Journal of Horticultural Science & Ornamental Plants 12 (3): 158-168, 2020
ISSN 2079-2158

DOI: 10.5829/idosi.jhsop.2020.158.168

Assessment of Genetic Characterization of Bean (*Phaseolus vulgaris* L.) Germplasm Collected from Erzincan of Turkey Using Microsatellite (SSR) Markers

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Abstract: The objective of this study was to explore the genetic diversity and relationship in some bean genotype collected from Erzincan with the SSR marker that emerged as a universal method. A total of 60 landraces and 4 commercial cultivars collected from different regions of Erzincan province were used in this study. Twenty polymorphic SSR primers yielded a total of 103 scorable bands. Twenty primers were studied and number of alleles in primers varied between 2-9 (with an average value of 5.15). Cluster analysis (UPGMA) based on SSR data, genotypes were divided into 2 groups. Polymorphic information content (PIC) values varied between 0.25 (BM114) and 0.80 (BM152 and BM156) with an average PIC value of 0.61. According to genetic structure analysis, genotypes were divided into 2 subpopulations. This is the first study conducted by the SSR to investigate the genetic diversity of local pinto and fresh bean genotypes in Erzincan. We believe that in future the results of this study will serve as a foundation for the development and breeding studies of pinto and fresh bean varieties.

Key words: Bean · Genetic diversity · PCA · SSR

INTRODUCTION

Phaseolus vulgaris L. is a member of the family of legumes (Fabaceae) genus Phaseolus. It's a crucial protein source in terms of both quantity and biological quality [1, 2]. Within this family, *Phaseolus vulgaris* L. constitutes majority (approximately 90%) of culture beans [3]. In addition, it is reported to have more genetic diversity both Turkey and world because of a widespread cultivation of the *Phaseolus vulgaris* [4]. Beans have two gene centers: Central America (Mesoamerica) and South America (Andean). Although there is no precise information about the time of entry in Turkey of beans, it is estimated that about 250-300 years ago came from Europe to Anatolia [5]. Turkey has an important place in the world in terms of plant genetic diversity as well as being the gene center of many plant species. Conservation and sustainable use of plant genetic resources play an important role in agricultural productivity. Identification and evaluation of genetic diversity is important for plant breeding programs. Some of the main objectives of growers are to develop new varieties with resistant to biotic and abiotic stress factors, high yield and quality while maintaining or improving nutritional value for humans [6]. Today, beans are grown in almost every region of Turkey and has shown spread to almost all regions with natural or artificial selection. There are some populations called with regional names [7]. The purpose of characterization of plant genetic resources is primarily to reveal the genetic variation between seed samples or populations and to determine the amount and distribution of genetic variation between these samples and populations, as well as to eliminate duplications and to establish a core collection. The degree of genetic diversity in plant germplasm are important sources for helps in planning breeding program for crop improvement [8, 9]. Genetic assessments through screening plant genetic sources with the molecular markers and identification of genetic relationships and similarities among the genotypes will construct the bases for further breeding studies [10]. Various methods have used molecular markers including AFLP (Amplified Fragment Length Polymorphisms) [11], RAPD (Random Amplified Polymorphic DNA) [12, 13],

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SCAR (Sequence Characterized Amplified Region) [14, 15, 16], ISSR (Inter Simple Sequence Repeat) [17, 18], SRAP (Sequence-Related Amplified Polymorphism) [19] and EST (Expressed Sequence Tag) [14, 20, 21] all to assess genetic diversity and relationships among several *Phaseolus vulgaris* species. SSR markers developed for beans are important genomic sources for genetic diversity analysis and plant breeding. SSR markers are frequently used and extremely useful tools in determining genetic variation. Due to its high degree of polymorphism, it is recommended to use SSR markers in genetic diversity analysis in many studies [2]. The SSR technique has successfully been used for assessment of genetic diversity in common beans [22, 23], faba bean [24], tomato [25], eggplant [26], cucumber [27], watermelon [28], pepper [29], cabbage [30], mango [31] and rice [32]. Microsatellites, also known as simple sequence repeats (SSR-Simple Sequence Repeats), are the smallest units repeated in DNA sequences and repeat patterns range from 1-6 bp [33]. In this study, genetic differences among the local pinto and fresh bean genotypes commonly grown in Erzincan (Turkey) were put forth with the aid of SSR marker method. No genetic characterization studies associated with pinto and green bean genotypes have been conducted in Erzincan using SSR marker. Therefore, revealing the genetic relationships of these genotypes is extremely important for both conservation of genetic diversity and breeding studies. Plant genetic resources are important for breeding efforts designed for the expose of new cultivars or for the improvement the characteristic of existing ones. Very little is known about genetic diversity and relatedness within the pinto and fresh bean germplasm in Erzincan province. Information in this area could be beneficial in the management of future germplasm, helpful in the selection of breeding material for bean and could provide essential information for breeding and genetics activities of pinto and fresh bean. The objective of this study was to investigate the genetic diversity with SSR markers among 60 local bean genotype and 4 commercial cultivars collected from various regions of Erzincan province. Additionally, the data obtained will provide an integrity in genetic identification studies on pinto and fresh bean and will also reduce workloads and costs of breeders.

