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Genetic Diversity and Population Structure of Barley Cultivars Released in Turkey and Bulgaria using iPBS-retrotransposon and SCoT markers

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ABSTRACT
To improve quantitative traits, it is essential to acknowledge genetic structure and diversity of the crop plants. In this study, 54 barley cultivars released from 1963 to date by different institutes in both Turkey and Bulgaria were screened with 18 iPBS and four SCoT markers to evaluate population structure and genetic diversity. According to the results, while total polymorphic band numbers was identified as 560, the polymorphic ones were found as 530 (438 and 92 amplified bands for iPBS and SCoT markers, respectively). In addition, the average polymorphic band number was found as 24.09. While the average polymorphism information content (PIC) value was 0.48, the average PIC value was 0.48 for iPBS and 0.48 for SCoT markers. The highest PIC value was determined as 0.50. The highest effective number of alleles, Shannon's information index, and Nei's genetic diversity were detected from the iPBS2271 marker at 1.61, 0.52 and 0.35, respectively among the iPBS markers while the highest values were obtained from SCoT-71 marker as 1.55, 0.32 and 0.48, respectively. As a result of a distribution of the 530 amplified bands in 54 barley cultivars, structure analysis showed that the subpopulations in the barley cultivars as a value of k=5. The average expected heterozygosity and fixation indices were identified as 0.234 and 0.322, respectively. Based on DICE similarity index, Martı and Zahir cultivars were found the most similar barley cultivars with 75% genetic similarity, whereas Özdemir and Karatay 94 and Tosunpaşa and Konevi cultivars were found 73% similar. On the other hand, Bayrak and Avcı-2002 were found the most diverse cultivars with 19.9% genetic similarity. As a result, the barley cultivars released in Turkey and Bulgaria were found varying and, the genetic diversity and statistics index analysis indicated that iPBS and SCoT markers are powerful markers to perform genetic diversity analysis.

Keywords: Barley, Genetic diversity, iPBS-retrotransposons, SCoT marker, Structure analysis

1. Introduction

Plant genetic diversity (PGD) is the major component for plant's life and crop improvement. In addition, genetic diversity becomes more crucial in response to climatic change and different biotic and abiotic stresses in nature. Intra and inter-specific differences in plant genetic resources (PGR) are the basic requirement for all crop improvement programs as well as providing preferred traits for farmers. Genetic diversity is affected primarily by sexual recombination as well as evolutionary forces such as genetic drift, mutation, migration etc. and domestication or artificial selection (Bhandari et al. 2017). Barley (*Hordeum vulgare*), derived from its wild progenitor *Hordeum vulgare* ssp. *spontaneum*, is vital crop for the malting and brewing industries (about 20% of global production) and it constitutes as an important animal feed (about 75% of global production). It is also using as a staple food in different parts of the world (5%) owing to adaptation to drought, soil salinity, and high altitudes (Baik & Ullrich 2008; Blake et al. 2011). Barley is a diploid crop (2n=14) with a genome size of 5.1 gigabases (Gb) and its genome contains 26.159 'high-confidence' genes (The International Barley Genome Sequencing Consortium, 2012).

Molecular (DNA) markers can be used for analyses of molecular variations that resulted by deletion, duplication, inversion, and/or insertion in the genomes (Govindaraj et al. 2015). Start Codon Targeted polymorphism (SCoT) is a dominant marker system and has been effectively employed to detect genetic variations. Single 18-mer primers are used to anneal short conserved region neighboring the ATG translation initiation (or start) codon. This method provides high polymorphism and reproducible markers related with characters of biological interest (Collard & Mackill 2009). SCoT marker system has been

successfully applied in various crops such as Tunisian citrus species (Mahjbi et al. 2015), landrace chickpea (Pakseresht et al. 2013), durum wheat (Etminan et al. 2016), cultivars of Egyptian wheat (Abdel-Lateif & Hewedy 2018), and 20 barley genotypes (Dora et al. 2017). Retrotransposons are repetitive and mobile sequences and observed in virtually all known eukaryotic genomes (Flavell et al. 1992). Particularly, retrotransposons are abundant in plant genomes and play important roles in genome evolution. In many cases, retrotransposons in plants cover more than 50% of the genome (Kumar & Bennetzen 1999). Retrotransposons are classified into two groups like long terminal repeat (LTR) and non-LTR retrotransposons. The inter-primer binding site (iPBS) method is a PCR-based marker system as a reverse transcriptase primer-binding site (PBS) based on complement of tRNA in LTR retrotransposons (Kalendar et al. 2010).

Shannon's diversity index (I), and Nei's genetic diversity (h) were used to evaluate the information of the markers including iPBS and SCoT markers by the researches. Diversity of the statistics index values of the markers with a value of zero or below zero than couldn't be used in the analysis, because of lower level of knowledge for genetic diversity. The highest value of the diversity index tends to a greater level of polymorphism of the primers and, thus, assisted to select the best marker loci in the genetic separation analysis (Tahir et al. 2019).

The iPBS markers have been used in numerous crops including Turkish okra (Yıldız et al. 2015), common bean (Nemli et al. 2015), pea, lens (Baloch et al. 2015a & 2015b, respectively) and tobacco (Yaldız et al. 2018). Thus, in the present study it was aimed to evaluate the genetic diversity of 37 Turkish and 17 Bulgarian barley genotypes using SCoT and iPBS-retrotransposon markers for the first time.

