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Genetic diversity analysis in the Turkish pepper germplasm using iPBS retrotransposon-based markers

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Abstract: *Capsicum* is one of the most important and diverse plant taxa, widely used as a spice and vegetable worldwide, including Turkey. Germplasm characterization is an essential step for crop breeding. In the present study, we characterized the genetic diversity and population structure of a collection of 94 pepper accessions using inter-primer binding site (iPBS) retrotransposon-based markers. A total of 20 iPBS primers were used that generated 172 bands (mean = 8.6 bands/primer), of which ~92% were polymorphic in the entire germplasm collection, whereas 83%, 69%, and 80% of the bands were polymorphic within the *C. annuum*, *C. chinense*, and *C. frutescens* subsets, respectively. All of the taxa analyzed were clearly differentiated by the iPBS markers. The polymorphism information content of the markers ranged between 0.15 and 0.99, with an average of 0.66. Cluster analyses by different methods (UPGMA, STRUCTURE, and principal coordinate analysis) revealed a clear separation of all of the *C. annuum* accessions from the other pepper species, with a few subclusters observed among the latter, including groups with accessions of both *C. frutescens* and *C. chinense*. At the interspecies level, the 3 clustering methods clearly discriminated *C. annuum* from *C. frutescens* and *C. chinense*. No clear association was found between the iPBS-based clustering and geographical origin or fruit characteristics of the accessions. This is the first report characterizing the genetic diversity and population structure in the Turkish pepper germplasm using iPBS markers. It is expected that these data will serve as a foundation for the development of new and improved pepper varieties.

Key words: *Capsicum*, genetic diversity, interspecies variation, iPBS, population structure, retrotransposon-based markers

1. Introduction

Peppers (*Capsicum* spp.) are grown worldwide for various purposes, including fresh and cooked vegetable consumption, spices, ornaments, and medicine (e.g., the compound capsaicin, present at high concentrations in hot peppers, has analgesic, antidiabetic, and anticancer effects), and because of its high content of phytonutrients (e.g., provitamin A, carotenoids, and vitamin C) (Bosland et al., 2012). The genus *Capsicum* belongs to the family Solanaceae, which originated in the tropical and subtropical regions of America, with Bolivia as its proposed center of origin (Eshbaugh, 1993; Olmstead et al., 2008). Currently, there are 27 recognized *Capsicum* species, 5 of which were domesticated through distinct events at different primary diversification centers of America (Pickersgill, 2007; Olmstead et al., 2008; Nicolai et al., 2013). These 5 species are *C. annuum*, *C. frutescens*, *C. chinense*, *C. pubescens*, and *C. baccatum*, and they include the world's most economically important *Capsicum* vegetables (Kumar et al., 2006). At least 2 regions are considered the centers of domestication for these 5 species: the tropical northwestern part of South America for *C. baccatum*, *C. pubescens*, and *C. chinense* and southern Mexico and Central America for *C. annuum* and *C. frutescens* (Kumar et al., 2006; Nicolai et al., 2013). On the basis of cytogenetic and cross fertility analyses, wild and cultivated *Capsicum* species have been grouped into 3 genetic pools: a first pool including *C. annuum*, *C. frutescens*, and *C. chinense*; a second pool with *C. baccatum* and its wild relative, *C. baccatum var. baccatum*; and a third pool that includes *C. pubescens* and the wild species *C. eximium* and *C. cardenasi* (Nicolai et al., 2013).

Among the 5 domesticated *Capsicum* species, *C. annuum* is the most widely distributed and economically

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important. It is a diploid and self-pollinating species with a chromosome number of 2n = 24 (Gyulai et al., 2000). *C. annuum* was domesticated from wild bird pepper or chiltepin (*C. annuum* var. *glabriusculum*) in Mexico (Perry et al., 2007; Kraft et al., 2014).

Peppers were introduced into Europe from America in 1493, following the first trip by Christopher Columbus, and they were rapidly distributed to Asia and Africa. *C. annuum* was the most widely adopted species in both Africa and Asia, although *C. chinense* and *C. frutescens* also became quite popular in these regions (Nicolai et al., 2013). Turkey, due to its geographical location, has historically played a vital role in the distribution of different crop species (Yildiz et al., 2015a; Baloch et al., 2017; Arystanbekkyzy et al., 2018; Nadeem et al., 2018a), including pepper (Yıldız, personal communication). Thus, to date, Turkey has maintained a huge and diverse collection of pepper genetic resources, including a large number of landraces, which represent a valuable source of locally adapted materials for breeding pepper (Bozokalfa et al., 2009).

In pepper germplasm collections, assessment of the level of genetic diversity and taxa relatedness, both within and between *Capsicum* species (especially among those belonging to the same gene pool), is essential for applying efficient germplasm conservation and management strategies, as well as for utilizing these resources in breeding programs (González-Pérez et al., 2014). Among the different techniques available for the assessment of diversity and population structures in crops, DNA molecular markers provide plenty of unbiased environmentally neutral genetic data (Nadeem et al., 2018b). Among them, several retrotransposon-based markers have been developed and successfully used for diversity analysis (reviewed by Kalendar et al., 2011). The inter-primer binding site (iPBS) is a retrotransposon-based molecular marker system based on the amplification of the region encompassed by the reverse transcriptase primer binding sites of 2 contiguous retrotransposons that are in opposite orientations (Kalendar et al., 2010). The iPBS method is applicable to all plant species due to the universal presence of a tRNA complement as the primer binding site of the reverse transcriptase in long terminal repeat (LTR) retrotransposons, and it requires no prior sequence information. Furthermore, the method has been successfully applied for assessing genetic diversity in different crop species, including pea (Baloch et al., 2015a), *Lens* (Baloch et al., 2015b), okra (Yildiz et al., 2015b), tobacco (Yaldız et al., 2018), and common bean (Nemli et al., 2015).

Molecular markers have been used previously for assessing genetic diversity, relatedness, and population structure in wild and domesticated *Capsicum* (Hill et al., 2013; González-Pérez et al., 2014; Hulse-Kemp et al., 2016). To date, the Turkish pepper germplasm has mainly been characterized based on morphological traits (Bozokalfa et al., 2009; Bozokalfa and Eşiyok, 2011), and only 2 studies evaluated these germplasms at the molecular level (Aktas et al., 2009; Akyavuz et al., 2018). Thus, the level of genetic diversity and the population structure in Turkish pepper germplasm remain largely uncharacterized. The goal of this study was to investigate the level of genetic diversity, phylogenetic relationship, and population structure of a germplasm collection of 94 Turkish pepper accessions (*Capsicum* spp.) using iPBS retrotransposon-based markers.

2. Materials and methods

2.1. Plant materials and DNA extraction

A total of 94 pepper accessions belonging to 3 species (85 *C. annuum*, 7 *C. frutescens*, and 2 *C. chinense*) from the Alata Horticulture Research Department were analyzed in this study (Table 1). Plants of these accessions were grown in pots under greenhouse conditions and fresh young leaves were collected and frozen at –80 °C until lyophilized and used for DNA isolation. The total genomic DNA was isolated from the freeze-dried leaves of individual plants following the CTAB protocol of Doyle and Doyle (1990), with minor modifications as incorporated by Boiteux et al. (1999). Concentration of the isolated DNA was measured with a NanoDrop spectrophotometer (DeNovix DS-11 FX, USA), and aliquots of the DNA samples were diluted to a final concentration of 5 ng/µL for further use in polymerase chain reaction (PCR) assays and were stored at –20 °C. All of the chemicals and reagents used in this study were purchased from Thermo Fisher Scientific (USA).

2.2. iPBS marker analysis

A total of 83 iPBS primers were initially screened on 8 randomly selected *C. annuum* genotypes using PCR amplification conditions, according to the method of Kalender et al. (2010). Based on the results of this first screening, 20 iPBS primers that yielded intense and polymorphic bands were selected for further analysis of the entire Turkish pepper germplasm collection. Information about the primer sequences and annealing temperatures is presented in Table 2. PCR reactions were performed in a final volume of 20 µL containing 3 ng/µL template DNA, 2 mM dNTPs, 0.2 U Taq DNA polymerase, 4 µM primer, 1X PCR buffer, and 7 µL distilled water. The thermocycler was programmed according to the method of Kalendar et al. (2010) as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 15 s, annealing temperature of 50–65 °C (depending on the primer) for 1 min, and a final extension step at 72 °C for 5 min. PCR products were detected by 2% (w/v) agarose gel electrophoresis using 0.5X TBE buffer for 3

Table 1. Characteristics of the 94 *Capsicum* genotypes.