MATERIALS AND METHODS

Collection of Plant Material: In this study, 60 bean genotypes (24 pinto beans and 36 fresh beans) commonly produced in Erzincan province were collected. Besides, 4 commercial cultivars (1 pinto bean and 3 fresh bean) in widely grown in the province used to compare with local genotypes (Table 1).

Genomic DNA Isolation: A greenhouse in the Erzincan Horticultural Research Institute was utilized to grow the sampled plants. Bulk DNA of 64 individuals per accession was prepared from young leaves of 2 weeks-old plants in Laboratory of Molecular Biology and Genetics, Agriculture Faculty, Ataturk University, Turkey in 2017. Slight modifications to the genomic DNA extraction model as described by Zeinalzadehtabrizi *et al*. [34] was performed. The concentrations of DNA, was determined through The NanoDrop® ND-1000 UV/V spectrophotometer (Thermo Fisher Scientific, U.S.A). A DNA concentration of 50 µg/ml was adjusted to give the final concentration. For SSR analysis, final DNA concentration was adjusted to 50 µg/ml. Diluted DNA samples were stored at -20°C for Polymerase chain reaction (PCR) reactions.

SSR Marker Analysis: In the study, 20 pairs of high molecular markers with high PIC values were selected from SSR markers developed by [35] and Blair [36, 37] (Table 2).

Data Analysis: The data obtained by scoring the SSR profiles with different primers individually as well as collectively were subjected to the construction of similarity matrix using Jaccard's coefficients. The similarity values were used for cluster analysis. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering was done and dendrogram was constructed using unweighted pair group method with arithmetic averages (UPGMA) for estimating genetic similarity based on Nei's coefficients among genotypes using NTSYS-2.02 [38]. Marker index for SSR markers was calculated in order to characterize the capacity of each primer to detect polymorphic loci among the genotypes. It is the sum total of the polymorphism information content (PIC) values of SSR markers produced by a particular primer. The PIC value was calculated using the formula $PICi = 1 - \Sigma P(i)^2$ [39], where pi is the frequency of the its allele. The PIC values provided an estimate of the discriminatory power of any locus by considering the number of alleles per locus and the relative frequencies of those alleles in the population. The genetic diversity within the genotypes was calculated from the following equations and Popgen program [40] using Nei's gene diversity index [41] and Shannon information index [42].

