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Dissection of Barley Landraces Originated From Twelve Different Countries by Using Simple Sequence Repeats Markers

Hülya SİPAHİ[a], Aysen YUMURTACI[b]

[a]*Sinop University, Faculty of Arts and Science, Department of Biology, Sinop, TURKEY*

[b]*Marmara University, Faculty of Science and Letters, Department of Biology, 34722, Istanbul, TURKEY*

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Corresponding Author: Hülya SİPAHİ, E-mail: hulyasipahi@hotmail.com, Tel: +90 (368) 271 55 16
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ABSTRACT

Landraces, as an important source of genetic diversity, are important for improvement of crop species. Investigating of genetic diversity among landraces is necessary to conserve genetic resources and develop future strategies on barley breeding. In this study, genetic diversity in barley landraces originating from twelve countries was studied using simple sequence repeat (SSR) markers. Sixteen SSR markers belong to the seven barley linkage groups revealed high genetic diversity. A total of 92 polymorphic alleles were scored and the number of alleles ranged from 1 to 7 per locus, with an average of 5.75. Genetic diversity was the highest in landraces from Turkey (0.66 ± 0.13) and the lowest in those from Ukraine (0.38 ± 0.24). While the highest percentage of polymorphic loci was found for landraces from Germany, Netherland, Russia, Turkey and USA as 100%, the lowest one was in England with 75%. Clustering analysis of landraces divided them into four main groups. The results provided additional genetic information about the barley landraces from different countries for future breeding process.

Keywords: Barley; Landraces; SSRs; Diversity

Basit Sıra Tekrarı İşaretleyicilerini Kullanarak On İki Farklı Ülkeden Köken Alan Arpa Yerel Çeşitlerinin İncelenmesi

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Hülya SİPAHİ, E-posta: hulyasipahi@hotmail.com, Tel: +90 (368) 271 55 16
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ÖZET

Genetik çeşitliliğin önemli bir kaynağı olan yerel çeşitler, tahıl türlerinin geliştirilmesi için önemlidirler. Yerel çeşitler arasındaki genetik çeşitliliğin araştırılması, genetik kaynakları koruma ve arpa ıslahında gelecek stratejileri geliştirme için önemlidir. Bu çalışmada, on iki ülkeden köken alan arpa yerel çeşitlerindeki genetik çeşitlilik, basit sıra tekrarları (BST) işaretleyicileri kullanılarak çalışılmıştır. Yedi arpa bağlantı grubuna ait onaltı BST işaretleyicisi yüksek genetik çeşitliği ortaya çıkarmıştır. Toplamda 92 polimorfik allel sayılmış ve allel sayısı 5.75 ortalama ile lokus başına 1 ila

7 arasında olmuştur. Genetik çeşitlilik en yüksek Türkiye yerel çeşitlerinde (0.66 ± 0.13), en düşük Ukrayna yerel çeşitlerinde (0.38 ± 0.24) belirlenmiştir. Polimorfik lokus yüzdesi, Almanya, Holanda, Rusya, Türkiye ve Amerika'da en yüksek yani % 100 iken, % 75 ile en düşük İngiltere'de bulunmuştur. Yerel çeşitlerin kümeleme analizleri onları dört ana gruba ayırmıştır. Sonuçlar gelecekteki ıslah çalışmaları için farklı ülkelerden arpa yerel çeşitleri hakkında ilave genetik bilgiler sağlamıştır.

Anahtar Kelimeler: Arpa; Yerel çeşitler; BST; Çeşitlilik

1. Introduction

Barley (*Hordeum vulgare* L.) is placed at the fourth rank among the top 10 crops. It is important for contributing to the world food supply as a human food, malt production, and livestock feed. Due to the nutritional quality, high yield and high tolerance level against to biotic or abiotic stresses, barley is an extensively studied cereal. Barley is accepted as one of the earliest domesticated crop and has been cultivated since the beginning of civilization. Breeder selection for resistance, quality or yield-contributed traits creates greater genetic uniformity and this genetic uniformity causes to narrow the structure of genetic material in time. However, genetic diversity has a fundamental importance for genetic improvement of crop plants. Landraces have evolved directly from their wild progenitors which lead to retain high level of genetic diversity and these are very important sources for barley improvement. Zeven (1998) stated that landraces have the ability to tolerate abiotic and biotic stress effects and they are capable of high yield stability and an intermediate yield level under a low input agricultural system.

