Article

iPBS-Retrotransposon Markers in the Analysis of Genetic Diversity among Common Bean (Phaseolus vulgaris L.) Germplasm from Türkiye

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Abstract: Beans are legumes that play extremely important roles in human nutrition, serving as good sources of protein, vitamins, minerals, and antioxidants. In this study, we tried to elucidate the genetic diversity and population structure of 40 Turkish bean (Phaseolus vulgaris L.) local varieties and 5 commercial cultivars collected from 8 different locations in Erzurum-Ispir by using inter-primary binding site (iPBS) retrotransposon markers. For molecular characterization, the 26 most polymorphic iPBS primers were used; 52 bands per primer and 1350 bands in total were recorded. The mean polymorphism information content was 0.331. Various diversity indices, such as the mean effective allele number (0.706), mean Shannon's information index (0.546), and gene diversity (0.361) revealed the presence of sufficient genetic diversity in the germplasm examined. Molecular analysis of variance (AMOVA) revealed that 67% of variation in bean germplasm was due to differences within populations. In addition, population structure analysis exposed all local and commercial bean varieties from five sub-populations. Expected heterozygosity values ranged between 0.1567 (the fourth sub-population) and 0.3210 (first sub-population), with an average value of 0.2103. In contrary, population differentiation measurement (Fst) was identified as 0.0062 for the first sub-population, 0.6372 for the fourth subpopulations. This is the first study to investigate the genetic diversity and population structure of bean germplasm in Erzurum-Ispir region using the iPBS-retrotransposon marker system. Overall, the current results showed that iPBS markers could be used consistently to elucidate the genetic diversity of local and commercial bean varieties and potentially be included in future studies examining diversity in a larger collection of local and commercial bean varieties from different regions.

Keywords: bean; breeding; genetic diversity; population structure

1. Introduction

It has been reported that the rate of disappearance of plant species has increased in recent years and it is thought that the rate of genetic erosion of plant species will increase in the coming years [1]. To minimize genetic erosion in agriculture and to ensure sustainability
in this field, many strategies have been developed for the protection of germplasm [2].
Germplasm refers to living tissues that are used in plant breeding studies and have a very
important place for the conservation of plant genetic resources. One of the important
tools in which plant germplasm is preserved is plant gene banks. These gene banks
contain different plant germplasms such as seeds, pollen, in vitro. These gene banks are
extremely important as they reflect the genetic diversity of both cultivated plants and their
wild relatives [3]. Genetic variation information is crucial to GenBank management and
breeding studies. This information assists in the creation of seed collections and facilitates
the use of desired local varieties in breeding programs [4]. Knowledge of the genetic
diversity between native species and improved varieties is crucial to supporting plant
breeding programs so that breeders can take advantage of existing local varieties adapted
to the climatic conditions of particular regions [5].

Bean (*Phaseolus vulgaris* L.) is one of the most valuable herbal products in the world due
to its nutritional properties, benefits to human health and economic importance [1]. Beans
are an important product that is widely grown and distributed in almost every region of
the world [2]. Beans show wide variation phenotypically, biochemically, and genotypically,
and are comprised of independent and differentiated gene pools, forming gene centers in
Central America and the Andes Mountains [6]. The contributions of these two gene pools
can generally be distinguished by seed size and certain other morphological characteristics.
The seeds of the Mesoamerican local varieties are small or medium in size, while those
of the Andean local varieties are larger [7]. The first bean cultivars corresponding to the
small-grained Mesoamerican local varieties s were identified in Spain, Portugal, and South
America in the early 16th century. Beans first came to Europe in the 16th-17th centuries [3].
It is reported that it reached Turkey from Europe in the 17th century [8]. Turkey is not the
homeland of the bean, but several studies have indicated the existence of wide variation
among local bean local varieties in Turkey [9]. The characterization of local varieties
provides an opportunity to determine genetic diversity and to identify new variations that
can be used in various breeding programs [10–14].

Genetic diversity studies have been carried out with bean varieties in many parts of
Turkey. However, these studies are not yet enough. Such diversity studies can support
breeding activities by both farmers and plant breeders. It is also crucial to the conservation
and sustainable use of the plant genetic resources needed to meet future food-security
demands [15].

Various morphological, chemical, biochemical, and molecular markers are widely
used to characterize bean genetic diversity [16]. The development of molecular markers
changed the fate of breeding studies and allowed these studies to accelerate. Molecular
markers provide direct estimation of genetic variation at the DNA level, reducing the
interference of environmental variation and being unaffected by the environment [17].
Molecular markers with different properties have been developed with studies by scientific
communities. Various methods have used molecular markers, including amplified fragment
length polymorphisms (AFLPs) [18], random amplified polymorphic DNA (RAPD) [19],
sequence characterized amplified region (SCAR) [20], single nucleotide polymorphism
(SNP) [21], inter simple-sequence repeat (ISSR) [4], simple-sequence repeats (SSR) [22],
and expressed sequence tag (EST) [23], all to assess the genetic diversity and associations
among several *Phaseolus* species.

