TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2020) 44: 417-427

doi:10.3906/tar-2002-9

Genetic diversity of pinto and fresh bean (*Phaseolus vulgaris* L.) germplasm collected from Erzincan province of Turkey by inter-primer binding site (iPBS) retrotransposon markers

Halil İbrahim ÖZTÜRK[1,*], Atilla DURSUN[2], Arash HOSSEINPOUR[3,4], Kamil HALİLOĞLU[3]

[1]Health Services Vocational School, Erzincan Binali Yıldırım University, Erzincan, Turkey
[2]Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum, Turkey
[2]Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum, Turkey
[4]Crop and Horticultural Science Research Department, Ardabil Agricultural and Natural Resources Research and Education Center, AREEO Ardabil (Moghan), Iran

Received: 03.02.2020 • **Accepted/Published Online:** 26.06.2020 • **Final Version:** 02.08.2020

Abstract: In this study, genetic diversity among 71 bean genotypes (41 pinto beans and 30 fresh bean genotypes) and 4 commercial cultivars (Aleyna, Gina, Perolar, and Serra) commonly grown at Erzincan province in Turkey were investigated. The iPBS (inter-primer binding site) markers were used to investigate the genetic diversity of the genotypes. Polymorphism ratio was identified as 100% in all primers. Twenty-seven primers were studied and number of alleles in primers varied between 13–69 (with an average value of 37.14). According to cluster analysis based on iPBS data, genotypes were divided into 2 groups. Polymorphic information content (PIC) values varied between 0.19 (iPBS 2077) and 0.42 (iPBS 2381, iPBS 2385, and iPBS 2389) with an average PIC value of 0.33. According to genetic structure analysis, genotypes were divided into 2 subpopulations. Majority of the genotypes of the regions from where beans were collected were placed in the same groups.

Key words: Genetic variation, molecular marker, *Phaseolus vulgaris*, population structure

1. Introduction

Beans (*Phaseolus vulgaris* L, 2n = 2x = 22), produced over the large fields both worldwide and in Turkey, are significant self-pollinating species belonging to *Leguminosae* family (Dursun et al., 2010; Öztürk and Dursun, 2018; Yeken et al., 2018). They have a quite large genetic diversity and this diversity needs genetic characterization. The primary objective in characterization of plant genetic sources is to put forth the genetic variations among seed samples or populations, and then use these samples to determine the quantity and distribution of genetic variation among the populations (Piergiovanni et al., 2006; Akbulut et al., 2013). 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 (Sarıkamış, 2014). Various methods have used molecular markers including Amplified Fragment Length Polymorphisms (AFLP) (Sustar-Vozlic et al., 2006), Random Amplified Polymorphic DNA (RAPD) (Erdinc et al., 2017), Sequence Characterized Amplified Region (SCAR) (Madakbaş et al., 2016), Inter Simple Sequence Repeat (ISSR) (Dagnew et al., 2014), Simple Sequence Repeats (SSR) (Sarıkamış et al., 2009; Bilir et al., 2019), Sequence-Related Amplified Polymorphism (SRAP) (Ceylan et al., 2014), and Expressed Sequence Tag (EST) (Garcia et al., 2011), all to assess genetic diversity and relationships among several *Phaseolus vulgaris* species. Inter-primer binding site (iPBS) is a universal method for DNA fingerprinting and retrotransposon isolation. It is an amplification technique and do not require sequence data. The iPBS technique has successfully been used for assessment of genetic diversity in common beans (Nemli et al., 2015; Aydın and Baloch, 2019) quinoa (Hossein-Pour et al., 2019a), adonis (Hossein-Pour, 2019b) and saffron (Gedik et al., 2017). However, there are very few studies about the use of iPBS-retrotransposon markers for assessment of genetic diversity in pinto and fresh bean genotypes. Also, inexistence of a genetic identification research on fresh bean with the aid of iPBS markers have made the present study unique in this sense. In this study, some local pinto and fresh bean genotypes commonly grown in Erzincan (Turkey) district were collected and genetic differences among the genotypes

* Correspondence: hiozturk@erzincan.edu.tr

were put forth with the aid of iPBS marker method. LTR-retrotransposons have primer binding sites (PBS) around 5' LTR. The 3' tip of cellular tRNA is bound to PBS and initiates mRNA synthesis of retrotransposon (Kumar and Bennetzen, 1999). In iPBS method, the regions so close as to have implications between 2 LTR retrotransposons are amplified in reverse directions. Primers are developed from the preserved regions of PBS. iPBS produces 15–50 bands with a length of between 100–5000 bp. Just because of high number of copies in investigated genomes of iPBS sequences, iPBS produces several numbers of net bands and it is easy to score them in standard agarose gels stained with ethidium bromide. iPBS method yields polymorphic fingerprints behaving as dominant marker (Kalendar et al., 2010). Present findings will reveal genetic variations among the genotypes and generate the bases for the further breeding studies. Such findings will also provide an integrity in genetic identification studies on pinto and fresh bean and will also reduce workloads and costs of breeders. The aim of the present study was to evaluate the population structure and genetic diversity of pinto and fresh bean genotypes collected from Erzincan province using iPBS markers.