Large numbers of barley landraces from all over the world have been gathered and stored in seed gene banks to secure a gene pool for future breeding process. Assessment of genetic diversity in landraces maintained in genebank is an essential step to detect the relationship among the landraces accessions, to understand the genetic structure of the landraces, to select the parents and design crossing process for breeding and to set priorities for genetic conservation strategies. Investigations about genetic relatedness between landraces or cultivated genotypes will contribute to understanding of the unknown part of crop evolution (Jones et al 2008). A number of molecular methods and bioinformatics tools are available for analysis of genetic diversity among and within germplasm populations and the characterization of germplasm collections. The availability of PCR-based markers, such as SSRs, offers an important opportunity for genetic characterization of germplasm collections. SSRs are one of the markers of choice for many plant breeding applications, due particularly to their co-dominant nature, transferability, reproducibility, amenability to high throughput and high level of polymorphism frequency. SSR markers are progressively used for the detection of genetic identities or fingerprints (Hokanson et al 1998; Becher et al 2000; Prasad et al 2000), the assessment of genetic diversity within a collection (Westman & Kresovich 1999; Macaulay et al 2001) and the determination of genetic relatedness between accessions (Lopes et al 1999; Li et al 2000). Potential of SSR markers is also displayed for several divergent plant species such as sweet potato (Veasay et al 2008), wheat (Ahmed 2002; Schuster et al 2009), buckwheat (Kishore et al 2012), barley, pea, oats and rye (Hagenblad et al 2012).

In the present research, 84 barley landrace accessions, collected from Turkey, Ukraine, Russia, Austria, Germany, Netherland, England, Polland, China, Ethiopia, Mexico and USA, were assessed with 16 SSRs to estimate regional genetic diversity, genetic relationship and population structure. In this way, geographical evolution will be studied according to the SSR marker profiles.

2. Material and Methods

2.1. Plant material

Eighty-four barley landrace accessions from twelve countries were included in this study (Table 1). Approximately 2 g of leaves from 5 plants of each accession were pooled for the extraction of genomic DNA. DNA samples were extracted by using GeneMark DNA isolation kit. DNA samples were then quantified using a Nanodrop ND-1000 UV-V spectrophotometer. Sixteen SSRs distributed throughout the seven linkage groups were assayed (Table 2).

2.2. Polymerase chain reaction and electrophoresis

PCR analysis was done using 16 primers (Table 2) as described by Sipahi 2011. PCR products were analysed on 6% PAGE gel in '1XTBE', stained by ethidium bromide (0.5 mg mL^{-1}) and visualized under UV light.

2.3. Data analysis

SSR markers are codominant, thus the banding patterns were scored as AA or BB (homozygote) and AB (heterozygote) genotypes. Estimates of genetic diversity index (H), the proportion of polymorphic locus (P), and the mean number of alleles per