Moreover, among them, retrotransposons are genetic elements capable of forming
major components of most eukaryotic genomes, constituting 50–90% of the plant genome.
Retrotransposons are divided into two: long terminal repeat (LTR) and non-LTR retrotrans-
posons. LTR-retrotransposons are more common in plants than the other group [24]. Due
to limitations in both LTR and non-LTR retrotransposons, inter primer binding site (ipBS)
retrotransposons have been developed as a universal marker used in the characterization
of both animal and plant species [25]. iPBS markers are the dominant markers and have
become a preferred marker in genetic diversity assessment in recent years due to their
universality [26]. The universality of the iPBS-retrotransposon marker has been proven and
molecular characterization and phylogenetic studies are available for these markers, also in beans [8,24,26]. In our previous studies [26] and in the studies of other researchers [8,9], it has been observed that retrotransposon markers are quite efficient for genetic diversity studies in terms of the total number of amplified and polymorphic bands. The local varieties evaluated so far represent only a small subset of the available resources. In addition, a comprehensive study has not yet been conducted to measure the genetic diversity of bean germplasm in Türkiye. Previous studies [7–9,20,26] allow the investigation of the genetic diversity of local bean varieties collected from a very narrow geographical region in Türkiye. There are no previous studies to reveal bean genetic diversity and population structure in Erzurum-Ispir district in the Northeastern Anatolia region of Türkiye using iPBS markers. Therefore, we here investigate the genetic diversity and population structure local bean varieties collected from the district of Ispir, using the iPBS marker system. It is necessary to identify, define, and use genetic resources for the continuity of breeding studies. We expect that our findings here will assist in the use, improvement, and preservation of local varieties that are well adapted to the changing environment.

2. Materials and Methods

2.1. Plant Materials

In this study, 45 Turkish bean (*Phaseolus vulgaris* L.) local varieties were used as plant material. The names and gathering places of the regional varieties are presented in Table 1 and Figure 1. Bean local varieties were collected in cultivated fields in form eight different Ispir districts of Erzurum in the Northeastern Anatolia region of Türkiye. The plants were grown for tissue sampling in the greenhouse of Atatürk University, Department of Field Crops, Faculty of Agriculture.

| Variety | Collected Location            | Latitude | Longitude | Altitude (m) |
|---------|-------------------------------|----------|-----------|--------------|
| G1      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G2      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G3      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G4      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G5      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G6      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G7      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G8      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G9      | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G10     | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G11     | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G12     | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G13     | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G14     | Ispir- Öztoprak village       | 40.518   | 41.052    | 1431         |
| G15     | Ispir-center                 | 40.485   | 41.002    | 1264         |
| G16     | Ispir-center                 | 40.468   | 40.983    | 1168         |
| G17     | Ispir-center                 | 40.468   | 40.983    | 1168         |
| G18     | Yesilyurt                     | 40.518   | 41.069    | 1549         |
| G19     | Yesilyurt                     | 40.518   | 41.069    | 1549         |
| G20     | Yesilyurt                     | 40.518   | 41.069    | 1549         |
| G21     | Maden village                 | 40.435   | 40.851    | 1226         |
| G22     | Maden village                 | 40.435   | 40.851    | 1226         |
| G23     | Maden village                 | 40.435   | 40.851    | 1226         |
| G24     | Maden village                 | 40.435   | 40.851    | 1226         |
| G25     | Ağıldere village              | 40.401   | 40.834    | 1470         |
### Table 1. Cont.

| Variety | Collected Location | Latitude   | Longitude  | Altitude (m) |
|---------|--------------------|------------|------------|--------------|
| G26     | Ağıldere village   | 40.401     | 40.834     | 1470         |
| G27     | Ağıldere village   | 40.401     | 40.834     | 1470         |
| G28     | Ağıldere village   | 40.401     | 40.834     | 1470         |
| G29     | Ağıldere village   | 40.401     | 40.834     | 1470         |
| G30     | Ağıldere village   | 40.401     | 40.834     | 1470         |
| G31     | Ulubel village     | 40.418     | 40.868     | 1424         |
| G32     | Ulubel village     | 40.418     | 40.868     | 1424         |
| G33     | Ulubel village     | 40.418     | 40.868     | 1424         |
| G34     | Ulubel village     | 40.418     | 40.868     | 1424         |
| G35     | Ulubel village     | 40.418     | 40.868     | 1424         |
| G36     | Ulubel village     | 40.418     | 40.868     | 1424         |
| G37     | Kirazlı village    | 40.436     | 40.887     | 1220         |
| G38     | Kirazlı village    | 40.436     | 40.887     | 1220         |
| G39     | Köprübaşı town     | 40.434     | 40.819     | 1286         |
| G40     | Köprübaşı town     | 40.434     | 40.819     | 1286         |
| G41     | Aras-98            |            |            |              |
| G42     | Elkoça-05          |            |            |              |
| G43     | Göynük-98          |            |            |              |
| G44     | Karaman-90         |            |            |              |
| G45     | Yakutiye-98        |            |            |              |
|         | Commercial cultivars |          |            |              |

**Figure 1.** Locations where local bean varieties were collected (Table 1; 1: Öztoprak village, 2: Ispir Center, 3: Yeşilyurt, 4: Maden Village, 5: Elmalı District Ağıldere village, 6: Ulubel village, 7: Kirazlı village, 8: Köprübaşı town. Commercial cultivars are not shown on the map).