2. Materials and methods

This study was carried out in the Molecular Biology and Genetics Laboratory of the Field Crops Department of Atatürk University in 2017.

2.1. Collection of plant material

In this study, 71 local bean genotypes (41 pinto beans and 30 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 were used to compare with local genotypes (Table 1).

2.2. Genomic DNA isolation

Sample plants were grown in a greenhouse. Bulk DNA of 71 individuals per accession was prepared from young leaves of 2-week-old plants in Laboratory of Molecular Biology and Genetics, Department of Field Crops, Agriculture Faculty, Ataturk University, Turkey in 2017. Genomic DNA extractions were performed as described by Zeinalzadehtabrizi et al. (2015) with slight modifications. DNA quality was affirmed through electrophoresis in 0.8% agarose gel. The NanoDrop ND-1000 UV/Vis spectrophotometer (Thermo Fisher Scientific, USA) was used to determine DNA concentrations. For iPBS analysis, final DNA concentration was adjusted to 50 µg/mL. Diluted DNA samples were stored at –20 °C for Polymerase chain reaction (PCR) reactions.

2.3. iPBS marker analysis

Among 71 *Phaseolus vulgaris* L. genotypes of the present study, initially 5 of them were randomly selected and were used in selection of polymorphic primers from 40 iPBS primers previously developed by Kalendar et al. (2010). Among these 40 iPBS primers, 27 of them with good/excellent PCR products were selected and were used for genotyping the whole set of *Phaseolus vulgaris* L. genotypes.

2.4. Data analysis

TotalLab TL120 software was used for analysis of iPBS band patterns. The iPBS amplification products were scored as present (1) or absent (0). Only the clear and strong bands were scored and were used in further analyses. Numerical Taxonomy and Multiware Analysis System (NTSYSpc version 2.0) was used based on Dice similarity matrix (Dice, 1945) to assess the genetic similarities among the genotypes. UPGMA (unweighted pair-group mean average) tree was constructed with the aid of the same software and a PCA (principle component analysis) was conducted (Rohlf, 2000). PIC (polymorphism information content) ($PIC=1-\Sigma pi2$, where Pij is the frequency of the pattern j for each marker i) was used to assess the diversity of each iPBS marker (Anderson et al., 1993). POPGEN1.32 was used to determine genetic parameters, effective number of alleles (ne), Nei's genetic diversity (h), and Shannon's information index (I) (Yeh et al., 1997). Model-based cluster analysis was used with the aid of Structure v. 2.2 software to determine genetic structure of the genotypes (Pritchard et al., 2000a; Pritchard et al., 2000b). Number of populations (K) expected to present in each 10 runs (generally varied between 2–10) characterized by a set of distinctive allele frequencies at each locus and the individuals were sited in K clusters. MCMC (Markov Chain Monte Carlo) posterior probabilities were estimated. The most expected value of K was predicted with the aid of Evanno's ΔK method (Evanno et al., 2005) and Structure Harvester (Earl, 2012).

3. Results and discussion

3.1. Polymorphism revealed by iPBS primers

Of 40 iPBS primers used in this study, 27 yielded sufficiently clear and scorable bands. With these 27 primers, 1003 visible and scorable bands were generated (Table 2). In similar studies conducted on beans, Nemli et al. (2015) screened 83 iPBS primers and reported that 47 iPBS primers (56%) yielded visible and scorable bands. In another study, average number of polymorphic iPBS-retrotransposon bands was reported as 3.8 and number of bands per primer was reported as 25 (Gedik et al., 2017). Additionally, iPBS-retrotransposon primers yielded greater data than IRAP (Boronnikova and Kalendar, 2010), RAPD (Bukhari et al., 2015) and AFLP (Sustar-Vozlic et al., 2006) methods. In present study, polymorphism ratio in all primers was identified as 100%. Number of alleles in primers varied between 13 (iPBS 2077) and 69 (iPBS 2384) (with an average value of 37.14) (Table 2). High

Table 1. List of bean genotypes collected from Erzincan in Turkey and their coordinates.