2. Material and Methods

2.1. Plant material

The plant materials used in the study consisted of 54 barley cultivars developed by Aegean Agricultural Research Institute Turkey (AARI), Bahri Dağdaş International Agricultural Research Institute Turkey (BDIARI), Field Crops Central Research Institute Turkey (FCCRI), Trakya Agricultural Research Institute Turkey (TARI), Transition Zone Agricultural Research Institute Turkey (TZARI) and Institute of Agriculture Karnobat, Bulgaria (IAK). Cultivar name, origin, developing institute, spike type, release date and pedigree of the cultivars are given in Table 1.

2.2. DNA isolation

Genomic DNA of the barley cultivars was isolated from single seeds. Three seeds from each cultivar were planted in a 10.5 cm pot and at two leaves stage one seedling per pot left. The leaves were collected from the seedling and fixed in 2 ml micro centrifuge tubes for DNA extraction. Genomic DNA of the cultivars was extracted using a cetyl trimethyl ammonium bromide method, indicated by Oliver et al. (2010).

Table 1- Pedigree, origin and spike type of the barley genotypes used in the study

No	*Cultivar*	*Origin*	*Institute*	*Spike Type*	*Release Date*	*Pedigree*
1	Bozhin	Bulgaria	IAK	Six-Rowed	1994	H280 - 7/ NaN_3 - mutant
2	Zemela	Bulgaria	IAK	Six-Rowed	2016	F2 2012/01 x Кт 2152 200 Gy - mutant
3	Aheloy 2	Bulgaria	IAK	Six-Rowed	1994	Hemus x №102/121
4	Zagoretz	Bulgaria	IAK	Two-Rowed	2008	4943 - 41 x 1023K-7
5	Alekssan	Bulgaria	IAK	Six-Rowed	2017	K-2169-01 x Кт 2145
6	Odisey	Bulgaria	IAK	Two-Rowed	2011	Obzor x Nutans 85242/64/ x Nutans 85242/64 (5)
7	Bul Perun	Bulgaria	IAK	Two-Rowed	1996	Alpha x Jet
8	Sladoran	Turkey	TARI	Two-Rowed	1998	Inrtoduced from Yugoslavia
9	Kıral-97	Turkey	BDIARI	Six-Rowed	1997	ADAIR/SL//WA1094-67
10	Erginel 90	Turkey	TZARI	Six-Rowed	1990	Escourgeon Hop 2171 (Fransa)
11	Tarm-92	Turkey	FCCRI	Two-Rowed	1992	Tokak / local population no 4875
12	Çetin 2000	Turkey	FCCRI	Six-Rowed	2000	Star (Iran) /line 4875
13	Tokak 157/37	Turkey	FCCRI	Two-Rowed	1963	Selection from Landraces
14	Avcı-2002	Turkey	FCCRI	Six-Rowed	2002	Sci/3Gi-72AB58, F1//WA1245141
15	Burakbey	Turkey	FCCRI	Two-Rowed	2013	Coss/OWB 71080-44-1H//Obruk 86
16	Ayrancı	Turkey	BDIARI	Two-Rowed	2018	Osk4.197/12-84//HB854/Astrix/3/Rod/4/Slad/3/Vict//Yrm/Lhfm
17	Akhisar 98	Turkey	AARI	Six-Rowed	1998	GEM*4/PİAST SEA-2636-4S-3S-2S-1S-0S
18	Aydanhanım	Turkey	FCCRI	Two-Rowed	2002	GK Omega / Tarm 92
19	Çıldır 02	Turkey	TZARI	Two-Rowed	2002	3896/28//284/28/3/Cum-50/4/624/682/5/WBQT12
20	Hazar	Turkey	TARI	Six-Rowed	2016	Osk4.39/2-84//Barbe-Rousse
21	Emon	Bulgaria	IAK	Two-Rowed	1998	137HS-21/M-21-H/3/Malta/M-20-H/M-21-H/4/111G-65
22	Sancak	Turkey	AARI	Six-Rowed	2014	1861112/ROBUR/7/ HLLA/EH 21B/6/MAN/HUIZ//M69.69/3/APAM/RL//H 272/4/CP/BRA/5/JOSO
23	Egebeyi	Turkey	AARI	Six-Rowed	2019	CEN-B/2*CA-I92//VIRINGA/3/ATACO/4/ Harma-02//11012-2/Cm67/3/Market semple Marageh /5/ ROHADES//TB//CHZO/3/GL/COPAL/3/BAR/RHODES//GL/COME
24	Martı	Turkey	TARI	Six-Rowed	2009	Flam/WM/5/Yky387/3/Api/Cm67//Manc/4/Yrm/Lhfm
25	Zahir	Bulgaria	IAK	Two-Rowed	2016	K10 x Кт 1206
26	Hasat	Turkey	TARI	Two-Rowed	2014	Rod/Scala
27	Asparuh	Bulgaria	IAK	Two-Rowed	2009	2119У-75 x Korten
28	Deviniya	Bulgaria	IAK	Two-Rowed	2011	Tamara x Aster
29	Vesletc	Bulgaria	IAK	Six-Rowed	1994	№102/121 x Karnobat
30	Kuber	Bulgaria	IAK	Two-Rowed	2009	2119У-41 x 2119У-165
31	Dariya	Bulgaria	IAK	Two-Rowed	2016	CRT 059 x Lambic
32	Orfej	Bulgaria	IAK	Two-Rowed	2007	Kjfi x Nutans 8486/40
33	IZ Bori	Bulgaria	IAK	Six-Rowed	2010	K 280-7 NaN3 - mutant
34	Konevi	Turkey	BDIARI	Two-Rowed	1998	CO55/OWB 710-80 (WBCB)
35	Tosunpaşa	Turkey	FCCRI	Two-Rowed	2016	Atlas/Zarjou
36	Zeynel Ağa	Turkey	FCCRI	Two-Rowed	2003	Antares/Ky63-1294//Lignee131
37	Yesevi 93	Turkey	FCCRI	Two-Rowed	1993	Tokak / local population 4857
38	Bülbül 89	Turkey	FCCRI	Two-Rowed	1989	13GTH / local population
39	IZ Sayra	Bulgaria	IAK	Two-Rowed	2010	Alfa x Nutans 85242/76 / x Yubileĭ 100
40	İnce-04	Turkey	TZARI	Two-Rowed	2004	4671/Tokak//4648/p12-119/3/WBCB-4
41	Bilgi-91	Turkey	TZARI	Two-Rowed	1991	Selection
42	Vamikhoca 98	Turkey	AARI	Six-Rowed	1998	GEM*3/3/CR 115/POR//BLANCO MA
43	Hilal	Turkey	AARI	Two-Rowed	2010	Melusine/Aleli/3/Matico/Jet//Shyri/4/Canela/5/Arupo/K8755//Mora/3/Canela CBSS 96M00698D-P-5M-1Y-1M-0Y
44	Kalaycı-97	Turkey	TZARI	Two-Rowed	1997	Erginel 90//364 TH / Tokak
45	Orza 96	Turkey	FCCRI	Two-Rowed	1996	Tokak 157-37/4857
46	Bolayır	Turkey	TARI	Two-Rowed	2007	Osk 4.197/12-84//HB854/Astrix/3/Alpha/Durra
47	Akar	Turkey	FCCRI	Two-Rowed	2012	Alpha/Durra//Antares/KY-63-1294/3/Tarm 92
48	Özdemir	Turkey	TZARI	Two-Rowed	2005	CUM/4060//P12-62/P169-2
49	Karatay 94	Turkey	BDIARI	Two-Rowed	1996	VONTAGE/GÜZAK//TAPLANİ/3/REKAL/CUM50/RIGIC
50	İmbat	Turkey	AARI	Six-Rowed	2020	80.5064//BOLDO/MJA/3/GEM
51	Larende	Turkey	BDIARI	Two-Rowed	2006	ALM(4652)/TOKAK//342TH/P12-119/3/W.BELT22
52	Ünver	Turkey	TZARI	Two-Rowed	2013	YEA389-3/YEA475-4//97-98DH8
53	Cumhuriyet 50	Turkey	TZARI	Two-Rowed	1973	No:28 (Kayseri) / Mansholt's-2 Rijige (Holland)
54	Bayrak	Turkey	AARI	Six-Rowed	2014	ARRAYAN/OLMO//LEO-B/3/Lignee527/Aths//Aths/Lignee686