Table 1- List of barley landraces studied

Çizelge 1- Çalışılan arpa yerel çeşitlerin listesi

No	*Code*	*Country*	*No*	*Code*	*Country*	*No*	*Code*	*Country*
C1	TUR 5809	USA	C29	TUR 4717	China	C57	TUR 1974	Russia
C2	TUR 1179	USA	C30	TUR 1179	Ethiopia	C58	TUR 1976	Russia
C3	TUR 1180	USA	C31	TUR 1415	Ethiopia	C59	TUR 5130	Russia
C4	TUR 1195	USA	C32	TUR 1414	Ethiopia	C60	TUR 1961	Russia
C5	TUR 4752	USA	C33	TUR 1880	Ethiopia	C61	TUR 5034	Ukraine
C6	TUR 5801	USA	C34	TUR 4770	Ethiopia	C62	TUR 1984	Ukraine
C7	TUR 5802	USA	C35	TUR 5725	Ethiopia	C63	TUR 1988	Ukraine
C8	TUR 5804	USA	C36	TUR 1081	The Netherlands	C64	TUR 5066	Ukraine
C9	TUR 5805	USA	C37	TUR 2025	The Netherlands	C65	TUR 5067	Ukraine
C10	TUR 1274	Germany	C38	TUR 2024	The Netherlands	C66	TUR 1108	Turkey
C11	TUR 1281	Germany	C39	TUR 2012	The Netherlands	C67	TUR 1443	Turkey
C12	TUR 1302	Germany	C40	TUR 2014	The Netherlands	C68	TUR 1453	Turkey
C13	TUR 1858	Germany	C41	TUR 1213	England	C69	TUR 1202	Turkey
C14	TUR 5069	Germany	C42	TUR 1220	England	C70	TUR 1203	Turkey
C15	TUR 4627	Germany	C43	TUR 1226	England	C71	TUR 1204	Turkey
C16	TUR 4678	Germany	C44	TUR 1363	England	C72	TUR 1294	Turkey
C17	TUR 1376	Austria	C45	TUR 1316	Poland	C73	TUR 1281	Turkey
C18	TUR 1256	Austria	C46	TUR 1318	Poland	C74	TUR 1609	Turkey
C19	TUR 1257	Austria	C47	TUR 1322	Poland	C75	TUR 5112	Turkey
C20	TUR 5091	Austria	C48	TUR 1331	Poland	C76	TUR 5083	Turkey
C21	TUR 5091	Austria	C49	TUR 4598	Poland	C77	TUR 5429	Turkey
C22	TUR 1706	China	C50	TUR 5791	Mexico	C78	TUR 1310	Turkey
C23	TUR 1741	China	C51	TUR 5802	Mexico	C79	TUR 1311	Turkey
C24	TUR 1744	China	C52	TUR 5792	Mexico	C80	TUR 1312	Turkey
C25	TUR 1749	China	C53	TUR 5786	Mexico	C81	TUR 1326	Turkey
C26	TUR 1759	China	C54	TUR 1961	Russia	C82	TUR 1582	Turkey
C27	TUR 4698	China	C55	TUR 1970	Russia	C83	TUR 1586	Turkey
C28	TUR 4706	China	C56	TUR 5015	Russia	C84	TUR 5799	Turkey

Table 2- Primer sequences, chromosomal locations, repeats and PCR conditions of 16 SSR markers used for barley germplasm screening

Çizelge 2- Arpa genetik kaynakların taranmasında kullanılan 16 BST işaretleyicilerinin primer dizinleri, kromozomal lokasyonları, tekrarları ve PCR koşulları