Accession name	Accession ID	Species	Geographical origin (collection site)	Comments/remarks
Hybrid1*	36x32C	*C. annuum*	Turkey	Hybrid
Hybrid2*	N269x32D	*C. annuum*	Turkey	Hybrid
283A	283A	*C. annuum*	Turkey	Inbred line
KmarasPepper1	71	*C. annuum*	Turkey	Pepper type
ChiliPepper1	221	*C. annuum*	Turkey	Chili pepper
FloridaVR2	56	*C. annuum*	Florida, USA	Sivri pepper, VR2 X, resistance to potato virus Y (PVY)
SivriPepper1	341	*C. annuum*	Turkey	Sivri pepper
LamiaType	1608	*C. annuum*	Russia	Lamia type
Charleston1	74	*C. annuum*	Turkey	Charleston
Kapia1	1570	*C. annuum*	Turkey	Kapia
ChiliPepper2	292	*C. annuum*	Turkey	Chili pepper
Chinense1	(PI 159241)	*C. chinense*	University of Georgia	Tolerance to tobacco etch virus
Perennial	67	*C. annuum*	-	Perennial, tolerance to CMV and ToMV
KmarasPepper2	1779	*C. annuum*	Turkey	Kahramanmaraş pepper type
BellPepper1	467	*C. annuum*	Turkey	Bell pepper
ChiliPepper3	320	*C. annuum*	Turkey	Chili pepper
SivriPepper2	409	*C. annuum*	Turkey	Sivri pepper
KmarasPepper3	16-1	*C. annuum*	Turkey	Kahramanmaraş pepper type
x7*	x-7	*C. annuum*	Turkey	Inbred line
x25*	x-25	*C. annuum*	Turkey	Inbred line
Kapia2	1121A	*C. annuum*	Turkey	Kapia
Charleston2	441	*C. annuum*	Turkey	Charleston
BellPepper2	405	*C. annuum*	Turkey	Bell pepper
Frutescens1	(PI 281418)	*C. frutescens*	University of California, USA	
Chinense2	(PI 159264)	*C. chinense*	University of Georgia	
SivriPepper3	215	*C. annuum*	Turkey	Short fruit
KmarasPepper4	111	*C. annuum*	Turkey	Kahramanmaraş pepper type
ChiliPepper4	47	*C. annuum*	India	Chili pepper, tolerance to high temperature and sensitive to low temperature
Frutescens2	(PI 281421)	*C. frutescens*	University of California, USA	
Frutescens3	(PI 281420)	*C. frutescens*	University of California, USA	
HatayPepper	1676	*C. annuum*	Turkey	Hatay pepper
YoloWonder	66	*C. annuum*	USA	Yolo Wonder, tolerance to PVY and TMV
SivriPepper4	343	*C. annuum*	Turkey	Sivri pepper
Frutescens4	(PI 281419)	*C. frutescens*	University of California, USA	
SanliurfaPepper1	302	*C. annuum*	Turkey	Şanlıurfa pepper
Frutescens5	(PI 281422)	*C. frutescens*	University of California, USA	
ChiliPepper5	390	*C. annuum*	Turkey	Chili pepper
ChiliPepper6	293A	*C. annuum*	Turkey	Chili pepper

Table 1. (Continued).

SivriPepper5	475A	*C. annuum*	Turkey	Sivri pepper
BellPepper3	468	*C. annuum*	Turkey	Bell pepper
SivriPepper6	164	*C. annuum*	Turkey	Sivri pepper
BellPepper4	458	*C. annuum*	Turkey	Bell pepper
HungarianPepper1	776-7	*C. annuum*	Hungary	Hungarian pepper
BellPepper5	244	*C. annuum*	Turkey	Bell pepper
YoloY	63	*C. annuum*	USA	Yolo Y, tolerance to PVY and TMV
ChiliPepper7	N50	*C. annuum*	Turkey	Chili pepper, tolerance to nematode
SivriPepper7	425	*C. annuum*	Turkey	Sivri pepper, high heterosis ability
SivriPepper8	24-A	*C. annuum*	Turkey	Sivri pepper
HungarianPepper2	765-4-1B	*C. annuum*	Hungary	Hungarian pepper
HungarianPepper3	765-4-2B	*C. annuum*	Hungary	Hungarian pepper
BellPepper6	15-A	*C. annuum*	Turkey	Bell pepper
SivriPepper9	342	*C. annuum*	Turkey	Sivri pepper
SivriPepper10	409	*C. annuum*	Turkey	Sivri pepper, high heterosis ability
ChiliPepper8	398-A	*C. annuum*	Turkey	Chili pepper
Charleston3	283	*C. annuum*	Turkey	Charleston
B7*	B-7	*C. annuum*	Turkey	Inbred line
SivriPepper11	N164	*C. annuum*	Turkey	Sivri pepper, tolerance to nematode
KmarasPepper5	KMH-2	*C. annuum*	Turkey	Kahramanmaraş pepper type
KmarasPepper6	107	*C. annuum*	Turkey	Kahramanmaraş pepper type
SivriPepper12	32	*C. annuum*	Turkey	Sivri pepper, high heterosis ability
KmarasPepper7	1452	*C. annuum*	Turkey	Kahramanmaraş pepper type
Charleston4	333	*C. annuum*	Turkey	Charleston, high heterosis ability
SanliurfaPepper2	İNAN	*C. annuum*	Turkey	Şanlıurfa pepper
SivriPepper13	336	*C. annuum*	Turkey	Sivri pepper, high heterosis ability
SivriPepper14	331	*C. annuum*	Turkey	Sivri pepper, high heterosis ability
SivriPepper15	414	*C. annuum*	Turkey	Sivri pepper
Kapia3	K34	*C. annuum*	Turkey	Kapia
Kapia4	K7	*C. annuum*	Turkey	Kapia
BellPepper7	244	*C. annuum*	Turkey	Bell pepper
ChiliPepper9	317	*C. annuum*	Turkey	Chili pepper
ChiliPepper10	35	*C. annuum*	Turkey	Chili pepper
ChiliPepper11	261	*C. annuum*	Turkey	Chili pepper
KmarasPepper8	KMH-1	*C. annuum*	Turkey	Kahramanmaraş pepper type
Frutescens7	287-A	*C. frutescens*	-	Rosy fruit shape
Kapia5	K25	*C. annuum*	Turkey	Kapia
ChiliPepper12	293	*C. annuum*	Turkey	Chili pepper
SM53	SM-5-3	*C. annuum*	Turkey	Inbred line
HungarianPepper4	774-4-2B	*C. annuum*	Hungary	High heterosis ability
Charleston5	363	*C. annuum*	Turkey	Charleston type, early blossoming, moderately tolerant to low temperature
Frutescens8	287	*C. frutescens*	-	Rosy fruit shape
SweetPickle	1895	*C. annuum*	Turkey	Sweet pickle

Table 1. (Continued).

KmarasPepper9	250	*C. annuum*	Turkey	Kahramanmaraş pepper type
BellPepper8	1530	*C. annuum*	Turkey	Bell pepper
SivriPepper16	195	*C. annuum*	Turkey	Sivri pepper
SivriPepper17	438	*C. annuum*	Turkey	Sivri pepper, high yield
SivriPepper18	407	*C. annuum*	Turkey	Sivri pepper, very high yield
Aricne	1882	*C. annuum*	USA	
Kapia6	953W	*C. annuum*	Turkey	Kapia, high yield
ChiliPepper13	202	*C. annuum*	Turkey	Chili pepper
SivriPepper19	32-D	*C. annuum*	Turkey	Sivri pepper
KmarasPepper10	1787	*C. annuum*	Turkey	Kahramanmaraş pepper type
KmarasPepper11	1780	*C. annuum*	Turkey	Kahramanmaraş pepper type
ChiliPepper14	47	*C. annuum*	India	Chili pepper
SivriPepper20	32-B	*C. annuum*	Turkey	Sivri pepper, high heterosis ability

*Unknown location and other characteristics.