#### 2.2. DNA Isolation and Quantification

Young leaves of beans (*P. vulgaris* L.) approximately 15-day-old plants were ground in liquid nitrogen at the molecular biology and genetics laboratory of Ataturk University. The collective DNA of 45 individuals per participation was then prepared, using the DNA
extraction method of Zeinalzadehtabrizi et al. [27], with modifications. The DNA quality was determined by electrophoresis, using agarose gel at 0.8% concentration. A NanoDrop ND-1000 UV/Vis spectrophotometer device (Thermo Fisher Scientific Company, Waltham, MA, USA) was used to determine the DNA concentrations. The final DNA concentration was selected for the iPBS analysis. The DNA samples for which the concentrations were determined were stored at ~20 °C for PCR (polymerase chain reactions) after further dilution.

2.3. PCR and iPBS Marker Analyses

Genetic diversity analyses were performed with iPBS primers available from Sigma Aldrich (Castle Hill, NSW, Australia). In the present study, 26 iPBS primers developed by Kalendar et al. [28] were used (Table 2). PCR Amplification was performed in a thermocycler (SensoQuest Labcycler) and were conducted in 10 µL reaction mixture comprising 25 ng template DNA, 0.5 U Taq polymerase, 0.25 mM dNTP, 1 µM (20 pmol) primer, 1X buffer; 2 mM MgCl2. The PCR thermal cycling profile is as follows: initial denaturation for 3 min at 95 °C, 38 cycles of 95 °C for 60 s, 50–60 °C for 60 s, 72 °C for 120 s and final extension at 72 °C for 10 min [29]. All PCR amplification products were resolved in agarose gel at 3% concentration at 200 V for 105 min. Finally, gels were visualized under UV light and photographed by digital camera (Model Nikon Coolpix500).

Table 2. List of 26 iPBS-retrotransposon primers with their sequence used to elucidate genetic diversity among 45 common bean varieties.

| Marker   | Primers Sequences (5′→3′) | Marker   | Primers Sequences (5′→3′) |
|----------|---------------------------|----------|---------------------------|
| iPBS-2074| GCTCTGATACCA              | iPBS-2377| ACGAAGGGACCA              |
| iPBS-2077| CTCACGATGCGGA             | iPBS-2378| GGTCCCTCATCCA             |
| iPBS-2078| GGCGGAGTCGGCA             | iPBS-2380| CAACCTGATCCA              |
| iPBS-2079| AGGTGGGCGCCA              | iPBS-2381| GTCCTACCTCCA              |
| iPBS-2080| CAGACGGCGCCA              | iPBS-2383| GCATGGGCTCCA              |
| iPBS-2095| GCTCGGATACCA              | iPBS-2384| GTAATGGGTCCA              |
| iPBS-2231| ACTTGGATGCTGATACCA        | iPBS-2385| CCATGCGGCTCA              |
| iPBS-2270| ACCTGGCGTGCCA             | iPBS-2386| CTGATCAACCCA              |
| iPBS-2271| GGCTCGGATGCCA             | iPBS-2389| ACATCTTCCCA               |
| iPBS-2274| ATGGTGGGGCGCCA            | iPBS-2390| GCAACAAACCCA              |
| iPBS-2276| ACCTCTGATACCA             | iPBS-2391| ATCTGTCAGCCA              |
| iPBS-2278| GCTCATGATACCA             | iPBS-2392| TAGATGGTGCCA              |
| iPBS-2298| AGAAGAGCTCCTGATACCA       | iPBS-2402| TCTAAAGCTCTTGATACCA       |

2.4. Data Scoring and Analysis

The PCR was performed in three replicates for each primer to verify the band pattern consistency. The DNA bands were scored, using TotalLab TL120 software (TotalLab Ltd., Gosforth, Newcastle upon Tyne, UK). For the iPBS amplification products, a band is scored “1” or absent “0” for each locus. Only clear, strong bands were scored, while faint, weak bands were ignored. The Numerical Taxonomy and Multivariate Analysis System for personal computer (NTSYSpc) V.2.0 programs based on the Dice similarity matrix [30] were used to determine the genetic similarities between the varieties. A UPGMA (Unweighted Pair-Group Method with Arithmetic mean) dendrogram was created with the NTSYSpc V.2.0 program. In addition, molecular variance (AMOVA) and PCoA (Principal Coordinate Analysis) analysis were performed using the Genalex 6.5 program [31]. A PIC (Polymorphism Information Content value) was used to assess the diversity of each iPBS marker [32]. The POPGEN v.1.32 program was used to determine the effective number of allele (ne), Nei genetic diversity (h), and Shannon’s information index (I) [33]. The Structure v.2.3.4 program was used to determine the genetic structures of the varieties [34,35]. Evanno’s ΔK [36] and Structure Harvester [37] methods were used to estimate the most expected K value. Using this method, Markov chain Monte Carlo (MCMC) posterior probabilities were estimated. The MCMC chains were run with a 10,000-iteration burn-in period, followed by
Principal coordinate analysis (PCoA) was performed with the GenALEx 6.5 program [38].