SSR	*Primer sequences (5′→3′)*	*Chromosome*	*Repeats*	*PCR[a]*	*n[b]*	*PIC[c]*
Bmac0213	ATGGATGCAAGACCAAAC CTATGAGAGGTAGAGCAGCC	1H	(AC)23	3	5	0.65
EBmac0501	ACTTAAGTGCCATGCAAAG AGGGACAAAAATGGCTAAG	1H	(AC)13	3	5	0.75
HVM20	CTCCACGAATCTCTGCACAA CACCGCCTCCTCTTTCAC	1H	(GA)19	3	6	0.77
WMC1E8	TCATTCGTTGCAGATACACCAC TCAATGCCCTTGTTTCTGACCT	1H	(AC)24	2	7	0.77
HVM36	TCCAGCCGACAATTTCTTG AGTACTCCGACACCACGTCC	2H	(GA)13	1	6	0.75
Bmac0209	CTAGCAACTTCCCAACCGAC ATGCCTGTGTGTGGACCAT	3H	(AC)13	3	5	0.84
Bmag0225	AACACACCAAAAATATTACATCA CGAGTAGTTCCCATGTGAC	3H	(AG)26	3	6	0.75
Bmag0013	AAGGGGAATCAAAATGGGAG TCGAATAGGTCTCCGAAGAAA	3H	(CT)21	3	7	0.82
Bmag0353	ACTAGTACCCACTATGCACGA ACGTTCATTAAAATCACAACTG	4H	(GA)22	3	6	0.81
HVM68	AGGACCGGATGTTCATAACG CAAATCTTCCAGCGAGGCT	4H	(AG)21	1	6	0.79
Bmac0310	CTACCTCTGAGATATCATGCC ATCTAGTGTGTGTTGCTTCCT	4H	(CT)11(AC)20	2	5	0.78
EBmac0679	ATTGGAGCGGATTAGGAT CCCTATGTCATGTAGGAGATG	4H	(AC)22	2	6	0.64
Bmag0387	CGATGACCATTGTATTGAAG CTCATGTTGATGTGTGGTTAG	5H	(AG)16	3	4	0.64
Bmac0113	TCAAAAGCCGGTCTAATGCT GTGCAAAGAAAATGCACAGATAG	5H	(AT)7(AC)18	3	5	0.64
Bmag0500	GGGAACTTGCTAATGAAGAG AATGTAAGGGAGTGTCCATAG	6H	(AG)29	3	6	0.75
Bmag0217	ATTATCTCCTGCAACAACCTA CTCCGGAACTACGACAAG	7H	(AG)17(AC)16	3	7	0.84

[a], the numbers represent one of the three PCR conditions described in the materials and methods section; [b], number of alleles; [c], PIC, polymorphism information content

locus (A) were calculated for each SSR locus and population using the software POPGENE version 1.32 (Yeh et al 1999). 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 i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers (Weber 1990). The genetic similarities (GS) were calculated according to Nei and Li (1979). Similarity coefficients were used to construct a

dendogram using UPGMA (unweighted pairgroup method using arithmetic averages; Sneath and Sokal 1973) by using the software NTSYS-pc version1.80 (Rohlf 1993).

3. Results and Discussion

In order to investigate genetic diversity of barley landraces from the twelve different country origin, sixteen SSR primer pairs covered the whole seven chromosomes were used (Table 1). Considering all 84 accessions, 16 SSR primer pairs generated a total of 92 alleles with an average of 5.75 polymorphic alleles per locus. The mean number of alleles is mostly dependent on diversity of populations analyzed. Some investigators have detected higher average numbers of alleles per locus in barley, ranging from 8 (Malysheva-Otto et al 2007; Varshney et al 2007) to 16.3 (Matus & Hayes 200). As given in Table 1, considering 12 countries, landraces originated from Russia, China, Germany, Turkey and USA amplified more than three alleles per locus, while remaining genotypes gave approximately 2 alleles as compared for the same criteria. Additionally, landraces from Russia and USA had the same mean number of alleles and also genetic diversity value despite the different number of landraces analysed for these countries. With a few exceptions, the mean number of alleles per locus was usually affected by the number of landraces used for each country. Total number of alleles displayed a range of 34 and 70 for Ukraine and Turkey respectively. It is also found that landraces from England and Mexico ensured the same allele numbers of 37. However, there was a 0.8 difference between the genetic diversity values of these two countries (Table 3). Saghai-Maroof et al (1994) reported the highest number of alleles per locus as 37 on 104 accessions of *subsp. vulgare* and 103 accessions of *subsp. spontaneum*. Matus and Hayes (2002) detected 10.3 average numbers of alleles per locus and the highest number of unique alleles in 147 barley progenitors (*subsp. spontaneum*). These authors explained this situation as a sign of large genetic diversity which exist in crop progenitors and as a potential reservoir of novel alleles for crop improvement. Considering 19 landraces from Turkey, among all SSR primers, Bmag217 caused an amplification of 6 alleles, while Bmac0213 had the least polymorphic results (Table 3). The number of allele observed at locus Bmag225 was higher than the value recorded in the study of Dizkirici et al (2008) who reported the number of allele as 4 at that locus for 80 Turkish barley cultivars.