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	Pinto Bean	Erzincan-Center-Bahçeliköy
7	Fresh bean	Erzincan-Center-Bahçeliköy
8	Fresh bean	Erzincan-Center-Bahçeliköy
9	Pinto bean	Erzincan-Center-Bahçeliköy
10	Pinto bean	Erzincan-Center-Ballıköy Village
11	Pinto bean	Erzincan-Center-Ballıköy Village
12	Pinto bean	Erzincan-Center-Ballıköy Village
13	Fresh bean	Erzincan-Center-Ballıköy Village
14	Fresh bean	Erzincan- Üzümlü- Bayırbağ
15	Pinto bean	Erzincan-Center-Cevizli Village
16	Pinto bean	Erzincan-Center-Cevizli Village
17	Fresh bean	Erzincan-Center-Cevizli Village
18	Fresh bean	Erzincan-Center-Cevizli Village
19	Fresh bean	Erzincan-Center-Cevizli Village
20	Fresh bean	Erzincan-Center-Cevizli Village
21	Pinto bean	Erzincan-Center-Cevizli Village
22	Fresh bean	Erzincan-Center-Cevizli Village
23	Pinto bean	Erzincan-Center-Cevizli Village
24	Pinto bean	Erzincan-Center-Çatalarmut Village
25	Pinto bean	Erzincan-Center-Çatalarmut Village
26	Fresh bean	Erzincan-Center-Çatalarmut Village
27	Fresh bean	Erzincan-Center-Çatalarmut Village
28	Fresh bean	Erzincan-Center-Çatalarmut Village
29	Fresh bean	Erzincan-Çayırlı-Balıklı Village
30	Pinto bean	Erzincan-Çayırlı-Balıklı Village
31	Pinto bean	Erzincan-Çayırlı-Balıklı Village
32	Pinto bean	Erzincan-Çayırlı
33	Fresh bean	Erzincan-Çayırlı
34	Pinto bean	Erzincan-Çayırlı
35	Pinto bean	Erzincan-Çayırlı
36	Fresh bean	Erzincan-Center
37	Fresh bean	Erzincan-Center-Ekmekli Village
38	Fresh bean	Erzincan-İliç
39	Fresh bean	Erzincan-Kemah
40	Fresh bean	Erzincan-Kemaliye
41	Fresh bean	Erzincan-Kemaliye
42	Pinto bean	Erzincan-Refahiye
43	Pinto bean	Erzincan-Tercan
44	Fresh bean	Erzincan-Üzümlü-Uluköy
45	Fresh bean	Erzincan-Üzümlü-Uluköy
46	Fresh bean	Erzincan-Üzümlü-Uluköy
47	Fresh bean	Erzincan-Üzümlü-Uluköy
48	Pinto bean	Erzincan-Üzümlü-Uluköy
49	Fresh bean	Erzincan-Üzümlü-Uluköy
50	Fresh bean	Erzincan-Üzümlü-Uluköy
51	Pinto bean	Erzincan-Üzümlü-Uluköy
52	Pinto bean	Erzincan-Üzümlü-Uluköy
53	Pinto bean	Erzincan-Üzümlü-Uluköy
54	Pinto bean	Erzincan-Üzümlü-Uluköy
55	Pinto bean	Erzincan-Üzümlü-Uluköy
56	Pinto bean	Erzincan-Üzümlü
57	Pinto bean	Erzincan-Üzümlü
58	Fresh bean	Erzincan-Üzümlü
59	Pinto bean	Erzincan-Üzümlü
60	Pinto bean	Erzincan-Üzümlü
61	Pinto bean	Erzincan-Üzümlü
62	Pinto bean	Erzincan-Üzümlü
63	Pinto bean	Erzincan-Üzümlü
64	Pinto bean	Erzincan-Üzümlü
65	Pinto bean	Erzincan-Üzümlü
66	Pinto bean	Erzincan-Üzümlü
67	Pinto bean	Erzincan-Üzümlü
68	Pinto bean	Erzincan-Üzümlü
69	Pinto bean	Erzincan-Üzümlü
70	Pinto bean	Erzincan-Center-Yaylabaşı
71	Fresh bean	Erzincan-Center-Yalnızbağ
72	Fresh bean (Aleyna)	Commercial cultivar
73	Fresh bean (Gina)	Commercial cultivar
74	Pinto bean (Serra)	Commercial cultivar
75	Fresh bean (Perolar)	Commercial cultivar

Table 2. Allele number, polymorphic allele number, polymorphism percentage, and PIC values of iBPS markers.