3. Results

3.1. Polymorphism Revealed by iPBS Primers

Sufficiently clear and scoreable bands were obtained from all primers included in the study. With these 26 primers, 1350 visible and scoreable bands were generated. The number of alleles in the primers varied between 23 (iPBS 2077 and 2383) and 80 (iPBS 2274) (Mean 37.14). When the analysis was performed with the iPBS markers, the PIC varied between 0.151 (iPBS 2298) and 0.495 (iPBS 2383) (Mean 0.331). Major allele frequency ranged from 0.528 (iPBS-2383) to 0.888 (iPBS-2298). The mean major allele frequency was 0.706 (Table 3).

Table 3. Twenty-six iPBS primers used in the detection of polymorphism among 40 local varieties and 5 commercial cultivars of beans (P. vulgaris L.).

| Marker      | Number of Alleles | Major Allele Frequency | PIC *  | Marker      | Number of Alleles | Major Allele Frequency | PIC *  |
|-------------|-------------------|------------------------|--------|-------------|-------------------|------------------------|--------|
| iPBS-2074   | 40                | 0.651                  | 0.430  | iPBS-2377   | 45                | 0.715                  | 0.309  |
| iPBS-2077   | 23                | 0.653                  | 0.387  | iPBS-2378   | 64                | 0.805                  | 0.241  |
| iPBS-2078   | 71                | 0.682                  | 0.323  | iPBS-2380   | 51                | 0.678                  | 0.336  |
| iPBS-2079   | 35                | 0.810                  | 0.226  | iPBS-2381   | 57                | 0.687                  | 0.359  |
| iPBS-2080   | 43                | 0.756                  | 0.316  | iPBS-2383   | 23                | 0.528                  | 0.495  |
| iPBS-2095   | 64                | 0.691                  | 0.352  | iPBS-2384   | 56                | 0.761                  | 0.252  |
| iPBS-2231   | 52                | 0.655                  | 0.398  | iPBS-2385   | 63                | 0.728                  | 0.313  |
| iPBS-2270   | 25                | 0.677                  | 0.153  | iPBS-2386   | 64                | 0.612                  | 0.397  |
| iPBS-2271   | 36                | 0.674                  | 0.311  | iPBS-2389   | 65                | 0.587                  | 0.396  |
| iPBS-2274   | 80                | 0.743                  | 0.342  | iPBS-2390   | 62                | 0.654                  | 0.431  |
| iPBS-2276   | 42                | 0.732                  | 0.329  | iPBS-2391   | 53                | 0.668                  | 0.341  |
| iPBS-2278   | 57                | 0.700                  | 0.338  | iPBS-2392   | 47                | 0.654                  | 0.379  |
| iPBS-2298   | 72                | 0.888                  | 0.151  | iPBS-2402   | 60                | 0.776                  | 0.292  |
| Mean        | 52                | 0.706                  | 0.331  |

* PIC: Polymorphism Information Content.

3.2. Genetic Diversity

The number of effective alleles (ne), genetic diversity of Nei (h) and Shannon’s information index (I) value of the bean varieties is presented in Table 4. The greatest ne (1.720), h (0.419), and I (0.609) values were observed in variety G36. The lowest ne (1.470), h (0.320), and I (0.500) values were observed in variety G27. The mean ne, h, and I value were calculated as 1.566, 0.361, and 0.546, respectively.

Table 4. Summary statistics for mean values for beans (P. vulgaris L.) varieties assessed with 26 iPBS primers.

| Variety | ne *  | h **  | I *  | Variety | ne *  | h **  | I *  |
|---------|-------|-------|-----|---------|-------|-------|-----|
| G1      | 1.491 | 0.329 | 0.511 | G24     | 1.530 | 0.347 | 0.531 |
| G2      | 1.538 | 0.350 | 0.534 | G25     | 1.586 | 0.369 | 0.556 |
| G3      | 1.540 | 0.351 | 0.535 | G26     | 1.550 | 0.355 | 0.540 |
| G4      | 1.601 | 0.376 | 0.563 | G27     | 1.470 | 0.320 | 0.500 |
| G5      | 1.521 | 0.343 | 0.526 | G28     | 1.658 | 0.397 | 0.586 |
| G6      | 1.568 | 0.362 | 0.548 | G29     | 1.696 | 0.410 | 0.601 |
| G7      | 1.609 | 0.379 | 0.566 | G30     | 1.642 | 0.391 | 0.580 |
| G8      | 1.604 | 0.377 | 0.564 | G31     | 1.688 | 0.408 | 0.598 |
| G9      | 1.593 | 0.372 | 0.560 | G32     | 1.588 | 0.370 | 0.557 |
| G10     | 1.591 | 0.372 | 0.559 | G33     | 1.586 | 0.369 | 0.556 |
| G11     | 1.576 | 0.365 | 0.552 | G34     | 1.524 | 0.344 | 0.528 |
| G12     | 1.589 | 0.371 | 0.558 | G35     | 1.476 | 0.322 | 0.503 |
| G13     | 1.549 | 0.354 | 0.539 | G36     | 1.720 | 0.419 | 0.609 |
| G14     | 1.568 | 0.362 | 0.548 | G37     | 1.648 | 0.393 | 0.582 |
| G15     | 1.562 | 0.360 | 0.546 | G38     | 1.520 | 0.342 | 0.526 |
3.3. Heterozygosity and Diversity of Varieties

