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Genetic screening of Turkish barley genotypes using simple sequence repeat markers

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Abstract

Thirty-four Turkish barley genotypes were differentiated and identified using barley simple sequence repeat (SSR) markers. Amplification of SSR loci were generated using 17 SSR primers. These SSR primers totally produced 67 alleles ranging from two to six alleles per locus with a mean value of 3.94 alleles per locus. Genetic similarity ranged from 0.507 to 1.000. Maximum genetic similarity was found between Efes-98-Başgül, and among Anadolu-86, Obruk-86, Anadolu-98, Tokak157/37 and Orza-96. Minimum genetic similarity was between Bolayır and Angora. Although SSR markers cannot classify 34 Turkish barley cultivars based on end use, growth habits and row groups, 27 Turkish barley genotypes could be identified uniquely using 17 SSR primers. These results will are useful proves for protecting breeder's rights and designing new crossings.

Keywords: Barley (*Hordeum vulgare* L.), genetic discrimination, simple sequence repeats, molecular markers, genetic similarity

Türk arpa genotiplerinin basit dizilim tekrarları işaretleyicileri ile genetik taranması

Özet

Basit Dizilim Tekrarları (BDT) işaretleyicileri kullanılarak, otuz dört Türk arpa genotipinin ayrımı yapılmış ve tanımlanmıştır. BDT lokuslarının çoğaltımı 17 BDT primeri kullanılarak yapılmıştır. Bu BDT primerleri lokus başına ortalama 3.94 olmak üzere 2 ila 6 arasında değişen toplam 67 allel üretmiştir. Genetik benzerlik 0.507 ila 1.000 arasında değişim göstermektedir. En yüksek genetik benzerlik Efes-98 ve Başgül ile Anadolu 86, Obruk-86, Anadolu-98, Tokak157/37 ve Orza-96 arasında bulunmuştur. En düşük genetik benzerlik ise Bolayır ve Angora arasındadır. BDT işaretleyicileri, 34 Türk arpa çeşidini, kullanım amaçlarına, yetişme koşullarına ve başak sıralarına göre sınıflandıramamasına rağmen, 27 Türk arpa çeşidi 17 BDT primeri kullanılarak tanımlanabilmiştir. Bu sonuçlar ıslahçı haklarını korunmasında ve yeni melezlerin tasarlanmasında faydalı olabilecek kanıtlardır.

Anahtar Sözcükler: Arpa (*Hordeum vulgare* L.*)*, basit dizilim tekrarları, genetik ayırım, moleküler markörler, genetik benzerlik

Introduction

Barley (*Hordeum vulgare* L.*)* genotypes are traditionally distinguished by morphological traits, such as hairiness of leaf sheaths, intensity of anthocyanin, number of rows, rachilla hair types, plant length. In most cases, genotypes are obtained from very similar parents. This makes the morphological differentiation rather difficult. Seed storage protein markers and molecular markers have been used as tools to enhance barley cultivar identification capabilities for several years. Among different classes of molecular markers, SSR markers have proved as markers of choice for several applications in breeding because of their multi-allelic nature, codominant inheritance, reproducibility, abundance and wide genomic distribution (Gupta and Varshney, 2000). SSRs are particularly attractive for distinguishing between cultivars because the level of polymorphism

detected at SSR loci is higher than that detected with any other molecular assay (Saghai Maroof *et al.*, 1994; Powell *et al.*, 1996).

So far, several investigations on the discrimination between barley genotypes using SSR markers have been carried out by Russell *et al.* (1997), Pillen *et al.* (2000), Turuspekov *et al.* (2001), and Chaabane *et al.* (2009). Limited information is available on genetic discrimination of Turkish barley cultivars. These research based on analysis of Inter Simple Sequence Repeats (ISSR) (Yalım, 2005), storage protein (hordein) and Random Amplified Polymorphic DNA (RAPD) (Sipahi *et al.,* 2010). The purpose of the present research was to distinguish 34 Turkish cultivars and estimate the genetic relations among these cultivars using SSR markers.

Materials and methods

Plant material

Thirty-four barley genotypes from Turkey used in the present study are listed in Table 1. Seed samples have been kindly provided by Central Research Institute for Field Crops (CRIFC) Ankara, Turkey. Barley seeds were germinated and grown under standard conditions (25±1°C, 16 hours of photoperiod for 14 days).

