCCAAACCGGAGC
Em11-Me6	GACTGCGTACGAATTCTA –	TGAGTCCAAACCGGACA
Em5-Me12	GACTGCGTACGAATTAAC–	TGAGTCCAAACCGGAGA
Em11-Me12	GACTGCGTACGAATTCTA –	TGAGTCCAAACCGGAGA

Table 5. PCR conditions for SRAP primers used in the study (Uzun, 2009)

Step		Temperature	Time	Number of cycles
1	Initial denaturation	95 ˚C	3 min	1
	Denaturation	94 ˚C	45 s	5
2	Annealing	35 ˚C	1 min	5
3	Elongation	72 ˚C	1 min	5
4	Denaturation	94 ˚C	45 s	35
5	Annealing	50 ˚C	1 min	35
6	Elongation	72 ˚C	1 min	35
	Last elongation	72 ˚C	5 s	1
7	Storage	4 ˚C	∞	1

Statistical analysis

In the study, the bands in the gel images obtained from the ISSR and SRAP marker methods were scored as present (1) and absent (0). The data were analyzed in the NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pcversion 2.11, Exeter Software, Setauket, N.Y., USA, Rohlf, 2000) computer package program. Similarity indices were calculated according to the DICE (1945) method and the dendrogram was created according to the UPGMA (Unweighted Pair-Group Method With Arithmetic Average) method. Key metrics such as the total number of bands, number of polymorphic bands, average polymorphism, effective number of alleles (Ne), allele frequency, Shannon's information index (I), expected heterozygosity (He), and the ratio of unbiased expected heterozygosity (uHe) were determined. Principal Component Analysis (PCA) was done based on the

variance-covariance matrix. The polymorphism rates of the primers were calculated according to the formula below. Polymorphism rate = (Number of polymorphic bands/total number of bands) x 100.

RESULTS

The data generated from the ISSR and SRAP marker systems used in the study were analyzed together and a similarity dendrogram was created with these data. In this study, scorable images (Figure 1) were obtained from 10 primers out of 16 ISSR primers and four primer pairs out of 18 SRAP primer pairs tested for 29 pepper genotypes. A total of 14 primers, 10 ISSR, and four SRAP, were evaluated and data were generated. For the data produced by ISSR and SRAP marker methods for 29 pepper genotypes, primer band length (bp), number of bands (number), polymorphic band number (number), polymorphism rate (%), monomorphic band number (number), and monomorphism rate (%) were presented in Table 6. A total of 62 bands were obtained, with an average of 4.4 bands per primer, and band lengths ranged from 150 to 1300 bp (Table 6). It was recorded that 47 of these bands were polymorphic, the average number of polymorphic bands was calculated as 3.3, while the average polymorphism rate was calculated as 66.3%. Primers with 100% polymorphism rate were BDBCA7C, Em11-Me2, and Em11-Me6, while primer combinations Em5-Me12 and Em11-Me12 produced monomorphic bands. A total of 15 monomorphic bands were obtained and the average number of monomorphic bands was calculated as 1.07.

Table 6. Band profiles and percent polymorphism rates of ISSR and SRAP primers in 29 pepper genotypes