Number	Primer name	Allele number	Number of polymorphic alleles	Percentage of polymorphism	PIC*
1	iPBS 2074	31	31	100%	0.27
2	iPBS 2077	13	13	100%	0.19
3	iPBS 2078	44	44	100%	0.26
4	iPBS 2079	33	33	100%	0.26
5	iPBS 2080	25	25	100%	0.37
6	iPBS 2095	33	33	100%	0.27
7	iPBS 2221	42	42	100%	0.28
8	iPBS 2231	36	36	100%	0.24
9	iPBS 2270	45	45	100%	0.32
10	iPBS 2271	31	31	100%	0.32
11	iPBS 2274	31	31	100%	0.37
12	iPBS 2276	46	46	100%	0.29
13	iPBS 2278	51	51	100%	0.34
14	iPBS 2298	42	42	100%	0.33
15	iPBS 2377	32	32	100%	0.40
16	iPBS 2378	44	44	100%	0.33
17	iPBS 2380	32	32	100%	0.34
18	iPBS 2381	21	21	100%	0.42
19	iPBS 2383	31	31	100%	0.34
20	iPBS 2384	69	69	100%	0.23
21	iPBS 2385	59	59	100%	0.42
22	iPBS 2386	41	41	100%	0.41
23	iPBS 2389	39	39	100%	0.42
24	iPBS 2390	43	43	100%	0.31
25	iPBS 2391	22	22	100%	0.40
26	iPBS 2392	36	36	100%	0.35
27	iPBS 2402	31	31	100%	0.27
Mean		37.14	37.14	100%	0.33

*The Polymorphism Information Content (PIC)

polymorphism ratio was mainly attributed to high number of genotypes used in this study.

With the analysis made through iPBS markers, PIC varied between 0.19 (iPBS 2077) and 0.42 (iPBS 2381, 2385, 2389) with an average value of 0.33. PIC scores the efficiency of polymorphic loci and designates the separation power of a primer (Guo and Elston, 1999). Nemli et al. (2015) in a similar study on beans reported the PIC value as between 0.03 (iPBS 2375) and 0.94 (iPBS 2394) with an average value of 0.73. Present findings comply with the values reported for different plant species such as common bean with a mean 0.73 PIC value (Nemli et al., 2015), guava with 0.28 PIC value (Mehmood et al., 2016), tea with PIC value 0.30 (Phong et al., 2016). Those values were similar with the present PIC value.

3.2. Genetic diversity

For 71 bean genotypes and 4 bean cultivars, ne, h, and I values are provided in Table 3. The greatest ne, h, and I values were respectively observed as 1.799, 0.444, and 0.636 in genotype ≠3; the greatest values were respectively observed as 1.618, 0.382, and 0.570 in genotype ≠63. For all genotypes, average values of ne, h, and I were calculated as

Table 3. Summary statistics for mean values for bean genotypes assessed by iBPS primers.

Code number (≠)	ne	h	I	Code number (≠)	ne	h	I
1	1.788	0.441	0.633	39	1.671	0.401	0.591
2	1.784	0.439	0.631	40	1.679	0.404	0.594
3	1.799	0.444	0.636	41	1.660	0.398	0.587
4	1.785	0.440	0.632	42	1.665	0.400	0.589
5	1.764	0.433	0.625	43	1.660	0.398	0.587
6	1.771	0.435	0.627	44	1.655	0.396	0.585
7	1.781	0.438	0.630	45	1.647	0.393	0.582
8	1.769	0.435	0.626	46	1.631	0.387	0.575
9	1.747	0.428	0.619	47	1.668	0.401	0.590
10	1.727	0.421	0.612	48	1.652	0.395	0.584
11	1.771	0.435	0.627	49	1.671	0.401	0.591
12	1.745	0.427	0.618	50	1.631	0.387	0.575
13	1.702	0.412	0.603	51	1.704	0.413	0.604
14	1.730	0.422	0.613	52	1.702	0.412	0.603
15	1.727	0.421	0.612	53	1.684	0.406	0.596
16	1.727	0.421	0.612	54	1.683	0.406	0.596
17	1.727	0.421	0.612	55	1.692	0.409	0.599
18	1.689	0.408	0.598	56	1.699	0.412	0.602
19	1.686	0.407	0.597	57	1.665	0.400	0.589
20	1.692	0.409	0.599	58	1.685	0.406	0.596
21	1.740	0.425	0.616	59	1.650	0.394	0.583
22	1.689	0.408	0.598	60	1.648	0.393	0.582
23	1.749	0.428	0.619	61	1.623	0.384	0.572
24	1.734	0.423	0.614	62	1.647	0.393	0.582
25	1.663	0.399	0.588	63	1.618	0.382	0.570
26	1.702	0.412	0.603	64	1.671	0.401	0.591
27	1.699	0.412	0.602	65	1.671	0.401	0.591
28	1.710	0.415	0.606	66	1.679	0.404	0.594
29	1.663	0.399	0.588	67	1.634	0.388	0.576
30	1.660	0.398	0.587	68	1.644	0.392	0.581
31	1.722	0.419	0.610	69	1.618	0.382	0.570
32	1.750	0.428	0.620	70	1.654	0.396	0.585
33	1.710	0.415	0.606	71	1.645	0.392	0.581
34	1.699	0.412	0.602	72	1.660	0.398	0.587
35	1.681	0.405	0.595	73	1.634	0.388	0.576
36	1.652	0.395	0.584	74	1.651	0.394	0.583
37	1.647	0.393	0.582	75	1.670	0.401	0.591
38	1.678	0.404	0.594				
Mean		1.692		0.408		0.599	