DNA extraction and SSR analysis

Total genomic DNA was isolated from seedlings of each cultivar according to Anderson *et al.* (1992). Seventeen microsatellite primer pairs were selected based on their chromosomal positions (Table 2). Polymerase chain reaction (PCR) reactions were performed in 25 µL of a mixture containing 20 ng DNA, 1X Taq Reaction Buffer, 5 units of Taq DNA Polymerase, 0.2 mM dNTPs and 0.25 µM of each primer. Depending on the primer used (Table 2), DNA amplifications were performed using one of the following amplification parameters: (1) Eighteen cycles of 1 min at 94°C for denaturation, 30 s at 64°C (decrease 1°C per 2 cycles until 55°C) for annealing, 1 min extension at 72 °C, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72° C and 5 mins final extension at 72°C. (2) 3 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C, followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C, and 5 mins final extension at 72°C. (3) 1 cycle of 3 min denaturation at 94°C, 1 min annealing at 58°C, 1 min extension at 72°C, followed by 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 58°C, 30 s extension at 72°C, followed by a single extension at 72°C for 5 mins.

PCR products were separated by electrophoresis using 3% agarose gel and 6% non-denaturating polyacrylamide gel in 1xTBE buffer, then stained with ethidium bromide and visualized under UV light. A 100 bp DNA ladder was used as a molecular size standard.

Data analysis

SSR data were scored for the presence (1) or absence (0) of clear bands. Only intense bands were scored visually. The genetic similarities (GS) among cultivars were calculated according to Nei and Li (1979). Based on the similarity matrix, a dendogram showing the genetic relationships between genotypes was constructed using unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) by using the software NTSYS-pc version1.80 (Rohlf, 1993).

Polymorphic information content (PIC) values were calculated for each primer according to the formula: $PIC = 1 - \sum(Pij)^2$, where Pij is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers (Anderson et al., 1993).

Results

Seventeen SSR primers were used for cultivar identification and estimation of the genetic relations among 34 Turkish barley genotypes. Table 1 lists the detail of the genotypes along with their breeding parents. All 17 SSR primers generated clear banding patterns with high polymorphism. The Figure 1 shows an example of two polymorphic bands between 150 and 200 bp generated by Bmag0500 primer. Seventeen SSR primers revealed a total of 67 alleles ranging from two to six alleles per locus with a mean value of 3.94 alleles per locus (Table 3). The effective number of alleles was less than observed alleles in all loci, with an average of 2.30. The PIC values ranged from 0.164 (Bmag353) to 0.747 (Bmac213) with an average value of 0.523 (Table3). Bmac213 and EBmac679 revealed the highest PIC values (0.747 and 0.714, respectively), which coincided with their highest number of polymorphic bands (5). The frequency of sixty percent of the 67 alleles was lower than 0.20 (Table 3). Five alleles showed frequencies higher than 0.70 and ten alleles had frequency of 0.03. These results revealed the distribution and representative aspect of the alleles in Turkish barley cultivars. The number of rare alleles, i.e. alleles found only in one genotype, was

determined. The frequency of rare alleles was 0.03. Two alleles (~130 bp) at the locus Bmag387 and Bmag500 and two alleles (~140 bp, 240 bp) at the locus Bmag013 and Bmag217 was fixed in Sladoran. The alleles (~140 bp, 220 bp, 200 bp, 150 bp, 230 bp, 130 bp) at locus EBmac501, HVM68, Bmac113, Bmag013, Bmag217, Bmag310 were fixed with only Kıral 97, Barbaros, Kalaycı 97, Angora, Bilgi 91 and Erginel genotypes, respectively.

The genetic similarity matrix was established using data generated by the seventeen SSR primers. Genetic similarity ranged from 0.507 to 1.000. Maximum and minimum similarities were found for Efes-98/Başgül, Anadolu-86/Obruk-86/Anadolu-98/Tokak157/37/Orza-96 and Bolayır/Angora, respectively.

Table 1. Turkish barley (*Hordeum vulgare* L.) genotypes used in this study along with their pedigrees.