Table 1: List of bean genotypes collected from Erzincan in Turkey

Code number (≠)	Name of Type	Collected location
1	Pinto Bean	Erzincan-Center-Bahçeliköy
2	Pinto Bean	Erzincan-Center-Bahçeliköy
3	Pinto Bean	Erzincan-Center-Bahçeliköy
4	Pinto Bean	Erzincan-Center-Bahçeliköy
5	Pinto Bean	Erzincan-Center-Bahçeliköy
6	Fresh bean	Erzincan-Center-Bahçeliköy
7	Fresh bean	Erzincan-Center-Bahçeliköy
8	Pinto bean	Erzincan-Center-Ballıköy Village
9	Pinto bean	Erzincan-Center-Ballıköy Village
10	Pinto bean	Erzincan-Center-Ballıköy Village
11	Fresh bean	Erzincan-Center-Ballıköy Village
12	Pinto bean	Erzincan-Center-Cevizli Village
13	Pinto bean	Erzincan-Center-Cevizli Village
14	Fresh bean	Erzincan-Center-Cevizli Village
15	Fresh bean	Erzincan-Center-Cevizli Village
16	Fresh bean	Erzincan-Center-Cevizli Village
17	Pinto bean	Erzincan-Center-Cevizli Village
18	Fresh bean	Erzincan-Center-Cevizli Village
19	Pinto bean	Erzincan-Center-Cevizli Village
20	Pinto bean	Erzincan-Center-Çatalarmut Village
21	Pinto bean	Erzincan-Center-Çatalarmut Village
22	Fresh bean	Erzincan-Center-Çatalarmut Village
23	Fresh bean	Erzincan-Center-Çatalarmut Village
24	Fresh bean	Erzincan- Çayırlı-Balıklı Village
25	Pinto bean	Erzincan- Çayırlı-Balıklı Village
26	Pinto bean	Erzincan- Çayırlı-Balıklı Village
27	Pinto bean	Erzincan- Çayırlı
28	Fresh bean	Erzincan- Çayırlı
29	Pinto bean	Erzincan- Çayırlı
30	Pinto bean	Erzincan- Çayırlı
31	Fresh bean	Erzincan- Center
32	Fresh bean	Erzincan- Center- Ekmekli Village
33	Fresh bean	Erzincan- İliç
34	Fresh bean	Erzincan-Kemah
35	Fresh bean	Erzincan- Kemaliye
36	Fresh bean	Erzincan- Kemaliye
37	Pinto bean	Erzincan- Tercan
38	Fresh bean	Erzincan-Üzümlü-Uluköy
39	Fresh bean	Erzincan-Üzümlü-Uluköy
40	Fresh bean	Erzincan-Üzümlü-Uluköy
41	Fresh bean	Erzincan-Üzümlü-Uluköy
42	Pinto bean	Erzincan-Üzümlü-Uluköy
43	Fresh bean	Erzincan-Üzümlü-Uluköy
44	Fresh bean	Erzincan-Üzümlü-Uluköy
45	Pinto bean	Erzincan-Üzümlü-Uluköy
46	Pinto bean	Erzincan-Üzümlü-Uluköy
47	Pinto bean	Erzincan-Üzümlü-Uluköy
48	Pinto bean	Erzincan-Üzümlü-Uluköy
49	Pinto bean	Erzincan-Üzümlü
50	Pinto bean	Erzincan-Üzümlü
51	Pinto bean	Erzincan-Üzümlü
52	Pinto bean	Erzincan-Üzümlü
53	Pinto bean	Erzincan-Üzümlü
54	Pinto bean	Erzincan-Üzümlü
55	Pinto bean	Erzincan-Üzümlü
56	Pinto bean	Erzincan-Üzümlü
57	Pinto bean	Erzincan-Üzümlü
58	Pinto bean	Erzincan-Üzümlü
59	Pinto bean	Erzincan-Üzümlü
60	Fresh bean	Erzincan-Center- Yanlızbağ Town
61	Fresh bean (Aleyna)	Commercial cultivar
62	Fresh bean (Gina)	Commercial cultivar
63	Pinto bean (Perolar)	Commercial cultivar
64	Fresh bean (Serra)	Commercial cultivar

Table 2: Information of SSR molecular markers

Primer No	SSR primer	Sequence 5'-3'
1	BM114	AGCCTGGTGAAATGCTCATAG
2	BM137	CGCTTACTCACTGTACGCACG
3	BM143	GGGAAATGAACAGAGGAAA
4	BM152	AAGAGGAGGTCGAAACCTTAAATCG
5	BM153	CCGTTAGGGAGTTGTTGAGG
6	BM154	TCTTGCGACCGAGCTTCTCC
7	BM156	CTTGTTCCACCTCCCATCATAGC
8	BM167	TCCTCAATACTACATCGTGTGACC
9	BM175	CAACAGTTAAAGGTCGTCAAATT
10	BM183	CTCAAATCTATTCACTGGTCAGC
11	BM188	TCGCCTTGAAACTTCTTGTATC
12	BM199	AAGGAGAATCAGAGAAGCCAAAAG
13	BM200	TGGTGGTTGTTATGGGAGAAG
14	BM210	ACCACTGCAATCCTCATCTTTG
15	BM211	ATACCCACATGCACAAGTTTGG
16	BMd1	CAAATCGCAACACCTCACAA
17	BMd15	TTGCCATCGTTGCTTAATTG
18	BMd18	AAAGTTGGACGCACTGTGATT
19	PVAG004	TTGATGACGTGGATGCATTGC
20	PVTTTC001	TTTAGCCACCGCAGCACCAC

STRUCTURE 2.2 program was used to determine the genetic structure of genotypes [43]. In many genetic diversity studies with beans, genotypes are successfully divided into groups using the STRUCTURE program [44, 45]. The F-statistics (FST) value reflects the difference between subpopulations [46]. Using the GenAlex program, basic coordinate analysis was carried out to better understand the diversity among genotypes. On the 2-dimensional diagram obtained by covering the total variance of the first two coordinates, groups were determined and compared with cluster analysis. Genetic variation within and between populations was examined with the GenAlex program [47] using the ANOVA method.