ne: Number of effective alleles; h: Genetic diversity of Nei; I: Shannon's information index

1.692, 0.408, and 0.599, respectively. In a previous study on beans, Shannon's information index values were reported as between 0.663–2.202 with an average value of 1.343 (Özkan, 2018). In another study conducted with 12 iPBS markers and 138 pea genotypes, I values were reported as between 0.24–0.58 with an average value of 0.39 (Baloch et al., 2015). Gedik et al. (2017) conducted a study to identify the genetic diversity and relativeness of the saffron genotypes and reported average h and I values respectively as 0.16 and 0.29. Yıldız et al. (2015) and Mehmood et al. (2013) found that I values as 0.12 and 0.27, respectively. In a study on fresh beans using the SCAR marker method, the genetic similarity index between green bean genotypes was found to vary between 0.52 and 0.98 (Ulukapı and Onus, 2012).

3.3. Cluster analysis and principal component analysis for iPBS-retrotransposon markers

According to cluster analysis based on iPBS data, genotypes were separated into 2 groups. There were 50 genotypes in the first group and 21 genotypes and 4 commercial cultivars in the second group (Figure 1). The first cluster was divided into 2 subgroups. All genotypes, except for ≠25 were placed in the first subgroup and the genotype ≠25 alone was placed in the second subgroup. Similarly, the second cluster was also divided into 2 subgroups. While the genotypes ≠51 was alone placed into the first subgroup, the other genotypes and commercial (Aleyna, Gina, Serra and Perolar) cultivars were all placed in the second subgroup (Figure 2). Geographical distribution area is an important factor for genetic diversity of the species (Zecca et al., 2012). Principle component analysis (PCA) presents spatial distribution of relative genetic distance between the populations (Klaedtke et al., 2017). In present study, PCA analysis was conducted for better and more detailed visualization of the variation within and between the populations. With the aid this method, a 2-D diagram is generated based on closeness or distance matrix between the genotypes and the distances between