Name of cultivars	Pedigrees	Row	End Use	Growth habit
1-Tokak 157/37	Selection from Turkish land races	2	Feed	Winter/Spring
2-Zafer 160	Selection from Turkish land races	6	Feed	Spring
3-Yeşilköy 387	Zafer160 / land race from Kırklareli (gene bank no 3351)	6	Feed	Spring
4-Yerçil 147	Strengs Frankengerste from Germany	2	Feed	Spring
5- Obruk 86	Selection from Tokak	2	Feed	Winter/Spring
6-Anadolu 86	Luther / BK 259-149/3 gün-82	2	Feed	Winter
7-Bülbül 89	13GTH / land race (Gene bank number 657)	2	Feed	Winter
8-Erginel 90	Escourgeon / Hop2171 (France)	6	Feed	Winter
9-Bilgi 91	Introduction from Mexico	2	Feed	Spring
10-Şahin 91	Unknown	2	Malting	Winter
11-Tarm 92	Tokak / land races no 4875	2	Feed	Winter/Spring
12-Efes 3	Unknown	2	Malting	Winter
13-Yesevi 93	Tokak / land race no 4857	2	Feed	Winter/Spring
14-Karatay 94	3896/I-3/Toplani/3/Rekal/1128/90 Manhaists	2	Feed	Winter
15-Orza 96	Tokak / land race no 4857	2	Feed	Winter/Spring
16-Balkan 96	Unknown	2	Malting	Winter
17-Kalaycı 97	Erginel 9 / Tokak	2	Feed	Winter/Spring
18-Kıral 97	Unknown	6	Feed	Winter
19-Sladoran	Introduction from Yugoslavia	2	Malt	Winter/Spring
20-Anadolu 98	Susuz selection / Berac (Turkey-Holland)	2	Malting	Winter
21-Efes 98	Tercan selection / Tipper (Turkey-England)	2	Malting	Winter
22-Angora	(Triax / line 818 no) / (Malta X Ungar) /2/ (lineno 818/Sultan)	2	Malting	Winter
23-Çetin 2000	Star (İran) / 4875 no line	6	Feed	Winter
24-Aydanhanım	GK Omega / Tarm 92	2	Malting	Winter/Spring
25-Avcı 2002	Sci/3/Gi-72AB58,F1//WA1245141	6	Feed	Winter/Spring
26-Çumra 2001	Tokak selection / Beka	2	Malting	Winter/Spring
27-Çatalhöyük 2001	S 8602 / Kaya	2	Malting	Winter
28-Zeynelağa	(Anteres x KY63-1249) x Lignee	2	Malting	Winter/Spring
29-Barbaros	Introduction from France	6	Feed	Winter
30-Larende	ALM (4652)/Tokak//342TP/P-12-119/3/W.BELT22	2	Feed	Winter/Spring
31-Çıldır	3896/28//284/28/CMM/14/624/682/5/WBQT12	2	Malting	Winter/Spring
32- Başgül	Severa/Tokak//Ad.Gerste/Clipper	2	Malting	Winter/Spring
33- İnce Arpa	4671/Tokak/4648/P12-119/3/WBCB-4	2	Malting	Winter/Spring
34- Bolayır	OSK4.197/12-84//HB854/Astix/3/Alpha/Durna	2	Feed	Winter

Table 2. Barley SSR primers, their sequences, the chromosomal location and repeat (F: Forward, R:Reverse)