Table 2. Name, sequence, and annealing temperature of the 20 selected iPBS primers used in this study.

iPBS primer	Sequence (5'–3')	Annealing temperature (°C)
2256	GACCTAGCTCTAATACCA	51
2272	GGCTCAGATGCCA	55
2274	ATGGTGGGCGCCA	63
2277	GGCGATGATACCA	52
2279	AATGAAAGCACCA	52
2240	AACCTGGCTCAGATGCCA	55
2251	GAACAGGCGATGATACCA	53
2253	TCGAGGCTCTAGATACCA	51
2077	CTCACGATGCCA	55
2400	CCCCTCCTTCTAGCGCCA	51
2241	ACCTAGCTCATCATGCCA	55
2249	AACCGACCTCTGATACCA	51
2228	CATTGGCTCTTGATACCA	53
2373	GAACTTGCTCCGATGCCA	55
2232	AGAGAGGCTCGGATACCA	55
2278	GCTCATGATACCA	50
2383	GCATGGCCTCCA	53
2229	CGACCTGTTCTGATACCA	52
2390	GCAACAACCCCA	55
2394	GAGCCTAGGCCA	55

h. After electrophoresis, ethidium bromide was used for gel staining and visualization under UV light, followed by photo documentation with the Imager Gel Doc XR+ system (Bio-Rad, USA). A 100-bp ladder (Thermo Fisher Scientific) was used as a molecular weight marker.

2.3. Data analysis

Only clear, intense, and reproducible polymorphic bands were scored as present (1) or absent (0) in all of the pepper accessions. Pop Gene v.1.32 software (Yeh et al., 2000) was used to estimate genetic diversity parameters of the Turkish pepper collection, including the effective number of alleles (n_e), gene diversity (h), Shannon information index (I), and polymorphic information content (PIC). To explore the genetic diversity at the intraspecific level, various diversity parameters like the effective n_e, h, I, and PIC were also calculated among the individuals of *C. annuum* using Pop Gene v.1.32. For calculation of the PIC values, the criterion of Baloch et al. (2015a) was followed. Pairwise genetic similarity (GS) values among the accessions were calculated using Jaccard's similarity coefficient (Jaccard, 1908), with the statistical software XLSTAT v.19.7.49007. Pairwise Nei's genetic distance (GD) (1972) values among the 3 pepper species were calculated using Pop Gene v.1.32. In addition, GD values were calculated among the accessions of *C. annuum* to gain insight into the intraspecific variation of this species using the same software. For visualization of the level of genetic diversity and relatedness among the 94 pepper accessions, clustering analyses were performed using principal coordinate analysis (PCoA), the unweighted pair-group method with arithmetic means (UPGMA), and model-based Bayesian algorithms. PCoA (multidimensional scaling) is

an eigenanalysis of a distance or dissimilarity matrix. The UPGMA dendrogram was constructed using pairwise genetic similarities (Jaccard coefficient) among the pepper accessions with XLSTAT software. Pop Gene v.1.32 was also used to construct the UPGMA-based dendrogram of the 3 pepper species (reflecting interspecies variation), as well as among the accessions of *C. annuum* (intraspecific variation). The population substructure in the Turkish pepper germplasm was examined using a Bayesian clustering model executed with STRUCTURE software (Evanno et al., 2005) and plotted with STRUCTURE PLOT (Ramasamy et al., 2014). The most likely number of clusters (K = number of subpopulations) in the STRUCTURE analysis was determined following the criteria of Evanno et al. (2005) and plotting the number of clusters (K) against the logarithm probability relative to the standard deviation (ΔK). For each K (from 1 to 7), ten independent runs were performed by applying the admixture model with the allele frequencies correlated, with a burn-in period of 10,000 and 100,000 Markov chain Monte Carlo repetitions.

3. Results

Of the 83 iPBS primers initially screened on the 8 pepper accessions, 13 primers (15.6%) failed to produce amplicons and were therefore discarded. Among the remaining 70 iPBS primers, which yielded PCR products of the expected size (in a range of 250–3750 bp), 52 primers (~63%) were polymorphic and the other 18 (~22%) produced only monomorphic bands. Based on these results, 20 iPBS primers yielding intense and polymorphic bands were selected for further analysis of the Turkish pepper germplasm collection.

The selected iPBS primers resulted in a total of 172 scorable bands, with a range of 3–15 and a mean of 8.6 fragments per primer (Table 3). Of the 172 scorable bands, 158 (~92%) were polymorphic in the germplasm collection, whereas 83%, 69%, and 80% of the bands were polymorphic within the *C. annuum*, *C. chinense*, and *C. frutescens* subsets, respectively. The average number of polymorphic fragments per primer was 7.9 and it ranged from 2 to 14. The PIC values of the markers varied broadly, from 0.15 (for primer iPBS2274) to 0.99 (iPBS2272), with a mean of 0.66. The n_e per marker ranged from 1.04 to 1.54, with a mean of 1.21, whereas the range and mean values for *h* were 0.04–0.33 and 0.15, respectively. The *I* per iPBS marker ranged from 0.10 to 0.49, with a mean of 0.25 (Table 3).

To investigate the genetic variations at an intraspecific level, various diversity indices were calculated for *C. annuum*, resulting in an average PIC value of 0.657. iPBS2400 was the primer with the highest PIC (0.781), whereas iPBS2274 had the lowest PIC (0.242) (Table 4). The average n_e was 1.153, with a range of 1.0 (for primer iPBS2272) to 1.510 (for primer iPBS2240). The mean *h* was 0.096, with iPBS2240 being the most polymorphic primer (*h* = 0.295) in the *C. annuum* germplasm. The average *I* value was 0.164, with a maximum of 0.445, observed for primer iPBS2240.

Pairwise GS values (Jaccard) among all of the Turkish pepper accessions revealed that Sivripepper2 and Chilipepper4 were the most similar accessions (GS = 0.97), followed by Kmaraspepper10 and Sanliurfapepper2 (GS = 096), and Kmaraspepper11 and Chilipepper13 (GS = 0.96), whereas the most genetically distant accessions were Frutescence7 and Chilipepper2 (GS = 0.21). Overall, the average GS value of the entire pepper collection was 0.73.

We further explored genetic variations within the species *C. annuum*, revealing an average GD among the *C. annuum* accessions of 0.0948. The 2 most unrelated accessions were Charleston5 and Chillipepper2 (GD = 0.303), followed by Hataypepper and Chillipepper2 (GD = 0.295), whereas the closest genetic accessions were Sivripepper20 and Chillipepper14 (GD = 0.117), followed by Kmaraspepper10 and Sanliurfapepper2 (GD = 0.017), and Kmaraspepper11 and Chillipepper13 (GD = 0.017).

Pairwise GS values were used to construct a dendrogram depicting genetic relatedness among the 94 Turkish pepper accessions. The UPGMA-based analysis clustered the pepper germplasm into 6 groups of accessions, with GS of >0.65, as defined by branches I to VI in Figure 1. The first branch (I) clustered the largest number of the accessions (81 accessions, representing 86% of the pepper collection), all belonging to *C. annuum*. The second (II) and third (III) branches contained 1 and 3 *C. annuum* accessions, respectively. Branch IV clustered 4 *C. frutescens* accessions, whereas branch V included accessions of both *C. chinense* and *C. frutescens*. Branch VI included 2 *C. frutescens* accessions. To visualize the variations in *C. annuum*, UPGMA-based clustering was performed, which divided the 85 *C. annuum* genotypes into 3 main groups: A, B, and C. Group A was composed of a single and very unique genotype, Charleston5 (Figure 2). Group B contained 3 genotypes (Chillipepper2, Sivripepper1, and Kmaraspepper1), and Group C was the largest main group, clustering 84 genotypes.

An analysis of genetic relatedness among the 3 pepper species revealed that *C. annuum* and *C. chinense* were the most genetically distant (GD = 0.47), whereas *C. annuum* and *C. frutescens* were the most closely related (GD = 0.247) (Table 5). The UPGMA analyses among the 3 pepper species confirmed these results, revealing a closer relation between *C. annuum* and *C. frutescens* than between any of the latter and *C. chinense* (Figure 3).