Primers	Primer sequence 5'-3'	Band length	Number of band	Polymorphic band number	Polymorphism rate (%)	Monomorphic band number	Monomorphism rate (%)
AGC6G	AGCGGGGGG	200-1200	9	8	% 88	1	% 11
AG7YC	AGAGAGAGAGAGAGYC	550-1000	2	1	% 50	1	% 50
TCC5RY	TCCTCCTCCTCCTCCRY	370-1020	5	4	% 80	1	% 20
CA6AC	CACACACACACAAC	560-1100	4	2	% 50	2	% 50
CAC3GC	CACCACCACGC	300-1300	7	6	% 85	1	% 14
GA8Y9	GAYGYGYGYGYGYGYGYG	150-1000	5	3	% 60	2	% 40
GACA4	GACAGACAGACAGACA	350-1200	6	4	% 66	2	% 33
AG8T	AGAGAGAGAGAGAGAGT	200-1200	8	6	% 75	2	% 25
CA8R	CACACACACACACACAR	450-1020	4	3	% 75	1	% 25
BDBCA7C	BDBCACACACACACACAC	400-1020	6	6	% 100	0	% 0
Em11-Me2	GACTGCGTACGAATTCTA (F) TGAGTCCAAACCGGAGC (R)	150-300	2	2	% 100	0	% 0
Em11-Me6	GACTGCGTACGAATTCTA (F) TGAGTCCAAACCGGACA (R)	300-430	2	2	% 100	0	% 0
Em5-Me12	GACTGCGTACGAATTAAC (F) TGAGTCCAAACCGGAGA (R)	350	1	0	% 0	1	% 100
Em11-Me12	GACTGCGTACGAATTCTA (F) TGAGTCCAAACCGGAGA (R)	200	1	0	% 0	1	% 100
Total			62	47		15	
Average			4.4	3.3	%66.3	1.07	%33.4

Table 7. Polymorphism values of studied primers

Primers	p	q	Ne	I	He	uHe
AGC6G	0.248	0.752	1.287	0.307	0.187	0.191
AG7YC	0.661	0.339	1.389	0.315	0.219	0.223
TCC5RY	0.557	0.443	1.464	0.432	0.287	0.292
CA6AC	0.791	0.209	1.473	0.340	0.243	0.249
CAC3GC	0.379	0.621	1.370	0.398	0.250	0.257
GA8Y9	0.543	0.457	1.340	0.325	0.215	0.219
GACA4	0.568	0.432	1.381	0.355	0.235	0.240
AG8T	0.579	0.421	1.488	0.424	0.286	0.292
CA8R	0.686	0.314	1.580	0.461	0.320	0.326
BDBCA7C	0.489	0.511	1.921	0.672	0.479	0.488
Em11-Me2	0.700	0.300	1.697	0.587	0.400	0.408
Em11-Me6	0.437	0.563	1.586	0.547	0.363	0.372
Em5-Me12	1.000	0.000	1.000	0.000	0.000	0.000
Em11-me12	1.000	0.000	1.000	0.000	0.000	0.000
Average	0.617	0.383	1.426	0.368	0.248	0.254

p and q: Allele frequency; Ne: Effective number of alleles; I: Shannon's information index; He: Expected heterozygosity; uHe: Unbiased expected heterozygosity.

Expected and observed allelic frequency values (p and q) ranged from 0.248 to 1.0 and from 0.00 to 0.752 (Table 7). The effective number of alleles (Ne) of the genotypes varied from 1.0 to 1.921 with a mean of 1.426, whereas the mean Shannon's Information index (I) was 0.368 with individual values ranging from 0.0 to 0.672. The expected heterozygosity (He) was found to be between 0.0 and 0.479 (average 0.248), and the unbiased expected heterozygosity (uHe) value varied from 0.0 to 0.488. The expected heterozygosity for all primers except Em5-Me12 and Em11-me12 was found to be lower than the unbiased expected heterozygosity.