Primer	Sequence	Reference	Location	Repeat	PCR[a]
Bmac0213	F:5'-ATGGATGCAAGACCAAAC-3' R: 5'-CTATGAGAGGTAGAGCAGCC-3'	Ramsay *et al.* (2000) Hearnden PR *et al.* (2007)	1H	(AC)23	3
EBmac0501	F:5'-ACTTAAGTGCCATGCAAAG-3' R:5'- AGGGACAAAAATGGCTAAG-3'	Ramsay et al. (2000) Varshney RK *et al.* (2007)	1H	(AC)13	3
WMC1E8	F:5'- TCATTCGTTGCAGATACACCAC-3' R:5'- TCAATGCCCTTGTTTCTGACCT-3'	Ramsay *et al.* (2000), Varshney RK *et al.* (2007) Hearnden PR *et al.* (2007)	1H	(AC)24	2
HVM20	F:5'- CTCCACGAATCTCTGCACAA-3' R:5'- CACCGCCTCCTCTTTCAC-3'	Liu *et al.* (1996)	1H	(GA)19	1
HVM36	F:5'-TCCAGCCGACAATTTCTTG-3' R:5'-AGTACTCCGACACCACGTCC-3'	Ramsay *et al.* (2000) Varshney RK *et al.* (2000) Liu *et al.* (1996)	2H	(GA)13	1
Bmag0013	F:5'-AAGGGGAATCAAAATGGGAG-3' R:5'-TCGAATAGGTCTCCGAAGAAA-3'	Ramsay *et al.* (2000) Varshney RK *et al.* (2007) Hearnden PR *et al.* (2007)	3H	(CT)21	3
Bmac0209	F:5'-CTAGCAACTTCCCAACCGAC-3' R:5'-ATGCCTGTGTGTGGACCAT-3'	Ramsay *et al.* (2000) Varshney RK *et al.* (2007) Hearnden PR *et al.* (2007)	3H	(AC)13	3
Bmag0225	F:5'-AACACACCAAAAATATTACATCA-3' R:5'-CGAGTAGTTCCCATGTGAC-3'	Ramsay *et al.* (2000) Varshney RK *et al.* (2007) Hearnden PR *et al.* (2007)	3H	(AG)26	3
Bmag0353	F:5'-ACTAGTACCCACTATGCACGA-3' R:5' -ACGTTCATTAAAATCACAACTG-3'	Ramsay *et al.* (2000) Varshney RK *et al.* (2007) Hearnden PR et al. (2007)	4H	(GA)22	3
HVM68	F:5'-AGGACCGGATGTTCATAACG-3' R:5'-CAAATCTTCCAGCGAGGCT-3'	Liu *et al.* (1996)	4H	(AG)21	1
Bmac0310	F:5'- CTACCTCTGAGATATCATGCC-3' R:5' -ATCTAGTGTGTGTTGCTTCCT-3'	Hearnden PR *et al.* (2007) Hayden MJ *et al.* (2008)	4H	(CT)11(AC)20	2
EBmac0679	F:5'-ATTGGAGCGGATTAGGAT-3' R:5'-CCCTATGTCATGTAGGAGATG- 3'	Hearnden PR *et al.* (2007)	4H	(AC)22	2
Bmag0337	F:5'-ACAAAGAGGGAGTAGTACGC-3' R:5'-GACCCATGATATATGAAGATCA-3'	Ramsay *et al.* (2000) Varshney RK *et al.* (2007) Hearnden PR *et al.* (2007)	5H	(AG)22	2
Bmag0387	F:5'-CGATGACCATTGTATTGAAG-3' R:5'-CTCATGTTGATGTGTGGTTAG-3'	Ramsay *et al.* (2000) Varshney RK *et al.* (2007) Hearnden PR et al. (2007)	5H	(AG)16	3
Bmac0113	F:5'-TCAAAAGCCGGTCTAATGCT-3' R:5'-GTGCAAAGAAAATGCACAGATAG-3'	Ramsay et al. (2000) Varshney RK *et al.* (2007) Hearnden PR *et al.* (2007)	5H	(AT)7(AC)18	3
Bmag0500	F:5'-GGGAACTTGCTAATGAAGAG-3' R:5'-AATGTAAGGGAGTGTCCATAG-3'	Hearnden PR *et al.* (2007)	6H	(AG)29	3
Bmag0217	F:5'-ATTATCTCCTGCAACAACCTA-3' R:5'-CTCCGGAACTACGACAAG -3'	Ramsay *et al.* (2000) Varshney RK *et al.* (2007)	7H	(AG)17(AC)16	3

[a] The numbers represent one of the three PCR conditions described in the materials and methods section.