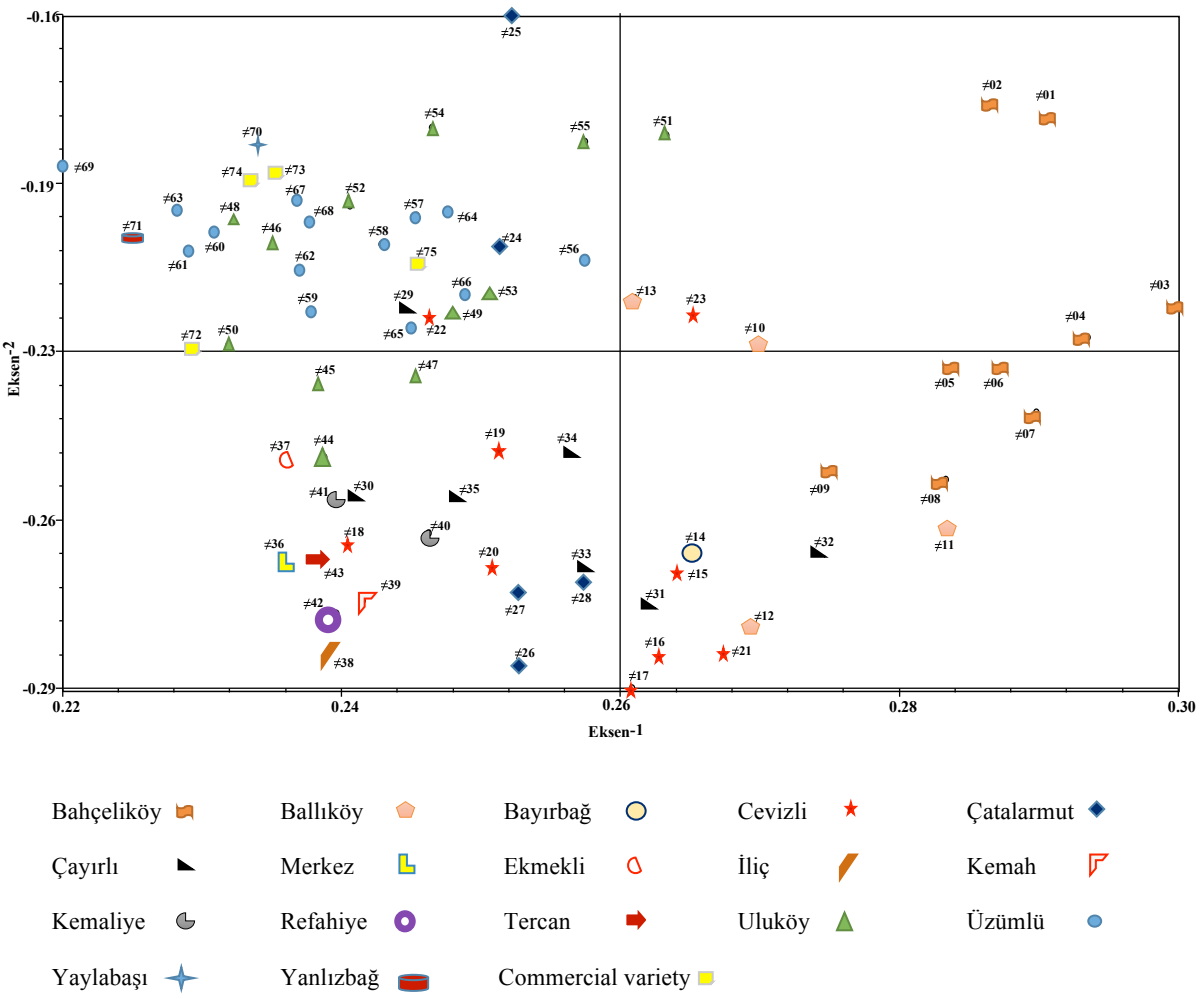

Figure 1. PCA created using the iPBS marker and separated on 2-dimensional diagram.