AARI: Aegean Agricultural Research Institute Turkey; BDIARI: Bahri Dağdaş International Agricultural Research Institute Turkey; FCCRI: Field Crops Central Research Institute Turkey; TARI: Trakya Agricultural Research Institute Turkey; TZARI: Transition Zone Agricultural Research Institute Turkey; IAK: Institute of Agriculture Karnobat, Bulgaria

2.3. iPBS and SCoT genotyping

A set of iPBS (18) markers reported by Kalendar et al. (2010) and SCoT (4) markers by Collard et al. (2009) and Luo et al. (2010) were used for diversity analysis in this study.

The information about the markers is shown in Table 2.

Table 2- DNA primers used in molecular characterization of the barley genotypes

Primer name	*Primer sequence (5'–3')*	*Tm (°C)*	*G/C ratio*
iPBS 2075	CTCATGATGCCA	50	50
iPBS 2083	CTTCTAGCGCCA	50	58.3
iPBS 2095	GCTCGGATACCA	44.8	58.3
iPBS 2219	GAACTTATGCCGATACCA	50	44.4
iPBS 2222	ACTTGGATGCCGATACCA	55	55.6
iPBS 2230	TCTAGGCGTCTGATACCA	50	50
iPBS 2244	GGAAGGCTCTGATTACCA	53.3	50
iPBS 2255	GCGTGTGCTCTCATACCA	57.1	50
iPBS 2271	GGCTCGGATGCCA	57.4	69.2
iPBS 2276	ACCTCTGATACCA	50	46.2
iPBS 2375	TCGCATCAACCA	45.1	50
iPBS 2378	GGTCCTCATCCA	44.2	58.3
iPBS 2387	GCGCAATACCCA	50	58.3
iPBS 2388	TTGGAAGACCCA	43.4	50
iPBS 2391	ATCTGTCAGCCA	48	50
iPBS 2394	GAGCCTAGGCCA	51.3	66.7
iPBS 2400	CCCCTCCTTCTAGCGCCA	57.4	66.7
iPBS 2415	CATCGTAGGTGGGCGCCA	50	66.7
SCoT-18	ACCATGGCTACCACCGCC	50	67
SCoT-39	CAATGGCTACCACTAGCG	50	56
SCoT-71	CCATGGCTACCACCGCCG	50	72
SCoT-74	CCATGGCTACCACCGGCA	50	67

Polymorphism information content (PIC) values were calculated for each iPBS and SCoT markers using the formula described by Weir (1996) using a web based (https://www.gene-calc.pl/pic) software. PIC=1-$\sum P_i^2$, where P_i is the frequency of the i^{th} allele in the 54 barley cultivars in the research.

Polymerase chain reactions (PCR) for the iPBS and SCoT markers were completed in a total volume of 20 µL, including iPBS and SCoT markers 10 µL primers (1 µM forward and reverse), 5 µL (150 ng) of genomic DNA, 5 µL of master mix (0.1 µL MgCl, 0.2 µL Taq polymerase enzyme, 2 µL reaction buffer, 1.2 µL dNTP mix (A+T+G+C) and 1.5 µL ddH_2O).