The summary statistics for nine populations (na: Observed number of alleles, ne: effective number of alleles, I: shannon’s information index, He: expected heterozygosity, uHe: and unbiased expected heterozygosity are listed in Table 5. We determined that the He value ranged from 0.173 (Av) to 0.052 (Kt) (Mean 0.110), while the uHe value ranged from 0.104 (Kt) to 0.208 (Av) (Mean 0.149). The I value among the nine populations ranged from 0.072 (Kt) to 0.286 (Iov) (Mean 0.161). The Percentage of Polymorphic Loci (PPL) for bean was lowest at 10.38% and 13.21%. Among the nine populations of bean, the PPL value ranged from 10.38% (Mv) to 84.30% (Ic) (Mean 28.05%). The h values of the nine bean populations are presented in Table 6. Among the nine populations of bean from Ispir, the smallest h values observed were in Av/Uv (0.068), while the greatest were observed in Ic/Kv (0.232).

Table 5. Summary statistics for 45 bean (P. vulgaris L.) varieties assessed with 26 iPBS primers.

| Population | n   | na  | ne  | I   | He  | uHe | PPL (%) |
|------------|-----|-----|-----|-----|-----|-----|---------|
| Av         | 6   | 0.908 | 1.305 | 0.253 | 0.173 | 0.208 | 43.40   |
| Iov        | 14  | 1.098 | 1.270 | 0.254 | 0.165 | 0.178 | 24.72   |
| Ic         | 3   | 0.519 | 1.166 | 0.132 | 0.092 | 0.138 | 53.58   |
| Kv         | 2   | 0.389 | 1.132 | 0.092 | 0.066 | 0.132 | 20.75   |
| Kt         | 2   | 0.336 | 1.104 | 0.072 | 0.052 | 0.104 | 13.21   |
| Mv         | 4   | 0.613 | 1.182 | 0.158 | 0.107 | 0.143 | 10.38   |
| Uv         | 6   | 0.781 | 1.218 | 0.195 | 0.130 | 0.156 | 26.98   |
| Yy         | 3   | 0.560 | 1.190 | 0.151 | 0.106 | 0.158 | 35.66   |
| Com        | 5   | 0.574 | 1.165 | 0.142 | 0.096 | 0.120 | 23.77   |
| Mean       |     | 0.642 | 1.192 | 0.161 | 0.110 | 0.149 | 28.05   |

n: number of sample size, na: number of distinct alleles, ne: effective number of alleles, I: shannon’s information index, He: expected heterozygosity, uHe: unbiased expected heterozygosity, PPL: percentage of polymorphic loci; Av: Ağıldere village, Iov: İspir-Öztoprak village, Ic: İspir-center, Kv: Kirazlı village, Kt: Köprübaşı town, Mv: Maden village, Uv: Ulubel village, Yy: Yeşilyurt, Com: Commercial variety.

Table 6. Pairwise population matrix of Nei genetic distance for nine groups of bean (P. vulgaris L.) varieties.

|       | Av     | Com    | Iov    | Ic     | Kv     | Kt     | Mv     | Uv     | Yy     |
|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Av    | 0.000  |        |        |        |        |        |        |        |        |
| Com   | 0.125  | 0.000  |        |        |        |        |        |        |        |
| Iov   | 0.124  | 0.179  | 0.000  |        |        |        |        |        |        |
| Ic    | 0.137  | 0.215  | 0.081  | 0.000  |        |        |        |        |        |
| Kv    | 0.128  | 0.072  | 0.209  | 0.232  | 0.000  |        |        |        |        |
| Kt    | 0.129  | 0.071  | 0.207  | 0.222  | 0.071  | 0.000  |        |        |        |
| Mv    | 0.099  | 0.202  | 0.114  | 0.109  | 0.215  | 0.211  | 0.000  |        |        |
| Uv    | 0.068  | 0.085  | 0.177  | 0.202  | 0.081  | 0.108  | 0.184  | 0.000  |        |
| Yy    | 0.119  | 0.207  | 0.104  | 0.086  | 0.229  | 0.212  | 0.087  | 0.197  | 0.000  |

Av: Ağıldere village, Com: Commercial variety, Iov: İspir-Öztoprak village, Ic: İspir-center, Kv: Kirazlı village, Kt: Köprübaşı town, Mv: Maden village, Uv: Ulubel village, Yy: Yeşilyurt.
3.4. Principal Coordinate Analysis (PCoA) and Dendrogram Generated from 26 iPBS Markers