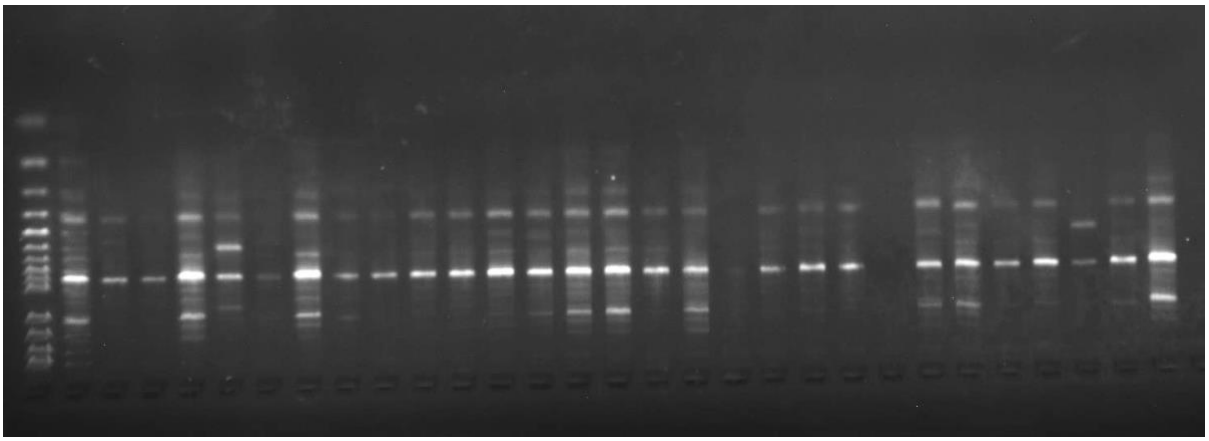

Figure 1. Gel image of AGC6G primer.

In the dendrogram created according to the Dice similarity matrix, the genetic similarity rate of Besni pepper varies between 0.68 and 0.95. According to the dendrogram, the genotypes were divided into two main groups at a similarity level of 68%: a large group with 24 genotypes and a small group with five genotypes. These groups also formed subgroups among themselves (Figure 2). Genotypes G5, G6, C1, C2, and C3 were included in the second (II) group, and the other 24 genotypes were grouped in the first (I) group. The large group was divided into two subgroups at 80% similarity level, and 11 genotypes collected from Besni district were grouped in the first subgroup (I-1), while the second subgroup (I-2) included 9 genotypes taken from Besni district and 4 genotypes taken from Gölbaşı district. I-1 and I-2 subgroups were divided into two groups (I-1-i and I-1-ii; I-2-i and I-2-ii) at 82% similarity level. When the dendrogram is examined, it is seen that B1 and C3 are the most distant genotypes, while genotypes G2 and G3 show 95% similarity and are the closest genotypes to each other among the other genotypes. Samples taken from the same village are located close to each other in the dendrogram.