The reactions were conducted in Sensoquest Thermocycler (Labcycler) with a first denaturing (94 °C, 5 min.), then 40 cycles of denaturing (94 °C, 1 min), annealing (44.2-57.4 °C, 1 min, gradient) and extension (72 °C, 1 min) afterwards a final extension (72 °C, 10 min). Products obtained from the PCR were fragmented by 3% agarose gel [100 ml 1xTBE {1 Lt H_2O + 10.8 g Tris + 5.5 Boric acid + 0.5 M 4 mL EDTA (pH: 8)} with 3 g agarose] electrophoresis in 1X TBE buffer after adding 5 µL loading dye to 10 µL PCR product and running for approximately 2.5 hours at 120V to 130V. The gels were stained with 1 µl/mL ethidium bromide. Gel images were captured using a Bio-Rad ChemiDoc (California, USA) gel documentation system and fragment sizes were determined by comparison with a 1 kb DNA ladder (Thermo Scientific Gene Ruler).

2.4. Data analysis

The presence or absence of iPBS and SCoT marker amplicons were scored as "1" or "0" to produce binary matrix data. The genetic similarity of the barley genotypes was calculated by Dice index (Dice 1945). A dendrogram was created based on an unweighted pair-group mean average (UPGMA) tree using NTSYSpc (Rohlf 1998) and effective allele numbers (ne), Nei's

genetic diversity (Kimura & Crow 1964) and Shannon's information index (Lewontin 1972) were calculated using POPGEN32 software (v3.2 Microsoft Windows-Based Freeware for Population Genetics Analysis) (Yeh et al. 2000).

A Bayesian model-based clustering algorithm named population structure was completed using STRUCTURE ver. 2.3.4 with almost default parameters to figure out the population structure of the barley cultivars. The admixture model (the ancestry and allele frequency model) which provides allele frequency correlations against a set of K genetics and shared allele frequencies were chosen to detect the populations numbers (K) with the range of 1 to 10, which are measured best in cases of complex population structure and the analysis repeated six times (Falush et al. 2003; Montilla-Bascon et al. 2013). The admixture alpha degree set to 1000 with 100000 Markov chain Monte Carlo (MCMC) and ten neutral simulations per K value were performed as indicated by Montilla-Bascon et al. (2013) and Earl & vonHoldt (2012). The ΔK method was implemented by STRUCTURE HARVESTER v0.6.94 web based software was used to determine K value that best fit the data (Evanno et al. 2005).

3. Results and Discussion

Molecular markers were used to determine genetic diversity and population structure for several plant species (Pasam et al. 2014; Dumlupinar et al. 2016; Güngör 2019; Hossein-Pour et al. 2019; Yıldız et al. 2020). Kalendar et al. (2010) indicated that retrotransposon-based molecular markers may be efficiently used to locate developmental issues, at the intragenus or intraspecific scale, as for their addition into the genome results polymorphic DNA regions. Among the retrotransposan markers, iPBS markers are used universally due to ease of usage, high reproducibility and relatively inexpensive compared to other technologies and they do not require prior sequence knowledge (Yıldız et al. 2020). Among the PCR-based gene target technologies, SCoT markers reported as low in cost and effective to use besides, highly reproducibility and high polymorphic bands per reaction (Luo et al. 2010). Barley cultivars released from 1963 to date by different institutes in both Turkey and Bulgaria were evaluated for their genetic diversity and structural backgrounds. Total and polymorphic band numbers was identified as 560 and 530, respectively. Also, the average polymorphic band number was found as 24.09. The polymorphism ratios of the iPBS and SCoT markers was identified as 93.9%and 97.8%, respectively. In addition, the highest ne, h and I values were obtained from iPBS2271 marker (Table 3).

Table 3- PIC values and total polymorphic band numbers of DNA markers

No	*Primer Name*	*Amplified Band Number*	*Polymorphic Band Number*	*Polymorphism Rate (%)*	*PIC Value*	*Effective number of alleles (ne)*	*Nei's genetic diversity (h)*	*Shannon's information index (I)*
1	iPBS 2075	24	23	95.83	0.49	1.51	0.29	0.44
2	iPBS 2083	23	23	100	0.49	1.48	0.31	0.48
3	iPBS 2095	15	15	100	0.40	1.51	0.29	0.44
4	iPBS 2219	20	18	90	0.47	1.46	0.28	0.43
5	iPBS 2222	29	27	93.1	0.50	1.42	0.26	0.40
6	iPBS 2230	23	18	78.26	0.49	0.95	0.12	0.21
7	iPBS 2244	27	27	100	0.50	1.37	0.25	0.41
8	iPBS 2255	26	26	100	0.50	1.48	0.28	0.43
9	iPBS 2271	28	28	100	0.50	1.61	0.35	0.52
10	iPBS 2276	33	33	100	0.48	1.57	0.32	0.48
11	iPBS 2375	22	20	90.9	0.48	1.34	0.22	0.35
12	iPBS 2378	26	20	76.92	0.50	1.33	0.20	0.31
13	iPBS 2387	26	26	100	0.50	1.54	0.31	0.48
14	iPBS 2388	22	21	95.45	0.48	1.27	0.22	0.36
15	iPBS 2391	22	20	90.9	0.48	1.29	0.21	0.34
16	iPBS 2394	25	23	92	0.50	1.45	0.29	0.44
17	iPBS 2400	39	34	87.17	0.40	1.38	0.26	0.40
18	iPBS 2415	36	36	100	0.44	1.39	0.25	0.39
19	SCoT-18	20	20	100	0.47	1.50	0.30	0.46
20	SCoT-39	30	30	100	0.49	1.54	0.31	0.47
21	SCoT-71	24	22	91.66	0.49	1.55	0.32	0.48
22	SCoT-74	20	20	100	0.47	1.53	0.31	0.47
Average		25.45	24.09	94.65	0.48	1.43	0.27	0.42
Total		560	530	-	-			