The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram placed the 45-bean variety into three clusters. There were only 18 (40%), 14 (31.11%) and 13 (28.88%) varieties in the first to three clusters, respectively (Figure 2). Cluster I contained 18 bean varieties including G36, G45, G44, G43, G42, G41, G40, G39, G38, G37, G35, G34, G33, G32, G30, G31, G29, and G28. Group II contained 14 bean varieties including G27, G26, G25, G24, G23, G22, G21, G20, G19, G18, G16, G15, G17 and G14. In addition, the third subcluster contained 13 bean varieties including G13, G12, G11, G10, G9, G8, G7, G4, G6, G5, G3, G2, and G1. Principal coordinate analysis (PCoA) spatially showed the relative h values between the varieties, revealing three distinct groups. All local varieties collected from Öztoprack Village of Ispir center and one local variety (G26) from Ağilere village are on the upper right, 2 varieties (G25, G27) from Ispir-Center, Maden village, Yeşilyurt and Ağıldere villages are on the lower left. The commercial varieties on the left of the axis and the varieties belonging to other locations are scattered in various parts of the diagram. The result showed the grouping pattern of the PCoA analysis corresponded with cluster analysis (Figure 3). The percentage of genetic diversity explained by each of the three main coordinates of the basic coordinate analysis was determined as 32.34, 6.35 and 5.23, respectively, and these first three components explained 43.92% of the diversity (Table 7). The group I contained G6, G8, G14, G7, G3, G9, G12, G13, G26, G1, G11, G4, G5, G2, G10, G15, G18, G27, G19, G16, G17, G21, G24, G20, G25, G22 and G23 where all of them consisted of Ispir-Öztoprak Village, Ağıldere Village, Ispir-Center and Maden Village. The varieties within this group showed higher variation and were scattered over a larger area. The group II comprised of G41 (commercial variety) and G34 (Ulubel village). The third group was composed of all other accessions including G37, G42, G36, G45, G38, G44, G43, G40, G31, G33, G39, G32, G29, G35, G28 and G30. The results showed that G45, G44, G42, G37 and G36 belong to groups II and III. AMOVA (Analysis of Molecular Variance) was used to detect the total variation and showed that the variation within populations was 67% and that between populations was 33% (Table 8).

Figure 2. Dendrogram of 45-bean varieties generated with data from 26 inter primer binding site (iPBS) primers.
Figure 2. Dendrogram of 45 bean varieties generated with data from 26 inter-primer binding site (iPBS) primers.

Figure 3. Principal coordinates analysis (PCoA) calculated from the pooled data of twenty-six inter-primer binding site (iPBS) primers in 45 bean varieties.

Table 7. PCoA analysis of bean varieties.

| Axis | 1     | 2    | 3     |
|------|-------|------|-------|
| %    | 32.34 | 6.35 | 5.23  |
| Cum %| 32.34 | 38.69| 43.92 |

Table 8. AMOVA of bean varieties, using inter primer binding site (iPBS) marker.

| Scheme           | Degree of Freedom (DF) | Sum of Squares (SS) | Variance Component | % Of Total Variance | p-Value |
|------------------|------------------------|---------------------|--------------------|---------------------|---------|
| Among Population | 8                      | 1150.70             | 21.439             | 33%                 | 0.332   |
| Within Population| 36                     | 1554.89             | 43.192             | 67%                 | 0.001   |
| Total            | 44                     | 2705.60             | 64.631             | 100%                |         |

3.5. Population Genetic Structure Analysis for iPBS Markers

To understand the population structure among the 45 bean varieties, we divided each entry into corresponding subgroups using the model-based approach in the STRUCTURE software. The ΔK value is used to calculate the optimum K value. The result of genetic structure analysis suggests that the greatest value of K was calculated as 5 (red [A], green [B], blue [C], yellow [D], pink [E]) (membership probability < 0.8) (Figure 4). At K = 5, group I included 1 variety containing G36 mixed with yellow and pink groups. Group II contained 7 varieties including G22, G23, G26, G25, G24, G20, G19. Group III included 12 varieties counting G8, G9, G11, G6, G3, G4, G7, G5, G10, G2 and G12. Group IV included 6 varieties counting G42, G41, G40, G38, and G43. Group V contains 4 varieties including G29, G30, G28 and G31. Furthermore, G21, G17, G16, G18, G27, G13, G14, G15, G1, G39, G44, G44, G37, G32, G33, G34 and G35 were placed in mixed groups (40.00%; membership probability < 0.8). The F-statistic (Fst) value was determined as 0.0002, 0.4371, 0.4061, 0.6372, and 0.5440 in the first to fifth subpopulations, respectively. Likewise, the expected heterozygosity values (He) were determined as 0.3210, 0.1858, 0.1947, 0.1567, and 0.1907 in the first to fifth subpopulations, respectively (Tables 9 and 10).
Table 9. Membership coefficients of five subpopulations of bean varieties.