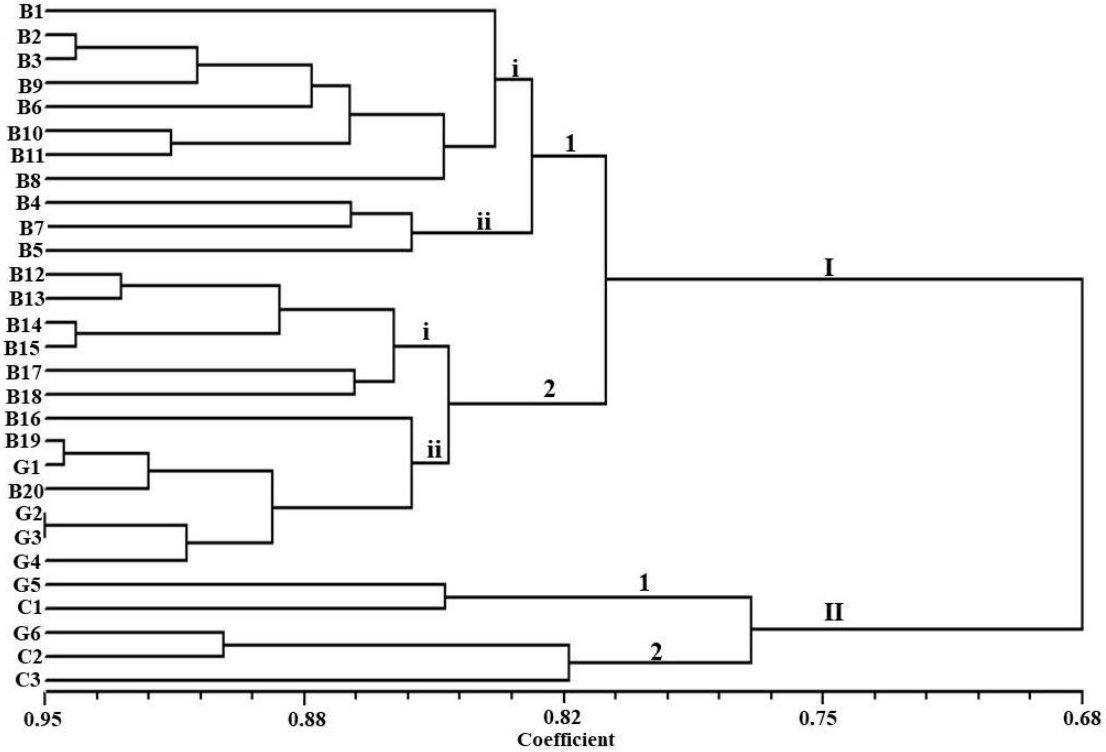

Figure 2. Dendrogram of the 29 genotypes using UPGMA method produced from SRAP and SSR markers.

According to the Two-Dimensional Principal Component Analysis graph, 29 pepper genotypes formed two different groups that were not close to each other. While one group consisted of genotypes taken from Besni district, the other group consisted of genotypes taken from Gölbaşı district and control varieties. Genotypes B1 (1) and B18 (18) collected from Oyratlı village were located in a different place than the other genotypes. It was observed that the genotypes collected from Oyratlı, Toklu, and Çamurcu villages of Besni district were separated from the other genotypes. In addition, it was observed that the genotypes obtained from Gölbaşı district were grouped with the genotypes used as control (Figure 3). In the Three-Dimensional Principal Component Analysis graph, it is seen that the genotypes form two groups. As in the Two-Dimensional Principal Component Analysis graph, it is seen that the genotype numbered B18 (18) is separated from the other genotypes. At the same time, the genotypes collected from Gölbaşı district and the control varieties are located close to each other and form a group (Figure 4).

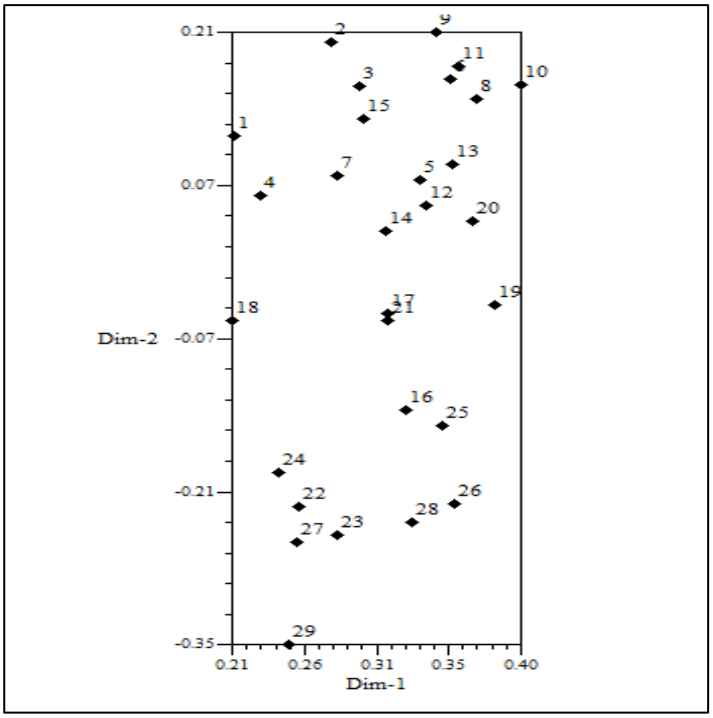

Figure 3. Two-dimensional graph created as a result of Principal Component Analysis with ISSR and SRAP data on 29 pepper genotypes.