Five hundred thirty polymorphic bands obtained from by iPBS (438 bands) and SCoT (92 bands) markers were used to create a dendrogram (Figure 1) and also used to calculate effective allele numbers (ne), Nei's genetic diversity and Shannon's information index for DNA markers (Table 3) and cultivars used in the study (Table 4). In current study, SCoT and iPBS markers confirmed useful in determination genetic diversity and population structure of barley cultivars. A ratio of 94.6% polymorphism was obtained from both SCoT and iPBS markers and the polymorphic band number was found as 24.09. In a recent study, Yildiz et al. (2020) reported 92% of polymorphic bands and 8.6 bands per iPBS marker reaction as 20 iPBS markers used and 158 polymorphic bands were generated and Hossein-Pour et al. (2019) stated 3.16 average band number per primer in iPBS markers. On the other hand, Luo et al. (2010) indicated 8.27 bands per SCoT primer and 76.19% polymorphism rate with 208 total polymorphic bands and Khodayari et al. (2012) reported 8.1 allele per locus in an Iranian barley landrace panel. Pasam et al. (2014) indicated a 5.74 average allele number in SSR markers in a spring barley set, while Elakhdar et al. (2018) indicated four allele number/locus in barley for SSR and SNP markers.

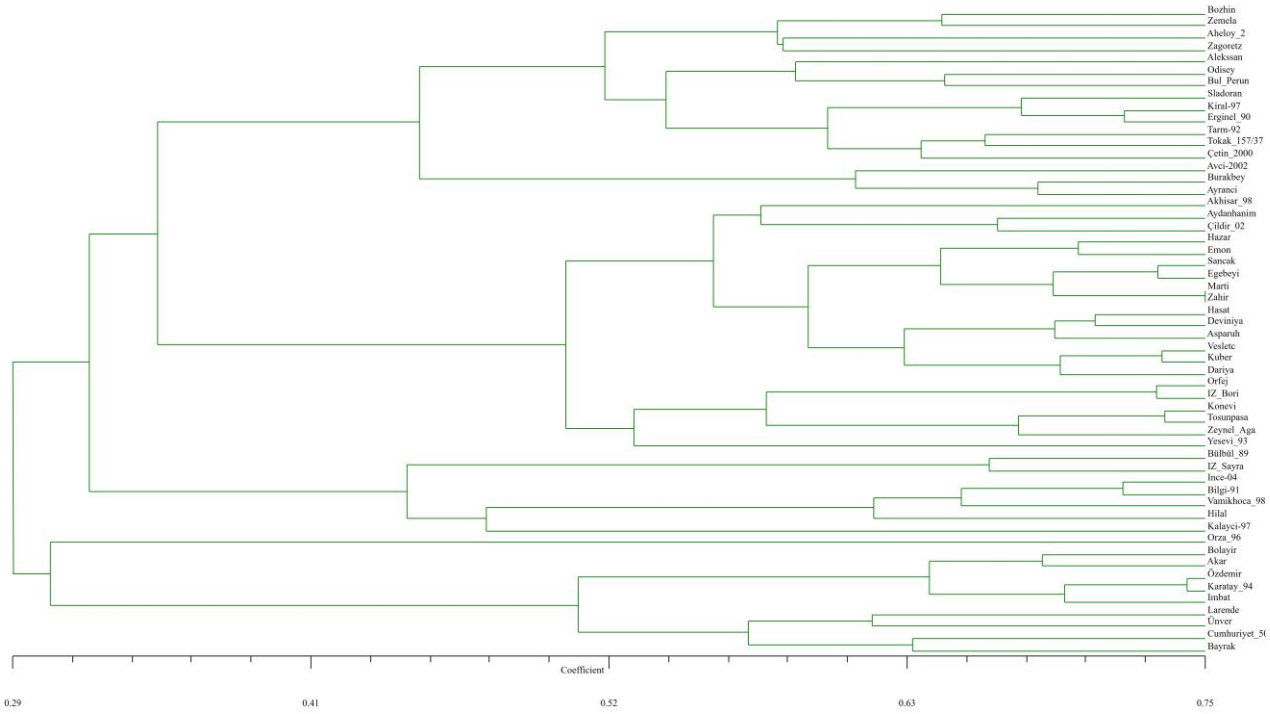

Figure 1- Dendrogram of 54 barley genotypes based on data of iPBS and SCoT markers according to UPGMA method with the Dice similarity index

Table 4- Summary statistics for 54 Barley genotypes assessed with DNA primers used in molecular characterization