| Varieties | Subpopulation | Subpopulation |
|-----------|---------------|---------------|
|           | I  | II | III | IV  | V  | I  | II | III | IV  | V  |
| G1        | 0.401 | 0.005 | 0.579 | 0.004 | 0.012 | G24 | 0.017 | 0.946 | 0.009 | 0.025 | 0.003 |
| G2        | 0.059 | 0.005 | 0.923 | 0.008 | 0.006 | G25 | 0.201 | 0.960 | 0.002 | 0.004 | 0.014 |
| G3        | 0.009 | 0.002 | 0.972 | 0.013 | 0.004 | G26 | 0.012 | 0.968 | 0.004 | 0.005 | 0.011 |
| G4        | 0.014 | 0.012 | 0.970 | 0.003 | 0.001 | G27 | 0.399 | 0.560 | 0.010 | 0.011 | 0.019 |
| G5        | 0.011 | 0.111 | 0.961 | 0.011 | 0.006 | G28 | 0.003 | 0.018 | 0.004 | 0.007 | 0.938 |
| G6        | 0.008 | 0.003 | 0.975 | 0.003 | 0.003 | G29 | 0.004 | 0.002 | 0.003 | 0.002 | 0.989 |
| G7        | 0.024 | 0.002 | 0.969 | 0.002 | 0.002 | G30 | 0.009 | 0.004 | 0.005 | 0.004 | 0.979 |
| G8        | 0.002 | 0.003 | 0.993 | 0.001 | 0.001 | G31 | 0.214 | 0.003 | 0.005 | 0.010 | 0.767 |
| G9        | 0.007 | 0.009 | 0.980 | 0.003 | 0.002 | G32 | 0.010 | 0.004 | 0.003 | 0.257 | 0.727 |
| G10       | 0.007 | 0.041 | 0.946 | 0.003 | 0.003 | G33 | 0.011 | 0.024 | 0.006 | 0.286 | 0.674 |
| G11       | 0.005 | 0.010 | 0.979 | 0.003 | 0.003 | G34 | 0.002 | 0.002 | 0.002 | 0.432 | 0.561 |
| G12       | 0.014 | 0.070 | 0.909 | 0.004 | 0.003 | G35 | 0.003 | 0.005 | 0.006 | 0.342 | 0.528 |
| G13       | 0.025 | 0.205 | 0.709 | 0.031 | 0.030 | G36 | 0.702 | 0.002 | 0.003 | 0.046 | 0.246 |
| G14       | 0.013 | 0.298 | 0.682 | 0.003 | 0.004 | G37 | 0.378 | 0.004 | 0.002 | 0.572 | 0.043 |
| G15       | 0.007 | 0.320 | 0.665 | 0.004 | 0.004 | G38 | 0.009 | 0.006 | 0.009 | 0.857 | 0.118 |
| G16       | 0.017 | 0.640 | 0.336 | 0.005 | 0.002 | G39 | 0.150 | 0.041 | 0.005 | 0.792 | 0.012 |
| G17       | 0.003 | 0.670 | 0.323 | 0.002 | 0.002 | G40 | 0.078 | 0.028 | 0.007 | 0.870 | 0.017 |
| G18       | 0.014 | 0.625 | 0.344 | 0.007 | 0.009 | G41 | 0.009 | 0.004 | 0.003 | 0.984 | 0.002 |
| G19       | 0.031 | 0.849 | 0.100 | 0.009 | 0.012 | G42 | 0.003 | 0.001 | 0.002 | 0.992 | 0.002 |
| G20       | 0.020 | 0.893 | 0.081 | 0.004 | 0.003 | G43 | 0.088 | 0.004 | 0.064 | 0.823 | 0.022 |
| G21       | 0.278 | 0.701 | 0.015 | 0.003 | 0.003 | G44 | 0.355 | 0.006 | 0.003 | 0.631 | 0.005 |
| G22       | 0.003 | 0.988 | 0.003 | 0.002 | 0.004 | G45 | 0.246 | 0.003 | 0.013 | 0.735 | 0.002 |
| G23       | 0.005 | 0.984 | 0.004 | 0.002 | 0.004 |
Table 10. Expected heterozygosity (He) and F_{ST} values in four squash subpopulations.

| Subpopulation (K) | Expected Heterozygosity (He) | F_{ST}  |
|-------------------|-------------------------------|---------|
| 1                 | 0.3210                        | 0.0002  |
| 2                 | 0.1858                        | 0.4371  |
| 3                 | 0.1947                        | 0.4061  |
| 4                 | 0.1567                        | 0.6372  |
| 5                 | 0.1907                        | 0.5440  |
| Mean              | 0.2103                        | 0.4049  |

4. Discussion

Determining the genetic diversity levels of the germplasm of a plant species is essential for the designing and structuring of plant breeding programs [39]. Molecular markers such as iPBS for determining the genetic diversity and associations of varieties and accessions play important roles in targeted parental selection independent of environmental influences. Along with a role of retrotransposons in the diversification of genetic material, retrotransposon activation is reported to be one of the key factors involved in host adaptation to environmental changes [40]. In our study, polymorphic iPBS markers enabled the identification of bean (P. vulgaris L.) species at the molecular level. This provided important information about the genetic associations between these varieties. The information produced by the iPBS marker system suggests that it can be used effectively for diversity studies and genetic analysis in bean varieties. Using this marker system, other researchers have successfully examined similar bean species in genetic diversity studies [6,8,24]. The genetic diversity observed in our study is higher than in similar studies performed on Turkish beans, using different molecular markers [7]. This result clearly indicates that iPBS retrotransposons are highly polymorphic markers. The PIC value is a crucial piece of information that scores the efficacy of polymorphic loci and indicates the discriminatory power of a primer [41]. In our study the PIC varied between 0.151 (iPBS 2298) and 0.495 (iPBS 2383) (Mean 0.331). In a similar study of beans in which iPBS markers were used, PIC values were reported between 0.19 and 0.42 (Mean 0.33) [26]. The results are different to those of [8], who found PIC values between 0.65 and 0.93 (Mean 0.80) in their study with iPBS retrotransposons in beans. The results of the researchers differed, probably due to the varieties being different, while other researchers used fewer markers.