Comparable clustering results were obtained using PCoA, which revealed 4 major groups, clearly separating the *C. annuum* accessions (all included in cluster I) from

Table 3. Performance of the 20 iPBS markers and estimates for the genetic diversity parameters of the Turkish pepper germplasm.

Primer	Amplified bands						
	Total	Polymorphic	% Polymorphism	PIC	n_e	h	I
2256	6	6	100	0.72	1.14	0.12	0.23
2272	3	2	66.7	0.99	1.04	0.04	0.10
2274	3	2	66.7	0.15	1.17	0.14	0.27
2277	10	9	90	0.72	1.26	0.17	0.29
2279	7	6	85.7	0.86	1.09	0.08	0.17
2240	8	8	100	0.66	1.54	0.33	0.49
2251	10	10	100	0.69	1.24	0.16	0.28
2253	14	14	100	0.57	1.35	0.22	0.37
2077	7	6	85.7	0.58	1.43	0.28	0.44
2400	9	9	100	0.75	1.22	0.16	0.28
2241	6	6	100	0.55	1.35	0.22	0.35
2249	12	12	100	0.58	1.14	0.11	0.21
2228	11	11	100	0.69	1.10	0.09	0.18
2373	9	9	100	0.62	1.19	0.15	0.27
2232	5	4	80	0.55	1.09	0.08	0.17
2278	3	2	66.7	0.99	1.14	0.11	0.23
2383	10	8	80	0.51	1.19	0.13	0.24
2229	15	14	93.3	0.59	1.17	0.12	0.23
2390	12	10	83.3	0.79	1.24	0.16	0.29
2394	12	10	83.3	0.58	1.26	0.17	0.28
Total	172	158					
Average	8.6	7.9	89.1	0.66	1.21	0.15	0.25

Effective number of alleles (n_e), gene diversity (h), Shannon information index (I), and polymorphic information content (PIC).

the accessions of *C. frutescens* and *C. chinense* (clusters II, III, and IV) (Figure 4).

A population substructure was found in the Turkish pepper germplasm, with 2 major genetic pools identified (K = 2): a *C. annuum* group (cluster I) and a *C. frutescens* + *C. chinense* group (cluster III) (Figure 1A). Additionally, a group of accessions presenting admixture from these 2 genetic pools was identified (cluster II). In general, the clustering results by UPGMA, STRUCTURE, and PCoA, at both the inter- and intraspecific levels, were highly concordant (Figures 1–4).

4. Discussion

Advancement in molecular marker technologies has boosted the success level of breeding activities for various crops worldwide (Nadeem et al., 2018b). Retrotransposons comprise a large fraction of most plant genomes, and their replication generates genomic diversity, which can be exploited as an excellent source of molecular markers for genetic diversity assessment in various crops (Andeden et al., 2013; Ali et al., 2019). Retrotransposon-based markers can be effectively used to address microevolutionary questions, at the intragenus or intraspecific level, as their insertion into the genome generates frequent and polymorphic DNA sites (Kalendar et al., 2011). Among the different retrotransposon-based marker systems available, iPBS systems have the advantage of being virtually universal in applicability (i.e. they may be used in any plant species), do not require prior sequence information, produce multiple polymorphic bands per reaction, are highly reproducible, and, compared to other markers, are inexpensive (Ali et al., 2019). Compared to other retrotransposon-based markers, iPBS-retrotransposons have higher reproducibility and are useful for a broader spectrum of organisms, since

Table 4. Various diversity parameters investigated in *C. annuum*.

iPBS primers	PIC	ne*	h*	I*
2256	0.774	1.060	0.050	0.100
2272	0.733	1.000	0.000	0.000
2274	0.242	1.008	0.008	0.021
2277	0.715	1.181	0.107	0.166
2279	0.776	1.007	0.007	0.016
2240	0.724	1.510	0.295	0.445
2251	0.751	1.166	0.107	0.180
2253	0.647	1.282	0.178	0.286
2077	0.600	1.356	0.228	0.362
2400	0.781	1.141	0.095	0.153
2241	0.619	1.266	0.166	0.273
2249	0.650	1.077	0.053	0.094
2228	0.745	1.031	0.029	0.066
2373	0.685	1.099	0.079	0.144
2232	0.538	1.010	0.009	0.026
2278	0.731	1.078	0.063	0.113
2383	0.522	1.103	0.074	0.123
2229	0.626	1.093	0.067	0.119
2390	0.720	1.150	0.097	0.157
2394	0.571	1.150	0.083	0.125
Average	0.657	1.153	0.096	0.164

Effective number of alleles (n_e), gene diversity (h), Shannon information index (I), and polymorphic information content (PIC).

they amplify regions not only of endogenous retroviruses but also of both *Gypsy* and *Copia* LTR retrotransposons (Melnikova et al., 2012). Very recently, Ali et al. (2019) investigated the genetic diversity of a world collection of safflower, confirming the universality of iPBS markers for diversity, taxonomic, and evolutionary studies. Demirel et al. (2018) used iPBS markers for the diversity assessment of a collection of 151 potato genotypes, concluding that iPBS markers were powerful and effective DNA markers for fingerprinting large samples. Baloch et al. (2015b) used iPBS and inter-simple sequence repeat (ISSR) markers for addressing the level of genetic variation in wild *Lens* species, reporting that these markers were effective and reproducible for establishing taxonomic and evolutionary relationships among cultivated and wild species. Similarly, Yildiz et al. (2015b) used iPBS markers to investigate genetic bottlenecks in Turkish okra, reporting iPBS markers as more informative and reliable than simple sequence repeats (SSRs). Results from the present study, revealing an average of 8.6 bands per iPBS marker and ~92% of polymorphic bands, coincided with the high level of polymorphism and informativeness reported for these markers in previous studies. It must also be noted that, under our experimental conditions, the iPBS band patterns obtained were highly reproducible, had intense signals, and were therefore easy to score.

4.1. iPBS markers for diversity analysis in pepper

In the present study, 20 iPBS markers generated 172 clear, unambiguous bands, of which 158 (92%) were polymorphic, indicating a high level of polymorphism in the pepper germplasm evaluated. In comparisons with other studies of pepper, the number of polymorphic bands obtained in this study was higher than that found by Ibarra-Torres et al. (2015) using ISSR and SSR markers to address inter- and intraspecific diversity in *C. pubescens* and *C. annuum* (24 and 36 polymorphic SSRs and ISSRs, respectively), by Aktas et al. (2009) using amplified fragment length polymorphisms (AFLPs) to assess the genetic diversity of Turkish *C. annuum* accessions (56 polymorphic AFLPs), and by Akyavuz et al. (2018) using peroxidase gene polymorphism markers for the genetic diversity of Turkish peppers (120 polymorphic bands). Conversely, Krishnamurthy et al. (2015) used the AFLPs of a collection of 59 *C. annuum* and *C. baccatum* accessions, reporting higher numbers of total (414) and polymorphic bands (389) when compared to the present study, although the level of polymorphism in their study (94%) was comparable to ours (92%). Nonetheless, it must be noted that the proportion of polymorphic bands found in both pepper studies using AFLP (Krishnamurthy et al., 2015) and iPBS markers (the present study) was remarkably high when compared to the results typically found using dominant multilocus markers (e.g., AFLP, random amplified polymorphic DNA (RAPD), transposon display). Thus, iPBS markers represent an excellent cost-effective marker option for detecting genetic variations in *Capsicum*.

4.2. Genetic diversity and population structure in the Turkish pepper germplasm

The mean PIC value, an indicator of the level of genetic diversity, of our pepper germplasm collection was 0.66, which was substantially higher than that reported by Naegele et al. (2016) (PIC = 0.40) using the SSR markers of a *C. annuum* collection from 9 countries and comparable to the mean PIC of 0.69 found by Rai et al. (2013) using the SSRs of a *Capsicum* collection from different geographical origins. The intraspecific PIC values of *C. annuum* ranged from 0.242 to 0.724, with an average of 0.657. These PIC values were higher than those obtained by Rana et al. (2014) using the RAPD and ISSR markers of *C. annuum*, and they reflect a good level of genetic variations within *C. annuum* that could be exploited for breeding purposes.