RESULTS AND DISCUSSION

Polymorphism Revealed by SSR Primers: A total of 20 most polymorphic SSR primers were used to characterize the pinto and fresh bean germplasm. In this study, all primer yielded sufficiently clear and scorable bands (Table 3). With these 20 primers, 103 visible and scorable bands were generated. In similar studies conducted on beans, Matondo *et al*. [2] reported that 12 SSR marker yielded visible and scorable bands. In present study, number of alleles in primers varied between 2 (BM154) and 9 (BM188) (with an average value of 5.15). With the analysis made through SSR markers, polymorphic information content (PIC) varied between 0.25 (BM114) and 0.80 (BM152, 156) with an average value of 0.61 (Table 3). PIC scores the efficiency of polymorphic loci and designates the separation power of a primer [48]. Ekbiç and Hasancaoğlu [5] in a similar study on beans reported the PIC value as between 0.06 (SSR-IAC63) and 0.82 (SSR-IAC116). In another study using 22 SSR primer on 41 common bean reported PIC value as between 0.16 (BMd16) and 0.77 (BM141) [49]. Present findings comply with the values reported for different studies with SSR markers in beans, a mean 0.61 PIC value [50] and 0.71 PIC value [51]. Those result were similar with the present PIC value. The mean level of heterozygosity per SSR marker was 0.47. This level of heterozygosity is quite higher than 0.19 and 0.24 to what was reported by Blair *et al*. [52] and Matondo *et al*. [2], respectively. Heterozygosity level ranged from 0.00 (BM143, BM152 and BM154) to 1.00 (BM153). BM152 marker had the highest gene diversity of 0.82 while BM114 marker had the lowest gene diversity of 0.02. The mean gene diversity value was 0.68.

Genetic Diversity: The ne, h and I values of bean genotypes and cultivars are shown in Table 4. The greatest ne, h and I values were respectively determined as 1.78, 0.44 and 0.63 in genotype ≠26. The greatest values were respectively observed as 1.61, 0.38 and 0.57 in genotype ≠8. Mean values (ne, h and I) for all genotypes were calculated as 17, 0.41 and 0.60, respectively. In a previous study using SSR markers on beans, Shannon's information index values were reported as between 0.663-2.202 with an average value of 1.343 [53]. Additional research conducted in a different study with 28 SSR markers and 138 *Vigna umbellata* genotypes, the values of I were reported as between 0.845-1.019 [54].

Table 3: Primer name, major allele frequency, allele number, gene diversity, heterozygosity and PIC value of the SSR primers used during this study

SSR primer	Major Allele Frequency	Allele Number	Gene Diversity	Heterozygosity	PIC
BM114	0.85	3	0.26	0.02	0.25
BM137	0.24	6	0.81	0.98	0.78
BM143	0.45	4	0.63	0.00	0.56
BM152	0.22	7	0.83	0.00	0.80
BM153	0.31	5	0.76	1.00	0.72
BM154	0.80	2	0.32	0.00	0.27
BM156	0.28	8	0.82	0.76	0.80
BM167	0.60	3	0.56	0.00	0.50
BM175	0.24	8	0.81	0.34	0.79
BM183	0.41	4	0.70	0.88	0.65
BM188	0.35	9	0.81	0.97	0.79
BM199	0.37	6	0.69	0.14	0.62
BM200	0.74	3	0.39	0.03	0.32
BM210	0.62	3	0.49	0.02	0.40
BM211	0.32	7	0.74	0.32	0.70
BMd1	0.42	5	0.70	0.98	0.65
BMd16	0.41	4	0.64	0.98	0.57
BMd18	0.36	7	0.74	0.27	0.70
PVAG004	0.31	4	0.74	0.85	0.69
PVTTTC001	0.35	5	0.74	0.92	0.70
Mean	0.43	5.15	0.66	0.47	0.61