No	*Genotypes*	*Effective number of alleles (ne*)*	*Nei's genetic diversity (h*)*	*Shannon's information index (I*)*
1	Bozhin	1.50	0.33	0.52
2	Zemela	1.55	0.35	0.54
3	Aheloy 2	1.43	0.30	0.47
4	Zagoretz	1.57	0.36	0.55
5	Alekssan	1.50	0.33	0.51
6	Odisey	1.44	0.30	0.48
7	Bul Perun	1.55	0.35	0.54
8	Sladoran	1.63	0.38	0.57
9	Kıral-97	1.65	0.39	0.58
10	Erginel 90	1.65	0.39	0.58
11	Tarm-92	1.59	0.37	0.55
12	Çetin 2000	1.57	0.36	0.55
13	Tokak 157/3	1.62	0.38	0.57
14	Avcı-2002	1.62	0.38	0.57
15	Burakbey	1.57	0.36	0.55
16	Ayrancı	1.59	0.37	0.55
17	Akhisar 98	1.59	0.37	0.55
18	Aydanhanım	1.53	0.34	0.53
19	Çıldır 02	1.56	0.36	0.54
20	Hazar	1.59	0.37	0.55
21	Emon	1.61	0.38	0.56
22	Sancak	1.67	0.40	0.59
23	Egebeyi	1.55	0.35	0.54
24	Martı	1.61	0.38	0.56
25	Zahir	1.55	0.35	0.54
26	Hasat	1.62	0.38	0.57
27	Asparuh	1.57	0.36	0.55
28	Deviniya	1.61	0.38	0.57
29	Vesletc	1.55	0.35	0.54
30	Kuber	1.61	0.38	0.56
31	Dariya	1.49	0.33	0.51
32	Orfej	1.56	0.36	0.54
33	IZ Bori	1.57	0.36	0.55
34	Konevi	1.56	0.36	0.54
35	Tosunpaşa	1.55	0.35	0.54
36	Zeynel Ağa	1.53	0.34	0.53
37	Yesevi 93	1.50	0.33	0.51
38	Bülbül 89	1.46	0.31	0.49
39	IZ Sayra	1.48	0.32	0.50
40	İnce-04	1.48	0.32	0.50
41	Bilgi-91	1.52	0.34	0.52
42	Vamikhoca 9	1.42	0.30	0.47
43	Hilal	1.48	0.32	0.50
44	Kalaycı-97	1.49	0.33	0.51
45	Orza 96	1.64	0.39	0.58
46	Bolayır	1.60	0.37	0.56
47	Akar	1.56	0.36	0.54
48	Özdemir	1.58	0.36	0.55
49	Karatay 94	1.52	0.34	0.52
50	İmbat	1.46	0.31	0.49
51	Larende	1.50	0.33	0.52
52	Ünver	1.49	0.33	0.51
53	Cumhuriyet 5	1.40	0.28	0.46
54	Bayrak	1.34	0.25	0.42
Average		1.54	0.35	0.53

PIC values were also calculated for each DNA markers and shown in Table 3. The average PIC value was 0.48 and the highest PIC value was 0.50, while the lowest one was 0.40. The highest ne, h, and I were obtained from the iPBS2271 marker at 1.61, 0.35 and 0.52, respectively, in contrast the lowest ones were found in the iPBS2230 marker at 0.95, 0.12 and 0.21, respectively. In addition, the total average ne, h, and I values were found as 1.43, 0.27 and 0.42, respectively (Table 3). A brief statistical results for each of the 54 barley genotypes was presented in Table 4. The highest ne, h, and I were obtained from Sancak cultivar at 1.67, 0.40 and 0.59, respectively, though the lowest values were observed in the Bayrak cultivar at 1.34, 0.25 and 0.42, respectively. In addition, the total average ne, h, and I were identified as 1.54, 0.35 and 0.53, respectively. Polymorphism information content is a measure of the primers used in a set of genotypes. In current study, the average PIC

value was 0.48 and PIC values of the iPBS markers ranked from 0.40 to 0.50 obtained from iPBS markers, while 0.46 to 0.49 for the SCoT markers. In earlier studies, Moragues et al. (2007) indicated an average PIC value 0.24 for AFLP and 0.70 for SSR markers in a durum wheat panel. Khodayari et al. (2012) determined a PIC value of 0.65 for SSR markers in barley landraces. Pasam et al. (2014) stated a 0.54 average PIC value for SSR markers in spring barley. Elakhdar et al. (2018) also reported a PIC value of 0.49 in barley genotypes. Hossein-Pour et al. (2019) reported an average PIC value with 0.20. Güngör (2019) reported an average PIC value of 0.72 on durum wheat cultivars. In addition, Kiraz et al. (2019) determined a PIC value of 0.79 in bread wheat mutant lines. Aydemir et al. (2020) calculated an average PIC value as 0.98 in a durum wheat population for DNA markers.

Based on a distribution of the 530 bands in 54 barley cultivars, STRUCTURE analysis was conducted with K=10. The subpopulations in the barley cultivars supported a value of k=5 (Figure 2) and the amount of admixture of each cultivar in the related subpopulation detected five barley subpopulations by STRUCTURE analysis is shown in Figure 3. According to STRUCTURE data, sub-population A, B, C, D and E indicated an admixture with 16.6%, 38.8%, 24%, 12.9% and 7.7% of the genotypes, respectively (Figure 3). Despite the maximum ΔK value was conducted at K=10, clusters at K=5 were best identified in terms of genotypic data. The sub-populations of the barley cultivars were grouped regardless to country, spike type and pedigree. In earlier works in different barley accessions the genetic diversity and population structure described two (Elakhdar et al. 2016), three (Elakhdar et al. 2018), five (Munoz- Amatriain et al. 2014), seven (Pandey et al. 2006), eight (Zhang et al. 2014) and 10 (Pasam et al. 2014) subpopulations.