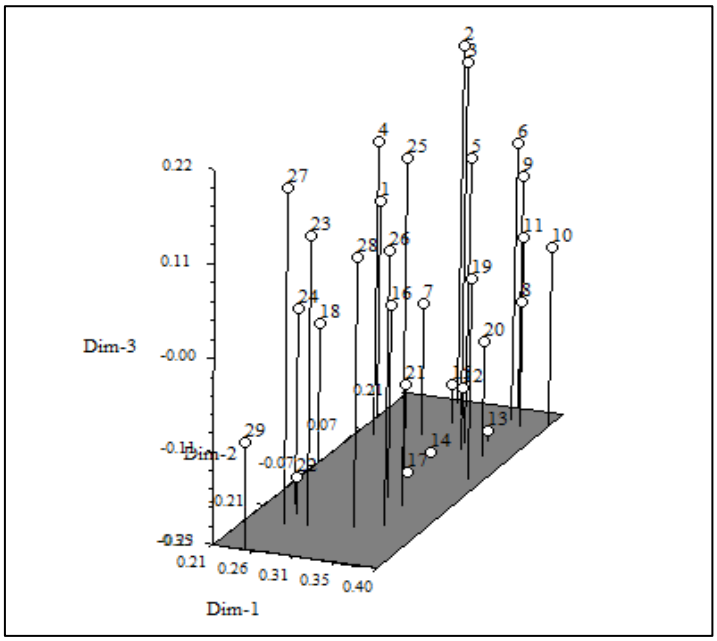

Figure 4. Three-dimensional graph created as a result of Principal Component Analysis with ISSR and SRAP data on 29 pepper genotypes.

DISCUSSION

In this study, the variation rate was determined in a total of 29 pepper genotypes, 26 Besni pepper genotypes, and 3 control varieties, using ISSR and SRAP marker methods. A total of 62 bands were obtained in the study, 15 of which were monomorphic and 47 were polymorphic. A variation between genotypes was found between 0.68-0.95 (32%). The groupings depending on the variation are shown in Figure 1, Figure 2, and Figure 3.

Numerous molecular characterization studies have been reported on peppers, with different results reported depending on the populations and primer systems used. There are several reports on the effectiveness of PCR-based techniques, including ISSR as well as other marker methods such as SRAP, in assessing the relationship or variability among different *Capsicum* genotypes (Ibarra-Torres et al., 2014; Ekbiç and Okay, 2024). In general, all ISSR markers used in the study produced clear and reproducible amplification profiles. When the marker efficiency was further quantified by heterozygosity values, it was shown that ISSR primers were effective in assessing genetic relatedness in cultivated *Capsicum* species cultivars. Oblatunji and Afloyan (2019) compared pepper genotypes and found higher He values (0.32 to 0.88) than our study. This is because they used genotypes from different *Capsicum* species while we used only *C. annuum* genotypes. Similarly, Cesur et al. (2020) and Ekbiç and Okay (2024) reported similar heterozygosity rates to our study using only *C. annum* genotypes. Yang et al. (2005) found a lower polymorphism rate than our study in their study in which 11 pepper genotypes were screened with 12 ISSR primers. In a study conducted using 18 SRAP primer pairs in 26 pepper genotypes, it was recorded that 90 polymorphic bands were obtained. The genetic similarity rate between all genotypes varied between 72% and 24%. In the study, it was reported that a high genetic diversity was observed in *C. annuum* genotypes (Adalı, 2017). In our study, lower rates of variation and polymorphic were detected. The difference in the number of polymorphic bands between two studies using approximately the same size population can be attributed to the number of SRAP combinations and genotypic differences (genotypes collected from a wider area and selected from different backcross populations). Similarly, in the study conducted by Okay (2019) with more genotype and primer combinations, the number of groups (5) and variation rates (35%-97%) were found to be higher than in our study. We thought that this difference was because the genotypes in our study were collected from a narrower area and the number of genotypes used was low. On the other hand, in a study conducted by Rana et al. (2014), 24 pepper genotypes were screened with 12 ISSR primers. A total of 59 bands were obtained, 29 of which were reported as polymorphic. While this study is parallel to our study in terms of the total number of bands obtained, the number of polymorphic bands was found to be higher in the Besni pepper population. This difference may be due to the higher number of genotypes used in our study. Bozokalfa et al. (2017) reported similar results to our study (0.62-0.94) in their study where 94 pepper genotypes were analyzed with 33 SRAP combinations. Although they used more genotypes and SRAP combinations in their studies, the fact that lower variation was reported compared to the studies given above can be explained by the low genetic diversity.