Table 4: Summary statistics for mean values of genotypes evaluated using SSR primers

Genotype code (≠)	ne*	h*	I*	Genotype code (≠)	ne*	h*	I*
1	1.76	0.43	0.62	33	1.74	0.42	0.62
2	1.73	0.42	0.61	34	1.66	0.40	0.59
3	1.71	0.42	0.61	35	1.74	0.42	0.62
4	1.76	0.43	0.62	36	1.71	0.42	0.61
5	1.66	0.40	0.59	37	1.69	0.41	0.60
6	1.69	0.41	0.60	38	1.66	0.40	0.59
7	1.71	0.42	0.61	39	1.69	0.41	0.60
8	1.61	0.38	0.57	40	1.64	0.39	0.58
9	1.73	0.42	0.61	41	1.69	0.41	0.60
10	1.76	0.43	0.62	42	1.69	0.41	0.60
11	1.69	0.41	0.60	43	1.69	0.41	0.60
12	1.69	0.41	0.60	44	1.71	0.42	0.61
13	1.73	0.42	0.61	45	1.69	0.41	0.60
14	1.69	0.41	0.60	46	1.69	0.41	0.60
15	1.71	0.42	0.61	47	1.69	0.41	0.60
16	1.69	0.41	0.60	48	1.69	0.41	0.60
17	1.71	0.42	0.61	49	1.66	0.40	0.59
18	1.66	0.40	0.59	50	1.73	0.42	0.61
19	1.64	0.39	0.58	51	1.71	0.42	0.61
20	1.64	0.39	0.58	52	1.69	0.41	0.60
21	1.68	0.40	0.59	53	1.69	0.41	0.60
22	1.64	0.39	0.58	54	1.73	0.42	0.61
23	1.71	0.42	0.61	55	1.73	0.42	0.61
24	1.73	0.42	0.61	56	1.64	0.39	0.58
25	1.69	0.41	0.60	57	1.69	0.41	0.60
26	1.78	0.44	0.63	58	1.66	0.40	0.59
27	1.71	0.42	0.61	59	1.69	0.41	0.60
28	1.69	0.41	0.60	60	1.71	0.42	0.61
29	1.71	0.42	0.61	61	1.69	0.41	0.60
30	1.76	0.43	0.62	62	1.69	0.41	0.60
31	1.66	0.40	0.59	63	1.66	0.40	0.59
32	1.69	0.41	0.60	64	1.73	0.42	0.61
Mean (G1-G64)					1.70	0.41	0.60