Five private alleles were found, one with primers Bmag0225, Bmag 0013, Bmag0353, HVM68, and EBmac0679. The countries with one of private allele were Ethiopia, China, Russia, The Netherlands, and Austria. Private alleles could be used as diagnositic markers for identification of specific regions or genotypes (Senior et al 1998). In addition, Liu et al (1996) suggested that unique alleles might be the possible results of high mutation rate at the SSR region. Private alleles can also be used to identify accessions with high genetic variability, whose selection can increase the allele richness of genebanks (Saavedra et al 2013).

The range limits for polymorphic information contents of markers were observed. The highest value was 0.84 for Bmag209 and Bmag217 markers and the lowest calculation was 0.64 for Bmac0113, Bmag0387, EBmac0679 markers (Table 2). The mean value of PIC content was 0.749, which was higher than as previously reported for EST-based SSR loci (Varshney et al 2007). As shown in Table 3, Turkish landraces had the highest level of genetic diversity (0.66), while the lowest levels of genetic diversity (0.38 and 0.39) were detected in landraces of Ukraine and England. The highest degree of polymorphism of SSR markers (0.75) detected in this study allowed a rapid and efficient identification of barley landraces originated from different countries. Similarly, Struss & Plieske (1998) reported high value of gene diversity (0.73) for barley accessions. These authors stated that the high diversity value of barley microsatellite markers makes them ideal markers for differentiating between barley genotypes.

The number of alleles detected in a locus is generally correlated with the gene diversity value of that locus. However, in this study, the number

Table 3- Summary of genetic diversity of barley landraces collected from different locations of the world

Çizelge 3- Dünyanın farklı lokasyonlarından toplanmış arpa yerel çeşitlerinin genetik çeşitliliğinin özeti

	Turkey (19)	*Ukraine (5)*	*Russia (7)*	*Austria (5)*	*Germany (7)*	*The Netherlands (5)*	*England (4)*	*Poland (5)*	*China (8)*	*Ethiopia (6)*	*Mexico (4)*	*USA (9)*
Bmac0213	2	2	3	3	3	2	1	1	2	3	1	3
EBmac0501	4	2	4	3	2	2	4	1	4	1	2	3
HVM20	5	2	4	3	3	3	2	2	4	2	3	4
WMC1E8	5	2	2	2	2	3	2	2	4	2	2	5
HVM36	4	2	3	3	3	3	2	1	2	2	3	4
Bmac209	4	3	4	3	4	3	3	2	3	3	2	3
Bmag225	4	1	3	2	3	2	1	3	2	3	2	2
Bmag013	5	4	5	2	4	3	3	3	5	3	1	5
Bmag353	5	3	2	3	4	2	3	3	4	3	3	4
HVM68	4	2	4	3	3	2	1	4	3	3	3	4
Bmac310	5	2	4	3	3	3	3	3	5	1	3	3
EBmac679	5	3	4	4	3	3	3	2	4	3	2	4
Bmag387	3	1	2	1	3	3	2	3	1	1	2	2
Bmac113	4	1	3	2	3	5	1	2	3	2	2	3
Bmag500	5	2	4	3	2	3	3	2	3	4	3	5
Bmag217	6	3	4	3	4	2	3	4	3	3	3	5
Total allele	70	34	55	43	49	44	37	38	52	39	37	59
The mean number of alleles per locus	4.38±0.96	2.19±0.83	3.44±089	2.69± 0.70	3.06 ±0.68	2.75±0.78	2.31±0.95	2.38±0.96	3.25±1.13	2.44±0.89	2.38±0.80	3.69±1.01
Genetic diversity	0.66±0.13	0.39±0.24	0.60±0.17	0.49 ±0.19	0.58 ± 0.13	0.55±0.12	0.39±0.26	0.44±0.25	0.56±0.19	0.45±0.2	0.47±0.21	0.63± 0.13
Number of polymorphic loci	16	13	16	15	16	16	12	13	15	13	14	16
Percentage of polymorphic loci	100	81.25	100	93.75	100	100	75	81.25	93.75	81.25	87.50	100

of alleles detected by SSR markers was not found correlated with the gene diversity. For example, the marker Bmag0013 showed an average gene diversity of 0.82, detecting seven alleles within 84 landraces, whereas Bmac0209 detected five alleles and showed a gene diversity value of 0.84.