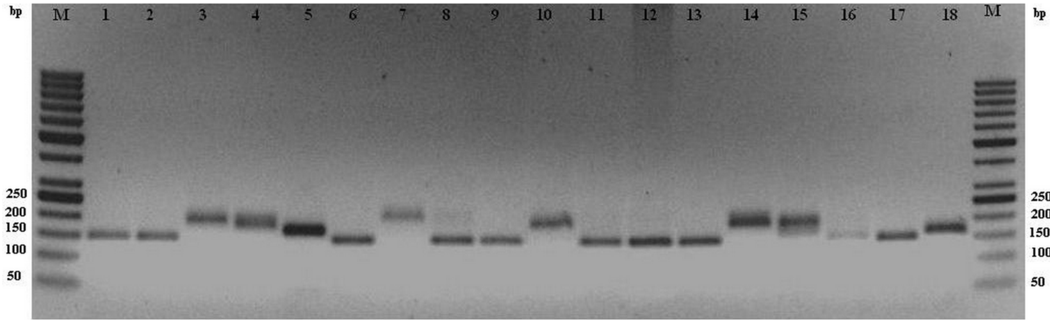

Figure 1. Agarose gel showing the alleles of the Bmag0500 SSR marker in Turkish barley cultivars. Tarm-92, 2. Yesevi-93, 3. Çetin-2000, 4. Yerçil, 5. Zeynelağa, 6. Çatalhöyük, 7.Kral-97, 8. Karatay-94, 9. Anadolu-86, 10. Çumra-2001, 11. Anadolu-98, 12. Tokak157/37, 13.Orza-96, 14. Erginel, 15.Yeşilköy, 16. Sladoran, 17. Bülbül-89, 18. Balkan-96. M:Molecular size standard 50bp DNA ladder.

A dendogram of the 34 barley cultivars was constructed by the UPGMA method (Figure 2). According to this dendogram, genotypes were divided in five different groups and two of them were also divided in two subgroups (Figure 2).

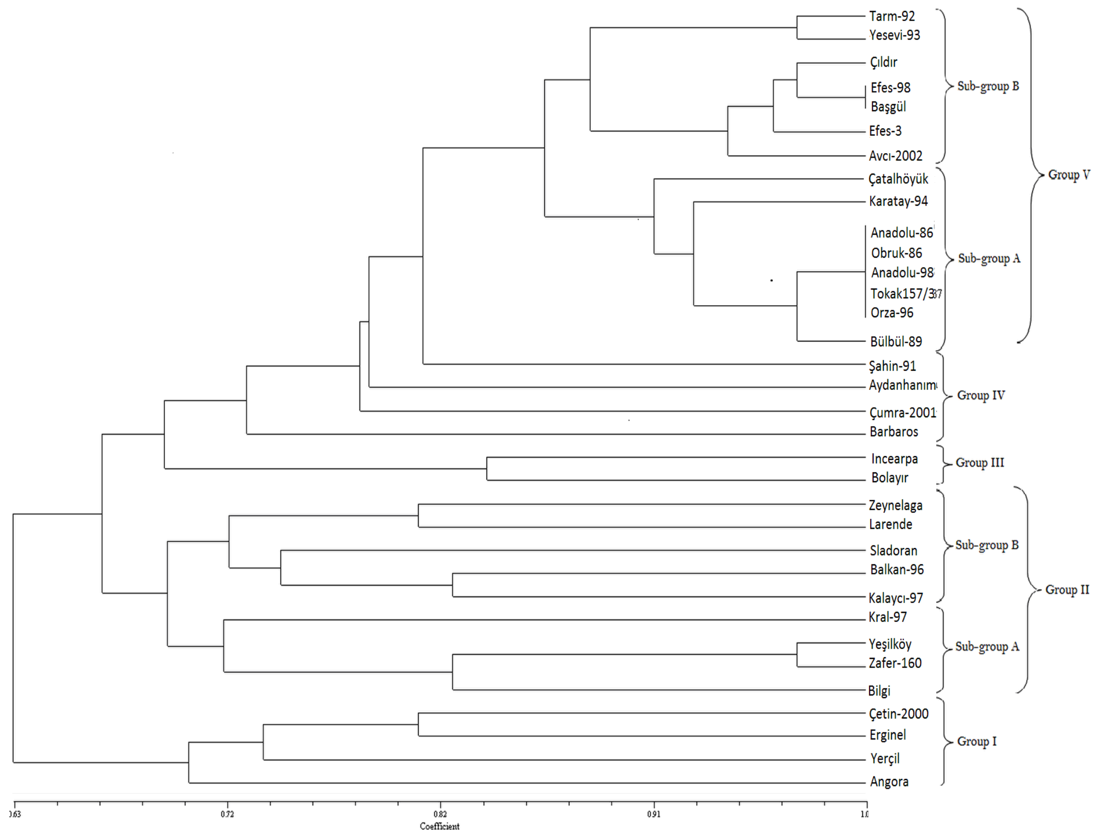

Figure 2. Dendogram constructed by the UPGMA method

The first group included two and six-row genotypes and genotypes of diverse end use and growth habit. The second group contained nine genotypes. These genotypes were divided two sub-groups. While the sub-group A comprised only feeding genotypes, the sub-group B was dominated by malting and two-row genotypes. The third group contained only two genotypes. The fourth group comprised majority of malting genotypes. The largest group was group five. This group was two-row type, except for Avcı, 2002.