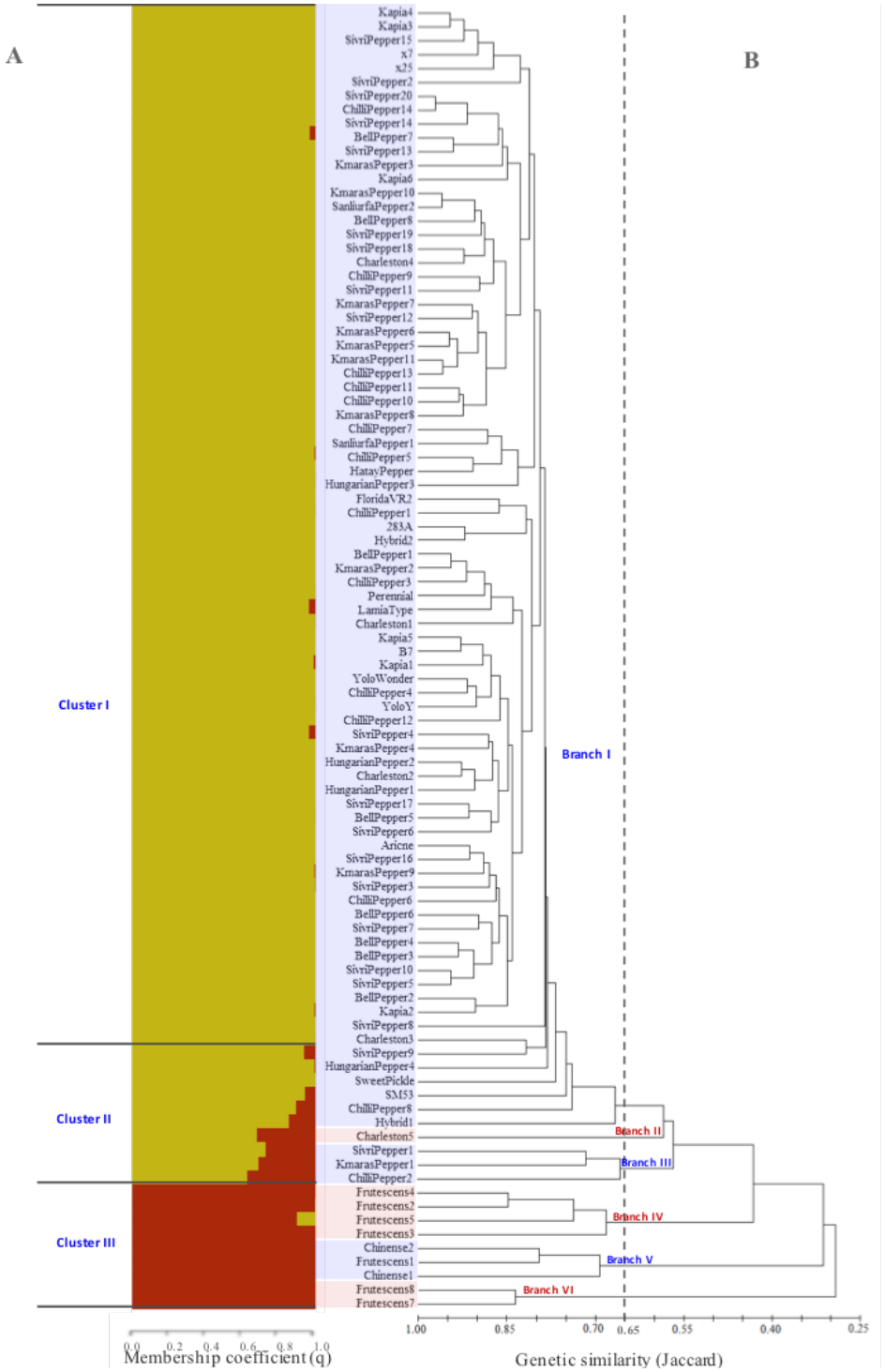

Figure 1. Estimate of genetic diversity in 94 *Capsicum* accessions using 158 iPBS markers. A) Population structure estimated by Bayesian clustering. Each individual is represented by a horizontal line, which is partitioned into K colored segments whose length is proportional to the estimated membership coefficient (q). The population was divided into 3 clusters (separated by black lines) based on the individual's level of admixture from 2 genetic pools (K = 2); clusters I and III include individuals from each of these pools with little or no admixture, whereas individuals in cluster II present a higher level of admixture. B) UPGMA dendrogram depicting genetic relationships among the pepper taxa. Six groups, originating in branches I to VI, were revealed by clustering the accessions at GS of >0.65.

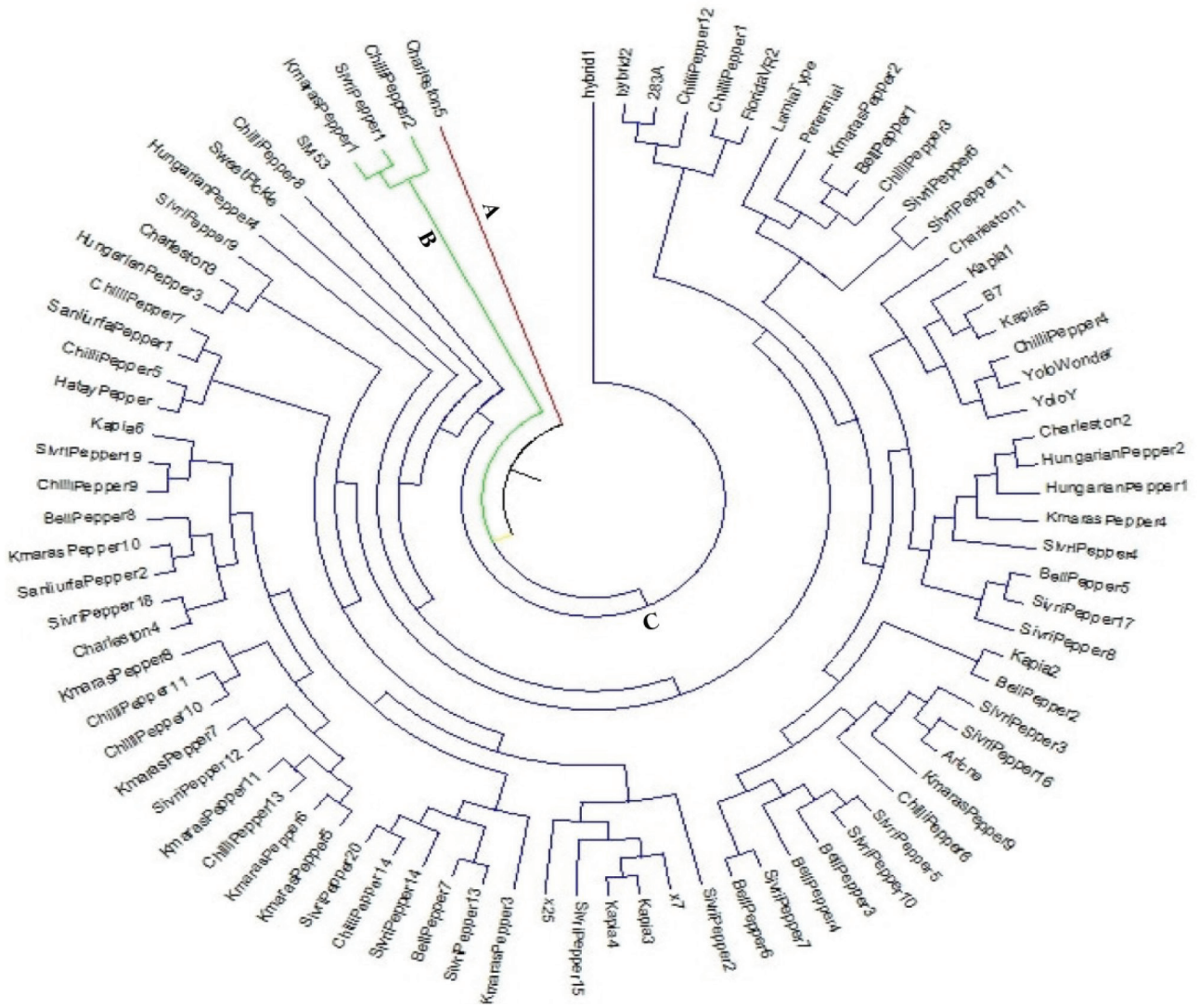

Figure 2. Genetic clustering of 85 *C. annuum* accessions based on UPGMA.