Cluster analysis divided the 84 landraces into several main groups, not always in accordance with their regional origins (Figure 1). These landraces are hugely different since we count four main different groups which contain two or three subgroups. 84 barley genotypes, except TUR 4698/TUR 4706 and TUR 1974/Tur 5790, could be distinguished. The landraces from Turkey were divided into two clusters. Subgroup A and B of cluster III and subgroup B and C of cluster IV, all had large numbers of landraces from Turkey. Landraces from USA were in cluster IV subgroup D, except one in cluster III subgroup D. The Russian landraces were spread across three clusters (I, II, III), with more lines in clusters III subgroup C than in the remainders. Most Chinese landraces were in cluster III, with only one exception in cluster II. Landraces from England were in cluster IV subgroup A. Mexican landraces were divided into cluster IV subgroup B and cluster II subgroup B. German landraces were in cluster II, III, and IV. Most of the Ukrainian and Polish landraces were in cluster IV, whereas most from Ethiopia were in cluster III, and most of Austria and the Netherland landraces were in cluster II. The results of our study support the ideas of Struss & Plieske (1998) who stated that cluster analysis could identify the genetic relationship of barley genotypes and demonstrates the potential and the ability of microsatellite markers for genetic discrimination in barley. It is also inferred that individuals with small exceptions representing different countries grouped together in the same root of dendrogram tree which were sequestered in the mixed clusters (Figure 1). Indeed, these findings can be easily figured out from the clustering positions of the countries of which were fully placed in the subgroupA of groupIV and subgroupB of groupII for England (C41-C44) and Austria (C17-C21), respectively (Figure 1). However, an opposite mixed clustering pattern occurred for landraces originated from the Netherlands (C36-C40) and these corresponding samples were separated into three different groups (Group I, II, III). Also, a misclassification was observed as a mixed clustering of Russian based seven barley (C54-60) (Figure 1). This might be due to the extended genetic profile of these seven Russian landraces. So, more detailed research might be provided by using large collections. Thus, results obtained from further imperative phylogenetic work will be effective while we are trying to construct the breeding material in line with our prospects. Additionally, our results showed that geographic distance alone may not explain genetic diversity between countries.

Genetic relationships among countries were further studied by cluster analysis (Figure 2). A cluster chart was drawn according to the similarity values which were calculated by using the amplification results of corresponded barley landraces. The coefficient of similarity ranged from 0.16 to 0.94**.** Countries are divided into seven clusters as shown in the dendrogram tree (Figure 2). While ten countries (Turkey, USA, China, Russia, Germany, Austria, Netherland, Ethopia, Ukraine and Poland) are placed in the distinct groups of dendrogram tree, England and Mexico are found in the same group,. This result strengthens previous reports on the correlation between eco-geographical distribution and SSR markers. As a supporting example, Fu & Horbach (2012) reported that continental distances between eastern and western countries caused a regional classification. Eco-geographical diversity analysis is typically applied to identify "centers of diversity". The monophyletic nature of barley domestication is demonstrated based on allelic frequencies at AFLP loci in wild and cultivated barley (Badr et al 2000). Israel-Jordan were considered as the region in which barley was brought into culture and Himalayas is a region of domesticated barley domestication. However, Morell and Clegg (2006) inferred at least two domestication of barley; one within Fertile Crescent and second 1,500-3,000 km farther east. In the other studies, origin of center for cultivated