Table 3. Number of observed, effective and polymorphic allele, frequencies of alleles and PIC values of 17 SSR loci in 34 Turkish barley genotypes.

Locus	Observed number of alleles	Number of polymorphic alleles	Effective number of alleles	Frequencies of alleles	Polymorphic information content (PIC)
Bmac213	5	5	3.90	0.15, 0.06, 0.15, 0.32, 0.32	0.747
EBmac501	4	3	2.12	0.03, 0.15, 0.64, 0.18	0.522
WMC1E8	2	2	1.49	0.21, 0.79	0.332
HVM36	3	3	1.61	0.76, 0.06, 0.18	0.371
Bmac209	2	2	1.78	0.68, 0.32	0.435
Bmag225	4	4	2.64	0.12, 0.15, 0.55, 0.18	0.617
Bmag353	2	2	1.19	0.09, 0.91	0,164
HVM68	5	4	2.20	0.09, 0.12, 0.64, 0.12, 0.03	0.540
Bmac310	4	3	2.50	0.03, 0.09, 0.41, 0.47	0.602
EBmac679	5	5	3.52	0.43, 0.15, 0.15, 0.21, 0.06	0.714
Bmag337	3	3	1.53	0.06, 0.79, 0.15	0.350
Bmag387	5	4	2.65	0.55, 0.03, 0.09, 0.12, 0.21	0.619
Bmac113	3	2	2.10	0.44, 0.03, 0.53	0.525
Bmag500	6	4	2.96	0.03, 0.53, 0.15, 0.15, 0.06, 0.08	0.663
HVM20	4	4	2.52	0.44, 0.06, 0.44, 0.06	0.606
Bmag013	5	3	2.70	0.03, 0.03, 0.50, 0.32, 0.12	0.631
Bmag217	5	3	1.76	0.06, 0.15, 0.74, 0.03, 0.03	0,425
Mean	3.94±1.25	3.29±0.99	2.30±0.73		0.523±0,56

Discussion

The average PIC value in this study was lower than what was reported in a previous study by Yalım (2005) who discriminated 28 Turkish barley genotypes using 10 ISSR primers. Ten ISSR primers produced an average PIC value of 0.611. The average PIC value of 0.523 detected in 34 Turkish cultivars is in accordance with Russell et al. (1997) who found an average PIC value of 0.50 using eleven microsatellite loci in 24 barley genotypes. The lower average PIC value was reported by Pillen *et al.* (2000). They detected average PIC value of 0.38 for 22 microsatellites in 25 German, 3 North American barley cultivars and 2 *H. vulgare* ssp. *spontaneum* accessions. Based on the genetic similarity dendogram of seventeen SSR primers, 27 Turkish cultivars could be distinguished uniquely. On the other hand, more SSR primers need to be used for reliable discriminating of seven Turkish cultivars (Efes-98, Başgül, Anadolu-86, Obruk-86, Anadolu-98, Tokak157/37, Orza-96). In general, the UPGMA cluster did not classify 34 Turkish barley cultivars corresponding to their pedigrees, the number of rows, end use and growth habits.

Yalım (2005) noticed that 10 ISSR primers were sufficient for separating 28 Turkish barley cultivars in which minimum and maximum genetic distances were between Efes-2/Yesevi-93 and Karatay-94/Aday-4 cultivars, respectively. In order to determine genetic variation and relationships among barley genotypes improved in Turkey using hordein and RAPD, Sipahi *et al.* (2010) screened 34 barley cultivars and observed 15 different hordein banding patterns twelve of which were cultivar specific. RAPD variation observed among cultivars higher than that of hordein and cluster analyses based on hordein data showed that most of the cultivars are genetically closely related.

Moreover, correspondence analysis by using these two marker systems showed that RAPD data could distinguish almost all barley cultivars except Tokak 157/37 and Bülbül 89, whereas hordein data were not able to discriminate the barley cultivars like RAPDs.

Our SSR analysis showed that this technique was time and labor saving, and effective approach for barley cultivar identification. Seven barley cultivars used in this study, which were not identified by seventeen SSR primers, should also be identified by combining different DNA based techniques such as RAPD, ISSR, STS, SNP or protein electrophoresis. Result of this investigation will benefit barley breeders when selecting potential parents to be used in crossing programs and will also facilitate the germplasm management.

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