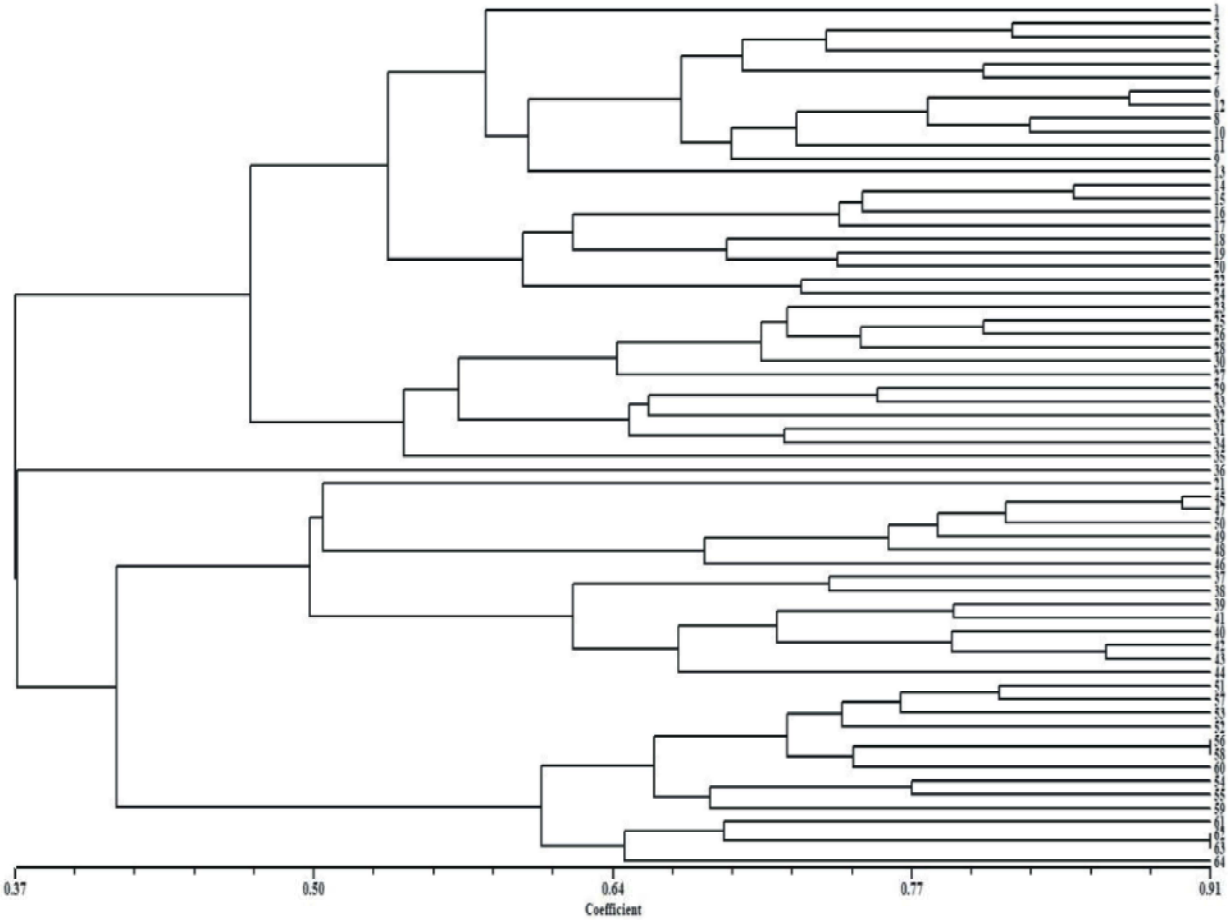

Fig. 1: Dendrogram generated by UPGMA method using SSR markers

Cluster Analysis and Principal Component Analysis for Microsatellite (SSR) Markers: Genotypes were divided into 2 main groups according to cluster analysis using SSR primers. 35 genotypes were in the first group, 25 genotypes and 4 commercial varieties were in the second group (Figure 1). The first cluster was divided into two sub-groups. All genotypes, except for ≠36 were placed in the first sub-group and the genotype ≠36 alone was placed in the second sub-group. Similarly, the second cluster was also divided into two sub-groups. While a single genotype (≠51) was placed into the first sub-group, the other genotypes and commercial cultivars were all placed in the second sub-group (Figure 1). The geographical distribution of the species is an important factor for genetic diversity [55]. Principle component analysis (PCA) shows the spatial distribution of the relative genetic distance of populations [56]. In this study, PCA analysis was applied for a more detailed visualization of the variation both within and between populations. With this method, a 2-D diagram is composed based on closeness or distance matrix between the genotypes and the distances between the resultant groups put forth the actual distance [57]. According to our results, the genotypes Çatalarmut (≠21), Uluköy (≠40), Üzümlü (≠58), were placed on upper left section of the Principle Axis-1. The genotypes Bahçeliköy (≠5, ≠6), Ballıköy ((≠8, ≠11), Cevizli (≠12, ≠14, ≠16, ≠18, ≠19), Çatalarmut (≠20, ≠22), Balıklı (≠25) Çayırlı (≠28, ≠29), Erzincan-Center (≠31), Kemah (≠34), Uluköy (≠38, ≠42, ≠43), Commercial variety (≠63) were gathered on lower left section of Axis-1. The genotypes Bahçeliköy (≠1, ≠3, ≠4), Ballıköy (≠9, ≠10), Cevizli (≠13, ≠15, ≠17), Çatalarmut (≠23), Balıklı (≠24, ≠26), Çayırlı (≠27, ≠30), İliç (≠33), Kemaliye (≠35), Uluköy (≠41) and Commercial variety (≠62, ≠64) were placed on lower right section of Axis -1. The genotypes Ekmekli (≠32), Kemaliye (≠36), Tercan (≠37) and Uluköy (≠39, ≠44, ≠45, ≠47, ≠48), Üzümlü (≠49, ≠50, ≠51, ≠52, ≠53, ≠54, ≠55, ≠57), Yanlızbağ (≠60)