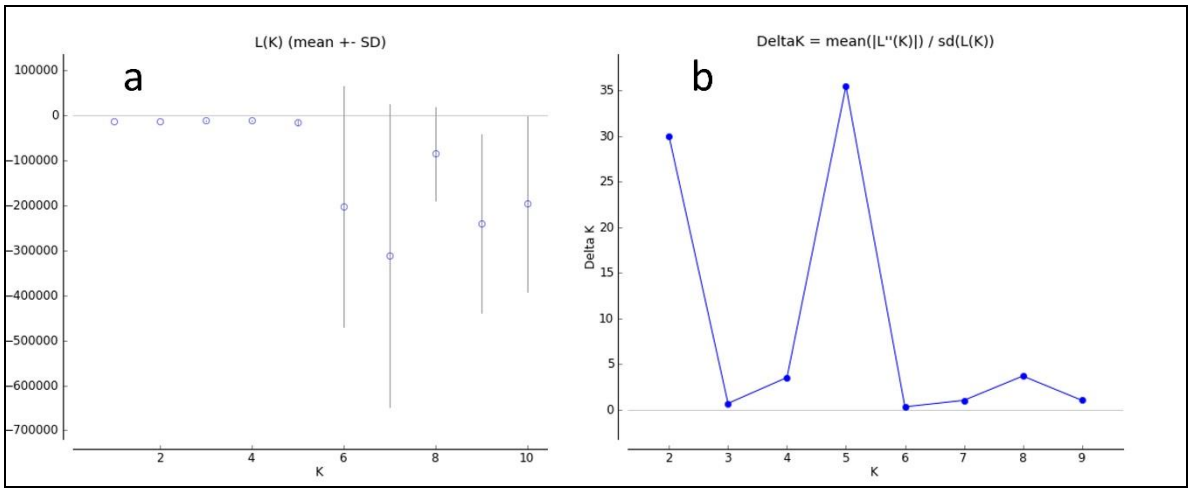

Figure 2- Admixture model of structure of Ln P (D) and ΔK for *Barley* subpopulations. a; Mean value of the statistic Ln P (D) b; DK

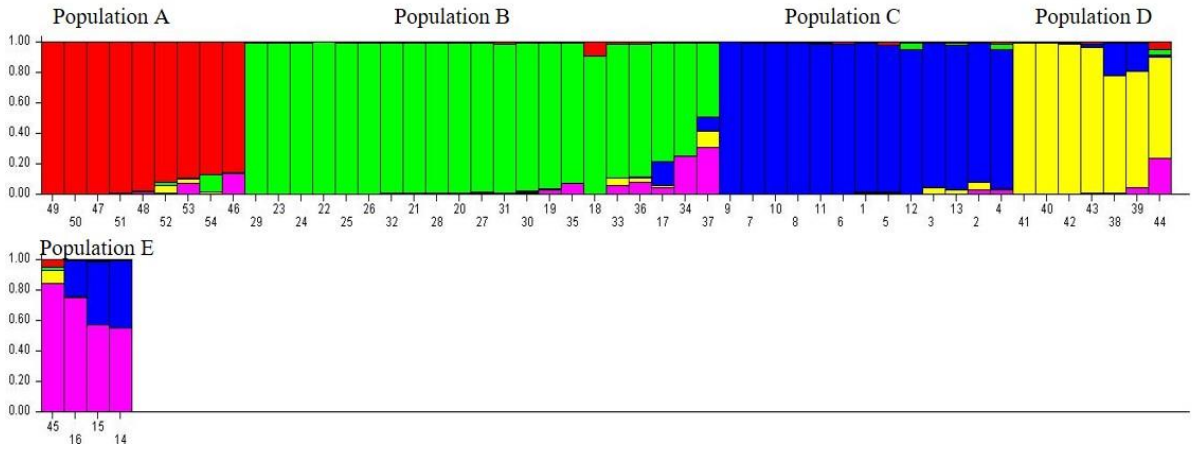

Figure 3- Population structure analysis of barley genotypes

To acknowledge about the genetic population structure of cultivars derived from different pedigrees. A Bayesian clustering modelling conducted in STRUCTURE software using 560 DNA markers. The number of groups (K) was arranged against ΔK to examine the most appropriate value of K. The highest ΔK value was detected at K = 5 (ΔK = 35.4787), including five sub-populations. At this K, most of the genotypes were linked to population B (Figure 1).

Expected heterozygosity (He) and fixation indices (Fst) values of sub-populations are shown in Table 5 and genetic differentiation measurement based on Fst values among sub-populations are indicated in Table 6. The barley sub-populations varied for Fst, determined to observe the relation within alleles drawn at varying scales of a hierarchically sub-divided population, however it was primarily modeled to measure the quantity of allelic fixation due to genetic alteration. The average He and Fst were found as 0.2335 and 0.3216, respectively (Table 5). The highest He was calculated from sub-population E with 0.3435, while the highest Fst value was obtained from sub-population A with 0.4697. Genetic differentiation based on Fst values among five barley sub-populations, sub-population A and B was found the most diverse populations with a value of 0.1225 (Table 6). The highest ne shows the existence of a high genetic variation suggesting the alleles capable to proceed in next-generation (Kimura 1965; Romero et al. 2019). It is concluded that ne is an important measure to assess markers with a high addition to variations in germplasms. In current study, the average ne was found 1.42 which was higher than a recent study reported as 1.26 by Barut et al. (2020) similar with a study for iPBS markers (Hossein-Pour et al. 2019). Shannon's information index is an important model to evaluate the diversity in a given set as it distinguishes the genetic variation in a germplasm combining plenty and equality (Yıldız et al. 2020). Shannon's information index and Nei's genetic diversity brought out the presence of satisfactory amount of genetic diversity in current germplasm. The average Shannon's information index was 0.42, which was consistent with previous works using different molecular markers (Barut et al. 2020). The average gene diversity was found 0.27 using SCoT and iPBS marker technologies in consistent with Hossein-Pour et al. (2019). In the study the average ne, h, and I of the cultivars were also calculated to evaluate cultivars with high ne, h and I. The average ne was 1.54, h was 0.35 and I was 0.53 (Table 4). Karagöz et al. (2020) reported the ne, I and h values for Oregano by iPBS marker respectively as 1.61, 0.37 and 0.55. Hossein-Pour et al. (2019) reported that ne, h and I values for quinoa by iPBS marker respectively as 1.52, 0.32 and 0.49. The expected heterozygosity values indicate the diversity amount of the primers, as those values are high; the primers variability is high (Pompanon et al. 2005). In previous works, He was reported as 0.28 in a barley set (Elakhdar et al. 2018) which was similar with our results, while (Zhang et al. 2014) indicated a value of 0.52 for a 80 wild and 16 commercial Tibetan barley genotypes. Elakhdar et al. (2018) explain the differences in expected heterozygosity as primer problems such as deletion of alleles and occurrence of inadequate alleles at annealing spots. Nevertheless, it is also concluded that heterozygote deficiencies might be due to Technical limitations (Elakhdar et al. 2018), while cleistogamy in barley may reduce the heterozygosity that flower sheds its pollen before opening makes it almost completely autogamous (Wang et al. 2013). In the research, the average Fst was 0.32. Elakhdar et al. (2018) reported an Fst value of 0.57 in an Egyptian barley set using SSR and SNP markers and stated that the difference between populations was higher than variation among population, which was in harmony with our findings.