The mean number of effective alleles (ne), genetic diversity of Nei (h) and Shannon’s information index (I) value of the bean varieties were calculated as 1.566, 0.361, and 0.546, respectively [42], in their study using iPBS markers in peas, reported I values between 0.24 and 0.58 (Mean 0.39). The mean PIC value (0.73) obtained in this study was higher than the studies performed on iPBS markers and guava (0.24) [43] and grape (0.44) [44]. According to the comparison, it can be said that the iPBS primers used in this study of beans are more suitable. The maximum number of effective alleles is always desirable as they indicate the presence of greater genetic variation. Moreover, Shannon’s index of knowledge is an important criterion for understanding variation, as it distinguishes genetic variation in a population combining abundance and uniformity. In a study to explore the genetic diversity and population structure of scarlet eggplant with iPBS markers, the average polymorphism information content was found to be 0.363. The mean effective number of alleles, mean Shannon’s information index and gene diversity values were reported as 1.298, 0.300 and 0.187, respectively [45]. The results differed, probably due to the plant species and the various locations studied. Knowledge of the genetic variation between populations of a plant species is crucial to breeding and conservation [46]. Population-specific traits within each bean strain or variety can also be used to optimize crossbreeding studies.

The population structure identified in this study was consistent with distance-based clustering from principal coordinate analysis (PCoA). In our study, we showed that intraspecific crosses, especially those between the Ic/Kv (0.232), Yy/Ky (0.229), and Kt/Ic (0.222) populations, may produce stronger hybrids, due to their greater genetic distance. We also
performed PCoA analysis to examine the genetic associations between bean varieties. In the first three axes, PCoA analysis explained 43.92% of the total variation. In PCoA analysis, cluster analysis data obtained from this matrix are generally considered reliable when the axes explain 25% or more of the total variation [47]. PCoA is a widely used method for assessing genetic diversity based on quantitative and qualitative traits that scales distance data to multidimensional planes to characterize diversity. However, the grouping based on population structure seems to be more accurate, as it could precisely differentiate the bean varieties. Molecular analysis of variance (AMOVA) revealed the presence of high variation within bean varieties, with the percentage of total variance being 67%. It has been stated that higher variations in varieties may be due to reasons such as selection, adaptation, gene flow, genetic drift, variation in ecotypes and pollination method [48].

The findings showed that the bean varieties were divided into five groups according to their genetic structures. Varieties accumulate several living mutations throughout the evolutionary process, which form the basis of genetic diversity. Moreover, recombination, random drift, natural selection, such forces shape the genetic makeup of populations. In the recent past, understanding population structure has become a feature of great interest, as it can be helpful in selecting various parents and mapping sign-trait relationships. As a tool, analysis of population structure can predict similarity levels between individuals, subpopulations, and contributions. When samples are plotted with different geographic origins, analysis of population structure shows the pattern of geographic distribution among populations [49]. In a similar study [9] reported that 67 bean varieties were divided into four subpopulations (K = 4). In a study by [50], SSR markers were used to determine the genetic diversity in 149 dry bean varieties, and the varieties were divided into three subpopulations (K = 3) according to genetic structure analysis. The markers used in the study are primarily effective in grouping the genotypes [17].

5. Conclusions

There are many tools for determining and revealing genetic diversity in plant breeding. However, in plant breeding programs, it is extremely important to know the genetic distance of the varieties that are not clearly defined are unknown in the germplasm. Although classical breeding studies have reached the desired rate in many plant species and varieties, molecular markers provide very important information in breeding programs in genotypes development studies. In addition, the determination of distance and proximity conditions between varieties by performing genetic analyses contributes to the creation of new populations and to obtaining high-yielding varieties with heterosis. Therefore, evaluation of the genetic diversity of local bean varieties is needed for the conservation and breeding of this genetic material. Molecular markers and genetic diversity studies provide the useful information that is so critically needed about population structure. More informative molecular markers, such as iPBS, are being increasingly used in the study of bean genetic diversity, and their power cannot be underestimated. In conclusion, we used the iPBS retrotransposon marker system to generate pre-breeding data that could potentially be applied to the identification of common bean (P. vulgaris L.) genetic re-sources, conservation, and selection of suitable parents to provide greater genetic diversity for use in breeding programs. We showed here that the iPBS marker system is a powerful and easy method for detecting variation among bean varieties. The current findings reveal the diversity in local bean varieties collected from Erzurum-Ispir and will provide a basis for subsequent bean breeding programs, as well as integrity in bean identification studies. According to the information obtained in the study, it was determined that the genetically most distant cultivars were the G1 and G36 local varieties. With future studies, it is thought that these varieties can be used in breeding and hybridization studies, taking into account their agro-morphological characteristics, their resistance to biotic and abiotic conditions.
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