Figure 3. UPGMA-based clustering among the different species of pepper.

In the present study, the average n_e was 1.21. Meng et al. (2017) reported a comparable average n_e (1.28) using the SSR markers of a *Capsicum* collection, despite the fact that their collection included accessions from 12 *Capsicum* species. The mean *h*, a parameter that reflects the level of genetic variation in a population, was 0.15, which was substantially similar to the value obtained (0.16) by Aktas et al. (2009) using AFLP markers in Turkish pepper germplasm. Moreover, we found a higher level of *I* than reported by Aktas et al. (2009), but lower than that reported by Lee et al. (2016) and Jung et al. (2010). The higher values obtained in the latter studies may be due to the fact that they used single nucleotide polymorphisms as a marker system, which are sometimes more informative markers. The level of heterozygosity found in our pepper collection was similar to that reported by Nimmakayala et al. (2014) using SSR markers in *Capsicum*.

Within *C. annuum*, we found lower values for n_e, mean *h*, and *I* (Table 4) when compared to the values obtained for these parameters in earlier studies (Aktas et al., 2009;

Table 5. Genetic distances among the different pepper populations.

Population	*C. annuum*	*C. chinense*	*C. frutescens*
C. annuum	****	0.6249	0.7810
C. chinense	0.4701	****	0.7770
C. frutescens	0.2472	0.2523	****

Nei's genetic identity (1972) (above diagonal) and genetic distance (below diagonal).

Jung et al., 2010; Meng et al., 2017). These differences may be due to the use of different marker systems and a larger number of accessions in their studies.

The average GS of our entire pepper collection was 0.73, ranging from 0.21 to 0.97. Sivripepper2 and Chilipepper4, both of which are *C. annuum*, were the most genetically similar accessions (GS = 0.97) and they were developed from a common progenitor (Keleş, personal communication). Similarly, cultivars Kmaraspepper10 and Sanlıurfapepper2 (GS = 0.96), and Kmaraspepper11 and Chilipepper13 (GS = 0.96), were developed using a common progenitor. Conversely, the most genetically dissimilar accessions were Frutescens7 (*C. frutescens*) and Chilipepper2 (*C. annuum*) (GS = 0.21), followed by Frutescens1 (*C. frutescens*) and Hybrid1 (*C. annuum*) (GS = 0.22).

An analysis of the intraspecific diversity of *C. annuum* revealed a mean GD of 0.0948. Charleston5 and Chillipepper2, and Sivripepper20 and Chillipepper14, were the farthest and closest related pairs of accessions, respectively. From a breeding and research perspective, Charleston5 and Chillipepper2 could be used as parental lines for developing mapping populations and generating new recombinant variants.

STRUCTURE analysis of the Turkish pepper germplasm revealed 3 major clusters. Cluster I was the largest and included only *C. annuum* accessions, all of which had member coefficient values of ≥0.5, as suggested by Habyarimana (2016) and Nadeem et al. (2018a). Cluster II also contained genotypes from *C. annuum*. However, cluster III was the most diverse cluster, with accessions from *C. chinense* and *C. frutescens*. UPGMA-based clustering grouped the *Capsicum* accessions into 6 branches (Figure 1). Branch I clustered the maximum number of accessions (81 accessions), all of which belonged to *C. annuum*. Within this group, no clear association was found between clustering and the geographical origin or fruit characteristics of the accessions. Very recently, Akyavuz et al. (2018) also found no association between the accessions and their geographical origin in Turkish pepper germplasm using peroxidase gene markers. Branches II and III included *C. annuum* accessions with some level of admixture with the *C. frutescens* + *C. chinense* gene pool (Figure 1). Branches IV, V, and VI were the outermost branches of the dendrogram and contained only accessions from *C. frutescens* and *C. chinense*. Branch IV contained 4 accessions of *C. frutescens*, while branch V harbored 2 accessions of *C. frutescens* and 1 accession of *C. chinense*. The latter branches included accessions of both species without a clear separation among them. According to Djian-Caporilano et al. (2007), *C. chinense* originated from *C. frutescens*. Thus, the close genetic relationship between the 2 species may explain why *C. frutescens* and *C. chinense* accessions clustered together in our STRUCTURE and UPGMA analyses. To broaden the picture, intraspecies variations were evaluated by constructing UPGMA-based clustering for the individuals of *C. annuum*, which divided the 85 accessions into 3 main groups, A, B, and C. Group A was a smaller group, clustering the single and very unique genotype named Charleston5. Group C was a bigger group, clustering 84 genotypes. The geographical provinces and fruit sizes and shapes explained the clustering results.

The interspecies UPGMA analysis separated *C. annuum* from the other 2 *Capsicum* species. The evolutionary history of the *Capsicum* genus proposes its origin in South American regions, and independent domestication events occurred in Mesoamerica and South America (Gonzalez-Perez et al., 2014). Based on cross fertility and cytogenetic studies, *Capsicum* species have been divided into 3 complexes. The *C. annuum* complex is the most common and cultivated complex, and includes *C. annuum*, *C. chinense*, and *C. frutescens*. However, many scientists have proposed combining *C. frutescens* and *C. chinense* into one species (Walsh and Hoot, 2001; Pickersgill, 2007). Morphological traits have commonly been used to discriminate among the 3 species of pepper; however, this method can be ambiguous. *C. frutescens* and *C. chinense* present similar morphological characteristics, and Djian-Caporilano et al. (2007) considered that *C. chinense* originated from *C. frutescens*. In this study, these 2 species clustered together, confirming their close phylogenetic relationship. Our results were in agreement with those of Gonzalez-Perez et al. (2014), reporting that *C. frutescens* and *C. chinense* clustered into one group and *C. annuum* into a separate group. Overall, these results suggested that iPBS-retrotransposons can be effectively used for addressing genetic and evolutionary relationships in plants. They may become a marker of choice for scientists with low budgets.

PCoA was performed to confirm the results of previous clustering analyses. Results from the PCoA and STRUCTURE analyses were in full agreement with those obtained by UPGMA regarding the relatedness of the