Similar to Besni pepper, Cırgalan local pepper variety, grown in limited areas in Kayseri province and some districts was also characterized with 16 ISSR primers, and it was found that 23% of the bands were polymorphic and the genetic similarity ratio was between 0.95-0.99. The reason for the low genetic diversity (5%) among Cırgalan pepper genotypes may be that it was grown in a very narrow area, pepper cultivation is low in the region, and the flower structure of Cırgalan pepper (Pınar et al., 2017). Ahmed (2013) reported that six hybrid pepper lines were characterized with 10 ISSR primers, and 60% (57) of the 87 bands produced were found to be polymorphic. In a study conducted in India with ISSR primers on 30 Bhut jolokia peppers, it was reported that pepper genotypes formed 3 groups and genetic differences were between 57% and 92% (Hazarika and Neog, 2014). López-Espinosa et al. (2018) reported that a total of 32 bands were obtained as a result of screening 60 Habareno peppers native to the Yucatan Peninsula of Mexico with 3 ISSR primers. The polymorphism rate was 98%.

As seen in the studies given above, ISSR and SRAP marker systems can be used to detect variations in pepper populations. They were also used successfully in our study and produced descriptive results. As a result of the analysis, although there were some minor exceptions, pepper genotypes were grouped according to where they were taken and fruit shape (Shain et al., 2022). As seen in the literature given, different variation rates were detected in different populations. These differences can be attributed to genotypic differences, the number of primers used, and the flower structure of the genotypes. Depending on the heterostyly observed in pepper species, cross-pollination or self-pollination can be encouraged. In populations where self-pollination is encouraged by flower structure, pollinating agents, and climatic conditions, diversity decreases, while in opposite conditions, cross-pollination is encouraged, and diversity increases (Zhang et al., 2023).

CONCLUSION

In this study, 26 Besni pepper genotypes and three cultivars that were morphologically characterized in our previous study were molecularly characterized. As a result of the analysis, a total of 62 bands were produced, 52 of which were polymorphic. In the dendrogram created, it was seen that the genotypes were divided into two main groups consisting of two subgroups. While the genotypes G2 and G3 were the most similar to each other, the two most dissimilar genotypes were B1 and C3. It was noted that the genotypes were grouped according to where they were collected from and shape of the fruit. Genotypes obtained from the same village but from different farmers

were mostly in the same group. This may be because farmers multiplied the seeds of genotypes whose characteristics they preferred and exchanged them among themselves. Molecular studies on local genotypes are an effective method to strengthen morphological studies. At the same time, the information produced about the genetic relationships (similarities and differences) of the studied material will provide important opportunities for plant breeders in breeding studies. Identifying genotypes with unique characteristics among local pepper genotypes, adding them to the base population, and protecting them are of vital importance for the sustainability of genetic diversity and plant production. The current population has not been evaluated in terms of yield, quality, and tolerance to stress conditions (biotic and abiotic). For these purposes, the Besni pepper population should be evaluated and prominent genotypes should be included in appropriate breeding programs.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Competing Interest

All authors have no conflicts of interest to disclose.

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