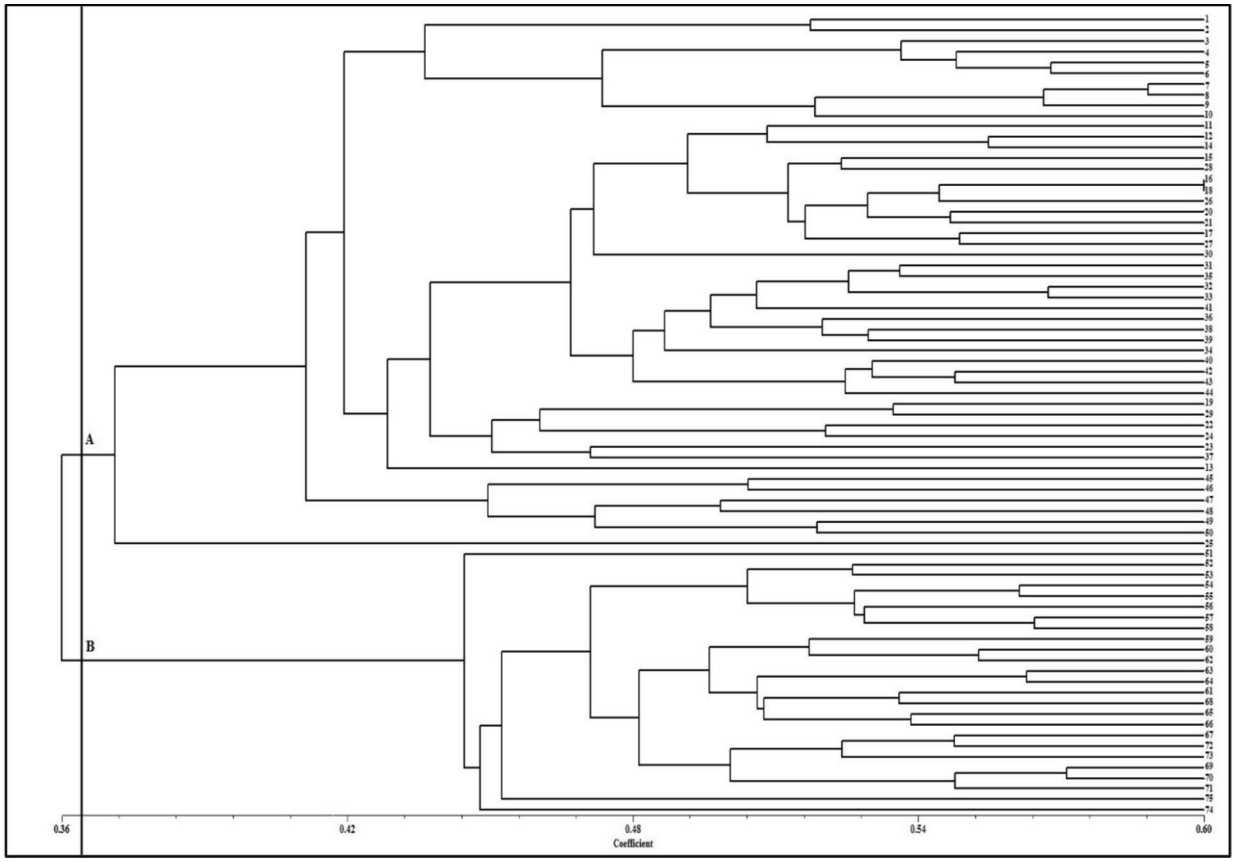

Figure 2. Dendrogram generated by UPGMA method using iPBS markers.

the resultant groups put forth the actual distances (Mohammadi and Prasanna, 2003). According to present findings, commercial cultivars (Aleyna, Gina, Serra and Perolar) and the genotypes Cevizli (≠22), Çatalarmut (≠24, ≠25), Çayırlı (≠29), Uluköy (≠46, ≠48, ≠49, ≠50, ≠52, ≠53, ≠54, ≠55), Üzümlü (≠56, ≠57, ≠58, ≠59, ≠60, ≠61, ≠62, ≠63, ≠64, ≠65, ≠66, ≠67, ≠68, ≠69), Yaylabaşı (≠70) and Yalnızbağ (≠71) were placed on upper left section of the Principle Axis-1. The genotypes Cevizli (≠18, ≠19, ≠20), Çatalarmut (≠26, ≠27, ≠28), Çayırlı (≠30, ≠33, ≠34, ≠35), Erzincan-Center (≠36), Ekmekli (≠37), İliç (≠38), Kemah (≠39), Kemaliye (≠40, ≠41), Refahiye (≠42), Tercan (≠43) and Uluköy (≠44, ≠45, ≠47) were gathered on lower left section of Axis-1. The genotypes Bahçeliköy (≠5, ≠6, ≠7, ≠8, ≠9), Ballıköy (≠11, ≠12), Bayırbağ (≠14), Cevizli (≠15, ≠16, ≠17, ≠21), and Çayırlı (≠31, ≠32) were placed on lower right section of Axis -1. The genotypes Bahçeliköy (≠1, ≠2, ≠3, ≠4), Ballıköy (≠10, ≠13), Cevizli (≠23), and Uluköy (≠51) were gathered on upper right section of Axis-1 (Figure 1). Our results showed that of pinto and fresh bean (*Phaseolus vulgaris* L.) germplasm has the least genetic diversity. In additional, our study naked that commercial cultivars such as Serra and Perolar and as well as Aleyna and Gina belonged to the same group. These results are supported by the results of Andeden et al. (2013), who reported the describing the narrow genetic base of the cultivated chickpea. This reinforces the necessity of broadening the genetic basis of the cultivated bean through the utilization of local varieties resources inbreeding programs for introducing favorable alleles into commercial varieties. Among its local relatives, ≠1 enjoyed highest genetic diversity, compared to the other genotype, according to iPBS marker.

3.4. Population genetic structure analysis for iPBS-retrotransposon markers

Results of genetic structure analysis are presented in Figure 3. ΔK is used to determine optimum values of K. In this study, the greatest value was determined as K = 2. According to present data, K value was considered as low. Such a low value was attributed to high gen flow between the regions from where genotypes were collected. Similar findings were reported in previous studies for population structure of bean genotypes (Nemli et al., 2015). Present findings revealed that there were 25 genotypes in the first subpopulation and 50 genotypes in the second subpopulation (Table 4). It was observed

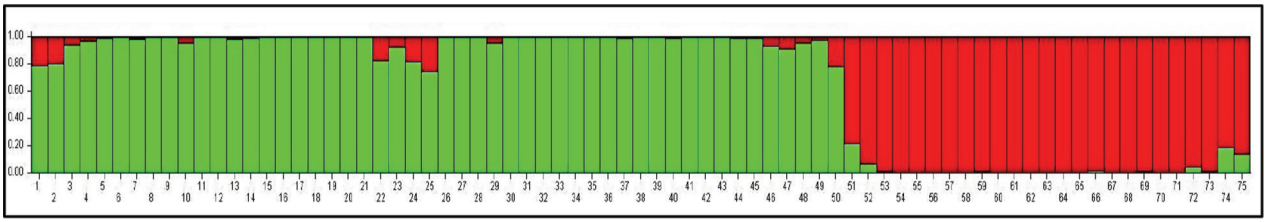

Figure 3. Genetic structure of genotypes according to iBPS data (the beans genotypes given in K = 2 are presented in Table 4).