Table 5- Heterozygosity and Fst values of 5 barley sub-populations

Sub-population (K)	*Expected heterozygosity (He)*	F_{ST}
A	0.1903	0.4697
B	0.2085	0.3560
C	0.2224	0.3294
D	0.2030	0.4429
E	0.3435	0.0098
Average	0.2335	0.3216

Table 6- Genetic differentiation based on Fst values among five Barley sub-populations identified by population structure analysis

Sub-populations (K)	*Sub-Pop A*	*Sub-Pop B*	*Sub-Pop C*	*Sub-Pop D*
Sub-Pop A	-			
Sub-Pop B	0.1225	-		
Sub-Pop C	0.1067	0.0981	-	
Sub-Pop D	0.1109	0.1033	0.0886	-
Sub-Pop E	0.0582	0.0456	0.0371	0.0459

The dendrogram generated by UPGMA clustered into two groups. The first group was consisted of 10 cultivars and the second one which was the major one included 44 cultivars. The first small group consisted of two sub-groups with 9 members in the first and one cultivar in the second one. The major group firstly divided into two and the first group contained 7 cultivars, the second sub-group clustered in two groups with 20 and 16 cultivars in each group, respectively. Martı and Zahir cultivars were found the most similar barley cultivars with 75% genetic similarity, whereas Özdemir and Karatay 94 and Tosunpaşa and Konevi cultivars were found 73% similar. On the other hand, Bayrak and Avcı-2002 were found the most diverse cultivars with 19.9% genetic similarity. Genetic diversity studies with 530 polymorphic bands obtained from four SCoT and 18 iPBS markers recommended that the commercial barley cultivars had high genetic variation due to different sources. The existence of genetic variation in a population may be described originated from the amount of various alleles, their position, the impacts they have on accomplishment and the complete characteristics of observable phenotypes of desired the ones that construct the population (Hamrick et al. 1992). Expanding the genetic base is one of the main goals of the breeders while cultivation is mostly done by genetically uniform cultivars thus raising the concerns about narrowing the genetic base. A dendrogram was created using genotypic data obtained from iPBS and SCoT markers. A wide genetic diversity

was observed on the commercial barley cultivars derived from different institutes of Turkey and Bulgaria. The genetic diversity of the barley cultivars ranked from 19.9% to 75%. As it is shown in Figure 1, the barley cultivars obtained from IAK were mostly grouped together (Bozhin, Zemela, Aheloy 2, Zagoretz, Alekssan, Odisey, Bul Perun) with the exceptions. Although the most similar cultivars Martı and Zahir derived from different institutes (TARI and IAK, respectively), they are neighbors and the most closest institutes geographically. Bayrak and Avcı-2002 were the most diverse cultivars obtained from AARI and FCCRI, even they are both six-rowed. Besides their different origination, the high dissimilarity might be caused by their growth habit since Bayrak is a spring barley, while Avcı-2002 is a winter type. In earlier studies, genetic diversity of the barley accessions was revealed using different molecular marker technologies. Orabi et al. (2009) indicated a high genetic diversity in wild barley accessions and barley landraces was nearly high as well. Khodayari et al. (2012) stated a genetic diversity in Iranian barley landraces and defined Iranian gene pool as a valuable source of new alleles for crop improvement. Pasam et al. (2014) indicated a genetic diversity in a wide barley accession including both two and six-rowed barleys. Bengtsson et al. (2017) reported a higher genetic diversity in two-rowed (SSR: 0.431; SNP: 0.305) barley lines compared to six-rowed ones (SSR: 0.386; SNP: 0.225). Our findings are in confidence with the previous works.

4. Conclusions

In the current study, 54 barley cultivars were characterized by 18 iPBS and four SCoT markers and 530 polymorphic bands were produced. Population structure analysis conducted with genotypic data revealed five subpopulations in the barley cultivars. Diversity analysis showed that cultivars clustered with regardless to the releasing institutes and country origins. However, Martı and Zahir cultivars originated from Trace region with different country origins were found the most similar barley cultivars with 75% genetic similarity. Nevertheless, Bayrak and Avcı-2002 were found the most diverse cultivars with 19.9% genetic similarity. The average effective number of alleles, Shannon's information index, and Nei's genetic diversity were found 1.43, 0.27 and 0.42, respectively. Structure analysis of barley cultivars derived from different pedigrees resulted in five sub-populations. In addition, the average expected He and Fst values were determined as 0.234 and 0.322, respectively. These results showed that the iPBS and SCoT markers are polymorphic, may be used for diversity analysis of barley.

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