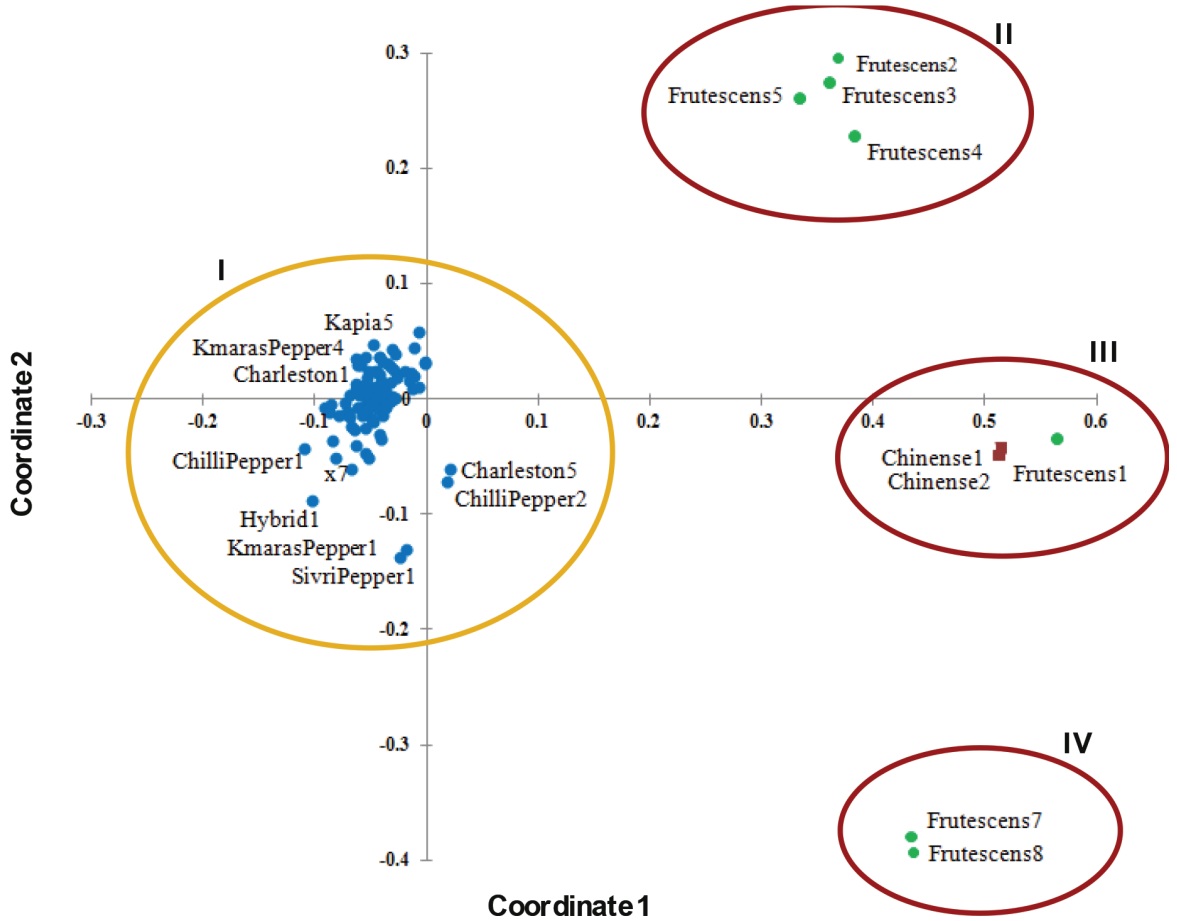

Figure 4. Genetic clustering of 90 *Capsicum* accessions based on principal coordinate analysis (PCoA) of 158 iPBS marker data. Four major clusters were revealed, separating all of the *C. annuum* (cluster I) from the *C. frutescens* and *C. chinense* accessions (clusters II, III, and IV). Accessions in cluster I (indicated in yellow circles) correspond to those in clusters I and II of the STRUCTURE results and branches I, II, and II of the UPGMA dendrogram (Figure 1), whereas clusters II, III, and IV (in red circles) correspond to cluster III of STRUCTURE and branches IV, V, and VI of the UPGMA dendrogram.

accessions at both the intraspecific (i.e. within *C. annuum*) and interspecific level.

Our results using all 3 clustering methods (UPGMA, PCoA, and STRUCTURE) revealed a clear separation of all the *C. annuum* accessions from *C. frutescens* and *C. chinense* materials. These results were in close agreement with those reported by Gonzalez-Perez et al. (2014), who found clear genetic separation between accessions belonging to *C. annuum*, *C. frutescens*, and *C. chinense*. Results from the PCoA and STRUCTURE analyses were in full agreement with those obtained by UPGMA, regarding the relatedness of the accessions at both the intraspecific (i.e. within *C. annuum*) and interspecific level.

In conclusion, the present study characterized, for the first time, the genetic diversity and population structure of the Turkish pepper germplasm using iPBS retrotransposon-based markers, revealing substantial variations both within *C. annuum* and among the 3 pepper species evaluated. The iPBS-retrotransposon markers were, under our experimental conditions, an excellent cost-effective and highly polymorphic marker option for addressing genetically related questions in *Capsicum*. We found that Chillipepper2 and Charleston5 were genetically diverse *C. annuum* accessions, while Frutescens7 and Frutescens1 were diverse *C. frutescens* genotypes. These genetically contrasting materials, belonging to *C annuum* and *C. frutescens*, could be used to develop segregating populations for mapping traits of interest, as well as to generate and select new phenotypic variants for breeding purposes. Based on our results, iPBS-retrotransposons should be regarded as a reliable and polymorphic marker system, allowing discrimination among and within pepper species. These universal markers can be utilized in any crop to investigate genetic diversity and relatedness. The data presented herein will be useful for pepper breeders and researchers.

References

Andeden EE, Baloch FS, Derya M, Kilian B, Özkan H (2013). iPBS-retrotransposon-based genetic diversity and relationship among wild annual *Cicer* species. J Plant Biochem Biotechnol 22: 453-466.

Aktas H, Abak K, Sensoy S (2009). Genetic diversity in some Turkish pepper (*Capsicum annuum* L.) genotypes revealed by AFLP analyses. Afr J Biotechnol 8: 4378-4386.

Akyavuz R, Taskin B, Koçak M, Yildiz M (2018). Exploring the genetic variations and population structure of Turkish pepper (*Capsicum annuum* L.) genotypes based on peroxidase gene markers. 3 Biotech 8: 355.

Ali F, Yılmaz A, Nadeem MA, Habyarimana E, Subaşı I, Nawaz MA, Chaudhary HJ, Shahid MQ, Ercişli S, Zia MA et al. (2019). Mobile genomic element diversity in world collection of safflower (*Carthamus tinctorius* L.) panel using iPBS-retrotransposon markers. PLoS One 14: e0211985.

Arystanbekkyzy M, Nadeem MA, Aktaş H, Yeken MZ, Zencirci N, Nawaz MA, Ali F, Haider MS, Tunc K, Chung G et al. (2019). Phylogenetic and taxonomic relationship of Turkish wild and cultivated emmer (*Triticum turgidum* ssp. *dicoccoides*) revealed by iPBS-retrotransposons markers. Int J Agric Biol 21:155-163.

Baloch FS, Alsaleh A, de Miera LE, Hatipoğlu R, Çiftçi V, Karaköy T, Yıldız M, Özkan H (2015a). DNA based iPBS-retrotransposon markers for investigating the population structure of pea (*Pisum sativum*) germplasm from Turkey.ˆ Biochem Syst Ecol 61: 244-252.

Baloch FS, Alsaleh A, Shahid MQ, Çiftçi V, de Miera LE, Aasim M, Nadeem MA, Aktaş H, Özkan H, Hatipoğlu R (2017). A whole genome DArTseq and SNP analysis for genetic diversity assessment in durum wheat from central fertile crescent. PLoS One 12: e0167821.

Baloch FS, Derya M, Andeden EE, Alsaleh A, Comertpay G, Kilian B, Ozkan H (2015b). Inter primer binding site (iPBS) retrotransposon and ISSR diversity among wild lens species. Biochem Sys Ecol 58: 162-168.

Boiteux LS, Fonseca MEN, Simon PW (1999). Effects of plant tissue and DNA purification methods on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. J Am Soc Hort Sci 124: 32-38.

Bosland PW, Votava EJ, Votava EM (2012). Peppers: vegetable and spice capsicums. Crops Prod Sci Hortic 12: 1-11.

Bozokalfa MK, Eşiyok D (2011). Evaluation of morphological and agronomical characterization of Turkish pepper accessions. Int J Veg Sci 17: 115-135.

Bozokalfa MK, Esiyok D, Turhan K (2009). Patterns of phenotypic variation in a germplasm collection of pepper (*Capsicum annuum* L.) from Turkey. Span J Agric Res 7: 83-95.

Demirel U, Tındaş İ, Yavuz C, Baloch FS, Çalışkan ME (2018). Assessing genetic diversity of potato genotypes using inter-PBS retrotransposon marker system. Plant Gen Res 16: 137-145.

Djian-Caporilano C, Lefebvre V, Sage-Daubeze AM, Palloix A (2007). *Capsicum*. In: Singh RJ (editor). Genetic Resources, Chromosome Engineering, and Crop Improvement: Vegetable Crops. Boca Raton, FL, USA: CRC Press, pp. 185-243.

Doyle JJ (1990). Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Eshbaugh WH (1993). Peppers: history and exploitation of a serendipitous new crop discovery. In: Janick J, Simon JE (editors). Advances in New Crops: Resources, Development, and Economics. New York, NY, USA: John Wiley, pp. 132-139.

Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-20.

González-Pérez S, Garcés-Claver A, Mallor C, de Miera LE, Fayos O, Pomar F, Merino F, Silvar C (2014). New insights into capsicum spp relatedness and the diversification process of *Capsicum annuum* in Spain. PLoS One 9: 12.