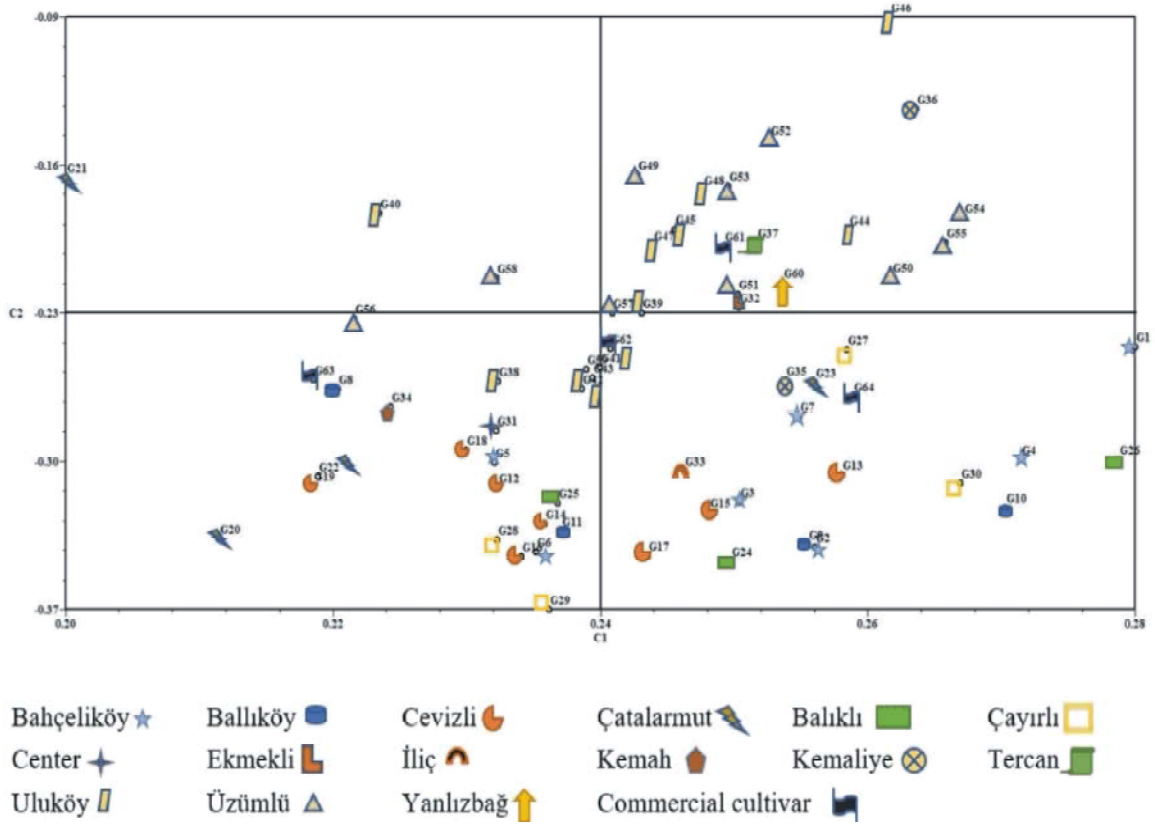

Fig. 2: PCA created using the SSR marker and separated on 2-dimensional diagram

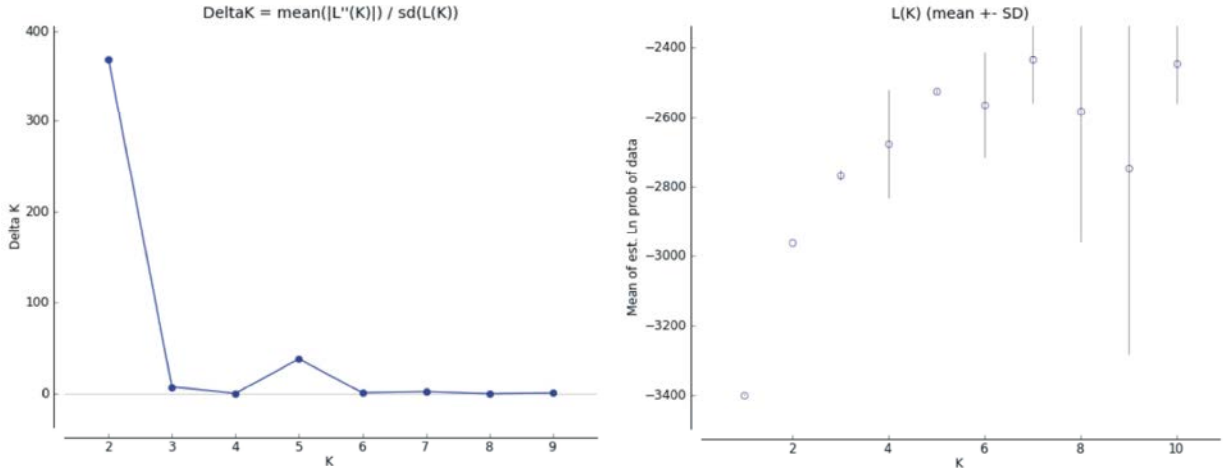

Fig. 3: Line graphs from the admixture model of structure of Ln P(D) (a measure of the natural logarithm of the posterior probability, P of the data, D) and ΔK for bean populations. a; Mean value of the statistic Ln P(D) produced by STRUCTURE at each value of K, b; DK

Table 5: Expected heterozygosity and FST values in 2 subpopulations of beans

Sub-population (K)	Expected heterozygosity	Fst value
A	0.25	0.38
B	0.28	0.31
Average	0.53	0.69

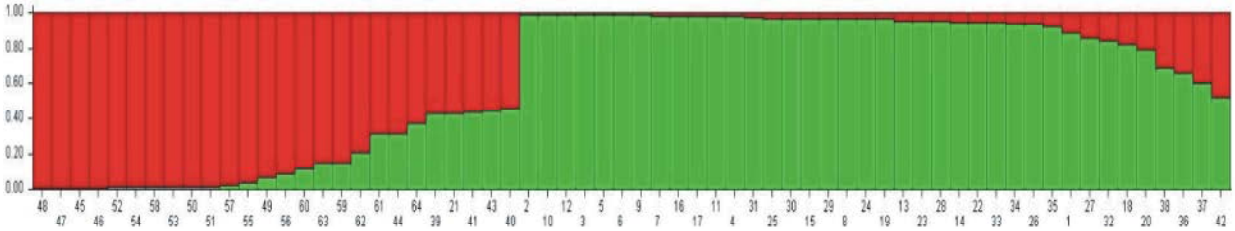

Fig. 4: Genetic structure of genotypes according to SSR data

and Commercial variety (≠61) were gathered on upper right section of Axis-1 (Figure 2). According to our findings, it was determined that the germplasm of the bean (*Phaseolus vulgaris* L.) has low genetic diversity. In addition, our study revealed that commercial varieties belong to the same group. Among its local cognates, ≠26 enjoyed highest genetic diversity, compared to other genotypes, according to SSR primers.

Population Genetic Structure Analysis for Microsatellite (SSR) Markers: The findings of the genetic structure analysis are shown in Figure 3. ΔK is an expression used to determine the optimum values of K. In this study, the largest K value was calculated as 2. According to the present findings, it was determined that the K value was low. Such a low value was attributed to both the proximity of the regions where the genotypes were collected and the high gene flow between the regions. While the first three groups (35 genotypes in total) were in the second subpopulation, the fourth group (29 genotypes in total) was in the first subpopulation (Figure 4). The FST (F-statistics) value was determined as 0.38 and 0.31 in the first and second subpopulations, respectively (Table 5).