Table 4. Membership coefficient of 2 subpopulations of bean genotypes.

Code number (≠)	Subpopulation		Code number (≠)	Subpopulation	
	I	II		I	II
1	0.209	0.791	39	0.003	0.997
2	0.197	0.803	40	0.011	0.989
3	0.058	0.942	41	0.004	0.996
4	0.034	0.966	42	0.002	0.998
5	0.008	0.992	43	0.002	0.998
6	0.004	0.996	44	0.008	0.992
7	0.016	0.984	45	0.008	0.992
8	0.004	0.996	46	0.065	0.935
9	0.003	0.997	47	0.086	0.914
10	0.05	0.95	48	0.045	0.955
11	0.005	0.995	49	0.027	0.973
12	0.004	0.996	50	0.218	0.782
13	0.015	0.985	51	0.785	0.215
14	0.011	0.989	52	0.93	0.07
15	0.003	0.997	53	0.987	0.013
16	0.002	0.998	54	0.993	0.007
17	0.007	0.993	55	0.998	0.002
18	0.002	0.998	56	0.993	0.007
19	0.005	0.995	57	0.998	0.002
20	0.003	0.997	58	0.996	0.004
21	0.005	0.995	59	0.989	0.011
22	0.176	0.824	60	0.996	0.004
23	0.076	0.924	61	0.998	0.002
24	0.182	0.818	62	0.999	0.001
25	0.254	0.746	63	0.995	0.005
26	0.003	0.997	64	0.997	0.003
27	0.004	0.996	65	0.996	0.004
28	0.003	0.997	66	0.98	0.02
29	0.044	0.956	67	0.998	0.002
30	0.007	0.993	68	0.998	0.002
31	0.003	0.997	69	0.988	0.012
32	0.007	0.993	70	0.997	0.003
33	0.002	0.998	71	0.996	0.004
34	0.007	0.993	72	0.956	0.044
35	0.004	0.996	73	0.988	0.012
36	0.004	0.996	74	0.814	0.186
37	0.01	0.99	75	0.86	0.14
38	0.004	0.996			

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

Subpopulation (K)	Expected heterozygosity	FST
1	0.2892	0.2535
2	0.3322	0.0718
Mean	0.6214	0.3253

that genetic structure analysis yielded similar results with cluster analysis. The 1st, 2nd, and 3rd groups (a total of 50 genotypes) were placed in the second subpopulation and the 4th group (a total of 25 genotypes) was placed in the first subpopulation. FST (F-statistic) value was determined as 0.2535 in the first subpopulation and as 0.0718 in the second subpopulation (Table 5). Nemli et al. (2015) used iPBS markers for 67 bean genotypes and reported 4 subpopulations (K = 4). In another study with SSR markers for 149 dry bean genotypes, genetic structure and diversity analyses revealed 3 subpopulations (K = 3) (Zargar et al., 2016).

In conclusion, the present study used the iPBS-retrotrasposon marker system to generate prebreeding data that can potentially be applied for selection of appropriate parents to introduce greater genetic variation in pinto and fresh bean (*Phaseolus vulgaris* L.) breeding programs. This study demonstrates that the iPBS marker system is a powerful and easy method for fingerprinting and distinguishing pinto and fresh bean (*Phaseolus vulgaris* L.) genotypes. In this study, 71 bean genotypes (41 pinto beans and 30 fresh bean genotypes) and 4 commercial cultivars (Aleyna, Gina, Perolar, and Serra) were used to identify their genetic diversity using 27 polymorphic iPBS-retrotransposon primers and stated that genotypes belonging to the same cultivar (pinto and fresh beans) were placed in different clusters of the dendrogram. The geographical distribution range is a major factor in determining the genetic diversity of varieties. The data provide an objective means of identifying and preserving the diversity of this germplasm. Furthermore, it would promote their utilization in future breeding in order to enhance genetic diversity of modern bean varieties through hybridization. Such similarities indicated potential gene flow between these locations. Present findings revealed the diversity in bean genotypes and may constitute the bases for further breeding studies in beans and will bring an integrity in bean identification studies.

Acknowledgment

This study was presented at 30th International Horticultural Congress, held from 12 to 16 August 2018 in İstanbul, Turkey. 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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