Gyulai G, Gémesné JA, Sági ZS, Venczel G, Pintér P, Kristóf Z, Törjék O, Heszky L, Bottka S, Kiss J et al. (2000). Doubled haploid development and PCR-analysis of F1 hybrid derived DH-R2 paprika (*Capsicum annuum* L.) lines. J Plant Physiol 156: 168-174.

Habyarimana E (2016). Genomic prediction for yield improvement and safeguarding of genetic diversity in CIMMYT spring wheat (*Triticum aestivum* L.). Aust J Crop Sci 10: 127-136

Hill TA, Ashrafi H, Reyes-Chin-Wo S, Yao J, Stoffel K, Truco MJ, Kozik A, Michelmore RW, Van Deynze A (2013). Characterization of *Capsicum annuum* genetic diversity and population structure based on parallel polymorphism discovery with a 30K unigene Pepper GeneChip. PLoS One 8: e56200.

Hulse-Kemp AM, Ashrafi H, Plieske J, Lemm J, Stoffel K, Hill T, Luerssen H, Pethiyagoda CL, Lawley CT, Ganal MW et al. (2016). A HapMap leads to a *Capsicum annuum* SNP infinium array: a new tool for pepper breeding. Hort Res 3: 16036.

Ibarra-Torres P, Valadez-Moctezuma E, Pérez-Grajales M, Rodríguez-Campos J, Jaramillo-Flores ME (2015). Inter- and intraspecific differentiation of *Capsicum annuum* and *Capsicum pubescens* using ISSR and SSR markers. Sci Hortic 181: 137-146.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bull Soc Vaud Sci Nat 44: 223-270 (in French).

Jung JK, Park SW, Liu WY, Kang BC (2010). Discovery of single nucleotide polymorphism in *Capsicum* and SNP markers for cultivar identification. Euphytica 175: 91-107.

Kalendar R, Antonius K, Smýkal P, Schulman AH (2010). iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theor Appl Genet 121: 1419-30.

Kalendar R, Flavell AJ, Ellis TH, Sjakste T, Moisy C, Schulman AH (2011). Analysis of plant diversity with retrotransposon-based molecular markers. Heredity 106: 520-30.

Kraft KH, Brown CH, Nabhan GP, Luedeling E, Ruiz JD, d'Eeckenbrugge GC, Hijmans RJ, Gepts P (2014). Multiple lines of evidence for the origin of domesticated chili pepper, *Capsicum annuum*, in Mexico. P Natl Acad Sci USA 111: 6165-6170.

Krishnamurthy SL, Prashanth Y, Rao AM, Reddy KM, Ramachandra R (2015). Assessment of AFLP marker based genetic diversity in chilli (*Capsicum annuum* L. & *C. baccatum* L.). Indian J Biotech 14: 49-54.

Kumar S, Kumar R, Singh J (2006). Cayenne/American pepper (*Capsicum* species). In: Peter KV (editor). Handbook of Herbs and Spices. Cambridge, UK: Woodhead, pp 299-312.

Lee HY, Ro NY, Jeong HJ, Kwon JK, Jo J, Ha Y, Jung A, Han JW, Venkatesh J, Kang BC (2016). Genetic diversity and population structure analysis to construct a core collection from a large *Capsicum* germplasm. BMC Genetics 17: 142.

Melnikova NV, Kudryavtseva AV, Speranskaya AS, Krinitsina AA, Dmitriev AA, Belenikin MS, Upelniek VP, Batrak ER, Kovaleva IS, Kudryavtsev AM (2012). The FaRE1 LTR-retrotransposon based SSAP markers reveal genetic polymorphism of strawberry (*Fragaria* x *ananassa*) cultivars. J Agric Sci 4: 111-118.

Meng C, Wei X, Zhao Y, Yuan Y, Yang S, Wang Z, Zhang X, Sun J, Zheng X, Yao Q et al. (2017). Genetic diversity analysis of *Capsicum* genus by SSR markers. Mol Plant Breed 8: 70-78.

Nadeem MA, Habyarimana E, Çiftçi V, Nawaz MA, Karaköy T, Comertpay G, Shahid MQ, Hatipoğlu R, Yeken MZ, Ali F et al. (2018a). Characterization of genetic diversity in Turkish common bean gene pool using phenotypic and whole-genome DArTseq-generated silicoDArT marker information. PLoS One 13: e0205363.

Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N et al. (2018b). DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip 32: 261-85.

Naegele RP, Mitchell J, Hausbeck MK (2016). Genetic diversity, population structure, and heritability of fruit traits in *Capsicum annuum*. PLoS One 11: e0156969.

Nei M (1972). Genetic distance between populations. Am Nat 106: 283-92.

Nemli S, Kianoosh T, Tanyolaç MB (2015). Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) accessions through retrotransposon-based interprimer binding sites (iPBSs) markers. Turk J Agric For 39: 940-894.

Nicolai M, Cantet M, Lefebvre V, Sage-Palloix AM, Palloix A (2013). Genotyping a large collection of pepper (*Capsicum* spp.) with SSR loci brings new evidence for the wild origin of cultivated *C. annuum* and the structuring of genetic diversity by human selection of cultivar types. Genet Resour Crop Ev 60: 2375-90.

Nimmakayala P, Abburi VL, Abburi L, Alaparthi SB, Cantrell R, Park M, Choi D, Hankins G, Malkaram S, Reddy UK (2014). Linkage disequilibrium and population-structure analysis among *Capsicum annuum* L. cultivars for use in association mapping. Mol Genet Genomics 289: 513-521.

Olmstead RG, Bohs L, Migid HA, Santiago-Valentin E, Garcia VF, Collier SM (2008). A molecular phylogeny of the Solanaceae. Taxon 57: 1159-1181.

Perry L, Dickau R, Zarrillo S, Holst I, Pearsall DM, Piperno DR, Berman MJ, Cooke RG, Rademaker K, Ranere AJ et al. (2007). Starch fossils and the domestication and dispersal of chili peppers (*Capsicum* spp. L.) in the Americas. Science 315: 986-988.

Pickersgill B (2007). Domestication of plants in the Americas: insights from Mendelian and molecular genetics. Ann Bot 100: 925-940.

Rai VP, Kumar R, Kumar S, Rai A, Kumar S, Singh M, Singh SP, Rai AB, Paliwal R (2013). Genetic diversity in *Capsicum* germplasm based on microsatellite and random amplified microsatellite polymorphism markers. Physiol Mol Biol Plants 19: 575-586.

Ramasamy RK, Ramasamy S, Bindroo BB, Naik VG (2014). STRUCTURE PLOT: A program for drawing elegant STRUCTURE bar plots in user friendly interface. SpringerPlus 3: 431.

Rana M, Sharma R, Sharma P, Bhardwaj SV, Sharma M (2014). Estimation of genetic diversity in *Capsicum annuum* L. germplasm using PCR-based molecular markers. Natl Acad Sci Lett 37: 295-301.

Walsh BM, Hoot SB (2001). Phylogenetic relationships of *Capsicum* (Solanaceae) using DNA sequences from two noncoding regions: the chloroplast atpB-rbsL spacer region and nuclear waxy introns. Int J Plant Sci 162: 1409-1418.

Yaldız G, Çamlıca M, Nadeem MA, Nawaz MA, Baloch FS (2018). Genetic diversity assessment in *Nicotiana tabacum* L. with iPBS-retrotransposons. Turk J Agric For 42: 154-164.

Yeh FC, Yang R, Boyle TJ, Ye Z (2000). Pop Gene 32, Microsoft Windows-based Freeware for Population Genetic Analysis. Version 1.32. Edmonton, Canada: University of Alberta.

Yildiz M, Cuevas HE, Sensoy S, Erdinc C, Baloch FS (2015a). Transferability of *Cucurbita* SSR markers for genetic diversity assessment of Turkish bottle gourd (*Lagenaria siceraria*) genetic resources. Bioch System Ecol 59: 45-53.

Yildiz M, Koçak M, Baloch FS (2015b). Genetic bottlenecks in Turkish okra germplasm and utility of iPBS retrotransposon markers for genetic diversity assessment. Genet Mol Res 14: 10588-10602.