CONCLUSION

Characterization of germplasm provides an opportunity to determine the genetic diversity and to identify the new variations that can be employed for breeding studies. Thus, it is expected that the results of this study will contribute bean breeding programs in Turkey as well as maintain the genetic integrity of the bean germplasm. During this study, significant numbers of local bean genotype were collected from Erzincan Province. To explore the higher level of diversity and relationship in the Turkish germplasm in the future, it is very important to collect local genotypes from other parts of Turkey and the SSR markers could be very beneficial to draw a clear picture of this.

In the future, there is also a need to perform genome-wide association studies using these genetic materials in order to determine the genes associated with different traits of interest.

Abbreviations:

≠ (Genotype code)
FST (The F-statistics)
h (Genetic diversity of nei)
I (Shannon's information index)
K (Number of populations)
ne (Number of effective alleles)
PCA (Principle component analysis)
PCR (Polymerase chain reaction)
PIC (Polymorphic information content)
SAHN (Sequential agglomerative hierarchical non-overlapping)
UPGMA (Unweighted pair-group method)

ACKNOWLEDGMENT

We would like to thank the Atatürk University, Coordinator of Scientific Research Projects, which supported this study (Project no: FBA-2017- 6192). We would like to thank the reviewers, editors and production team for providing invaluable contributions.

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