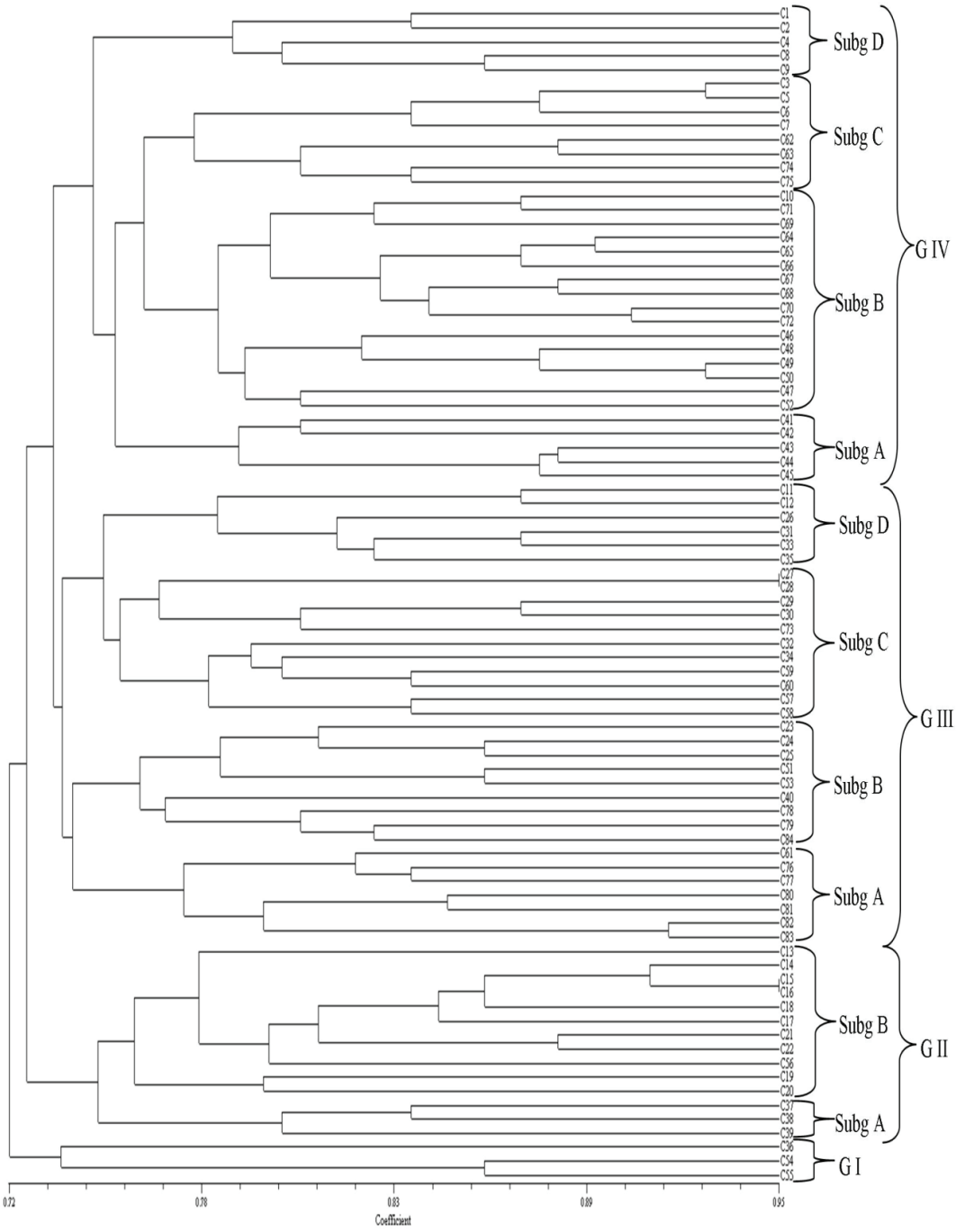

Figure 1- Dendrogram tree of 84 barley landraces; *Subg, subgroups; G, groups*

Şekil 1- 84 arpa yerel çeşidin dendogram ağacı; Subg, altgruplar; G, gruplar

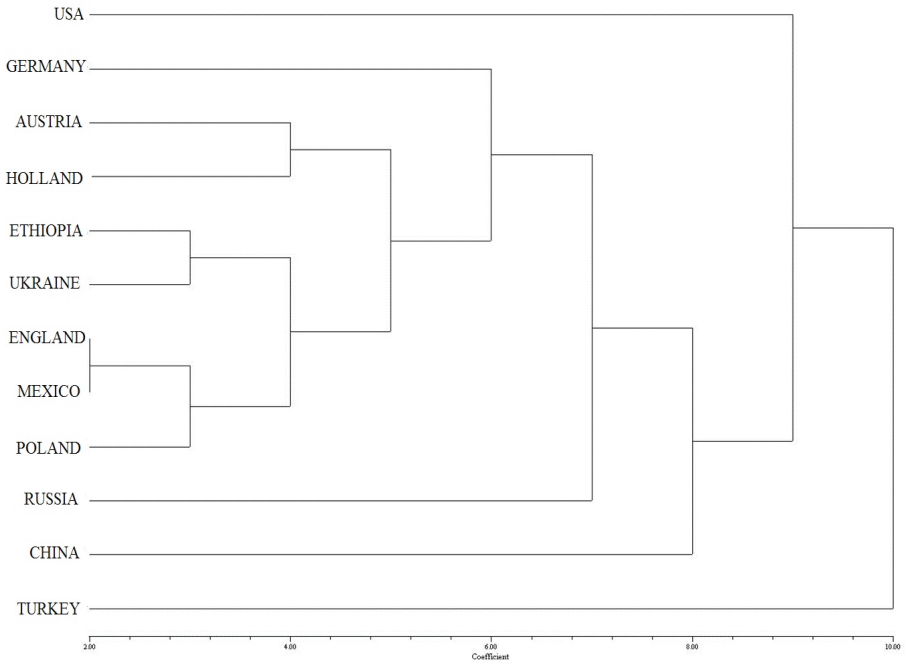

Figure 2- Country profiles of barley landraces

Şekil 2- Arpa yerel çeşitlerinin ülke profilleri

barley was stated as Tibet (Yang et al 2008; Sun et al 2011). So, genetic diversity of barley has been mostly studied in Asian derived varieties. It was continued with the Africa, Europe, America and Australia (Jilal 2011). However, these origin centers can change over time, as breeding and germplasm exchange continues (Peeters 1988). This might be formed within the population due to the genetic shift in time (Malysheva-Otto et al 2007).

In this study, the highest genetic diversity was found in landraces from Turkey. This may be explained by a much longer evolutionary history of barley landraces in Turkey. In most of the phylogenetic studies, it was primarily designed to trace both genetic diversity and geographic origin of a plant. It is one of the best practical ways to clarify the germplasm sources during domestication. There are several reports regarding to determine the country of origin and relatedness of crops for several kinds of cereals such as wheat (Zhang et al 2006), broomcorn millet (Hu et al 2009). At different time intervals, there are number of reports released about barley evaluation. In a research by Leišová et al (2007), the authors screened a core collection of 176 barley accessions originated from European, eastern (America) and western (Asia and Australia) part of the world by using 26 microsatellite loci and indicated that Czech varieties and varieties from America, Australia and Asia are clustered in the different roots of dendrogram. These authors concluded that it was one of the evidence about the distinction of European based genotypes from the remaining group of samples originated from eastern and western countries and this type of large extension between varieties might be associated with age, botanical characters and especially country of origin for barley.

4. Conclusions

The result of our present study revealed that the barley landraces preserved in CRIFC seed genebank has great genetic diversity. Especially accessions from Turkey possess higher genetic diversity than foreign accessions. Assessment of genetic diversity in landraces preserved in genebank by SSR markers is important for barley improvement effort. Namely, genotyping data for landraces provide a general guide not only for designing crossing in breeding programme, but also for detecting duplications of accessions in collection.

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