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Full Length Research Paper

Genetic variation of barley germplasm from Turkey assessed by chloroplast microsatellite markers

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Five chloroplast DNA microsatellite loci were tested to analyze cytoplasmic variation in a set of 94 barley accessions; 23 *Hordeum vulgare ssp. spontaneum* (wild) from Southeastern Anatolia, 47 *Hordeum vulgare ssp.vulgare* landraces and 24 Turkish barley cultivars. Fifteen (15) haplotypes were detected. Within wild barley, two of the five loci were polymorphic with two or three alleles and four haplotypes were found, giving a gene diversity value of 0.31. One of these haplotypes was found in 13 Turkish cultivars. Two loci were polymorphic in the landraces, giving three haplotypes and a gene diversity value of 0.33. One of haplotypes found in landraces was observed in a Turkish cultivar. All loci were polymorphic in 24 Turkish cultivars and the numbers of alleles per locus ranged from two to three alleles, with a gene diversity value of 0.61. Of the six haplotypes detected in Turkish cultivars, three were unique to three cultivars. The chloroplast microsatellite loci were found multiallelic and variable within *H. vulgare ssp. vulgare* and *H. vulgare ssp. spontaneum* intraspesific accessions and useful to compare degrees of genetic variation between accessions. The results improved the knowledge in genetic variation of CRIFC seed genebank's barley accessions and could be informative for broadening the genetic base of barley.

Key words: Barley, chloroplast, genetic diversity, germplasm, microsatellite.

INTRODUCTION

The chloroplast DNA (cpDNA) has been an attractive source for studies in the field of molecular systematic, evolution and population genetics. The characteristic features of chloroplast genome are the absence of recombination and the conservation of gene order, which facilitates inference of plant phylogenies. In addition, chloroplast genome has considerable advantage for tracking gene flow via seeds or pollen in natural populations due to its uniparental inheritance (Petit et al., 2005).

Various studies using restriction fragment analysis showed considerable amount of variation within chloroplast DNA at the interspecific and intergeneric levels (Bukhari, 1999; Panda et al., 2003; Warwick and Sauder, 2005; Ahmad et al., 2009; Jadwiszczak et al., 2012). Shawet et al. (2005, 2007) has highlighted the potential of noncoding regions of the chloroplast genome for genus and species-level phylogenetic analyses. Population geneticists have recognized that noncoding chloroplast DNA can provide informative variation at the species and population level (Ebert and Peakal, 2009).The discovery of hybervariable chloroplast microsatellite loci also known as simple sequence repeats (cpSSRs) allowed the detection of intraspecific cytoplasmic diversity in natural plant populations. Population geneticists have also used chloroplast microsatellites to detect population-level polymorphism. Chloroplast microsatellites are short mononuc-

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Table 1. Barley accessions used in this study.

Geographical origin	Type of material	Number of accessions
Turkey	Landraces	47
Southeastern Anatolia (Adıyaman)	Wild barley	5
Southeastern Anatolia (Gaziantep)	Wild barley	4
Southeastern Anatolia (Şanlıurfa)	Wild barley	6
Southeastern Anatolia (Hatay)	Wild barley	5
Southeastern Anatolia (Diyarbakır)	Wild barley	3
Turkey	Cultivars	24
Total		94

leotide tandem repeats, generally located in the non-coding regions of the chloroplast genome, that extensively show variation in repeat number (Provan et al., 2001; Jakobsson et al., 2007).

Provan et al. (1999) have developed primers for analysis of chloroplast microsatellite loci in the genus *Hordeum*, which includes cultivated barley (*Hordeum vulgare* ssp. *vulgare*) and its wild progenitor *Hordeum vulgare* ssp. *spontaneum*. Seven pairs of primers designed for the am-plification of mononucleotide repeats were polymorphic and intraspecific variation was detected in both cultivated and wild barley. In the other study, chloroplast micro-satellite primer pairs designed by Provan et al. (1999) were used to examine genetic variability in barley land-races, sampled from five different ecogeographical regions of Syria and Jordan (Russell et al., 2003). Only one primer pair (hvcptrnLF) was found polymorphic, resulting in two length variants. The authors concluded that the distribution of chloroplast polymorphism is structured and not distributed randomly across the barley landraces sampled.

Genetic diversity in barley is progressively being lost in farmers' fields and in nature due to domestication and industrialization. Threatened landraces, cultivars that were former and wild relatives of cultivated species are impor-tant for barley improvement because breeding gains rely on access to the genetic variation in the germplasm. Central Research Institute for Field Crops (CRIFC) seed genebank has a rich resource of landraces, cultivars as well as wild barleys collected from Turkey. In order to gain valuable information on the barley genetic resources of Turkey, we used chloroplast microsatellite markers to investigate the intraspecies genetic diversity in wild bar-ley, landraces and cultivars and to make a genetic diver-sity comparison.

MATERIALS AND METHODS

Plant materials

Twenty three (23) accessions of *H. vulgare* ssp. *spontaneum* (wild barley), 47 accessions of *H. vulgare* ssp. *vulgare* landraces and 24 *H. vulgare* ssp. *vulgare* cultivars, all from the germplasm collection of CRIFC seed genebank were analysed (Table 1). The 24 *H. vulgare* ssp. *spontaneum* accessions were collected from five provinces of Southeastern Anatolia, a part of the primary centers of diversity of wild barley. The cultivars represent the majority of barley varieties grown in Turkey.

DNA isolation and simple sequence repeats analysis of chloroplast DNA

DNA was extracted from leaves according to Ahmed et al. (2009). Five primer pairs were used for amplification chloroplast micro-satellite loci: hvcppsbK, hvcptrnS1, hvcptrnS2, hvcprpoA, hvcprps12 (Provan et al., 1999; Molina-Cano et al., 2005) (Table 2). Microsate-llite loci were amplified as described previously (Provan et al., 1999). PCR analysis were carried out in a total volume of 10 µl with 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCL, 1.5 mM $MgCl_2$, pH 8.3), 200 µM dNTPs, 0.5 U *Taq* polimerase, 10 pmol forward primer, 10 pmol reverse primer and 50 ng of genomic DNA. Ampli-fication was performed in a thermocycler Techne as follows: one cycle of 3 min at 94°C; 30 cycles of 15 s at 94°C', 15 s at 60°C and 60 s at 72°C; final extension 5 min at 72°C. Sizing of PCR products was carried out using an ABI 3130XL Genetic Analyzer.

Data analysis

As the chloroplast genome is haploid and does not recombine, a unique combination of size length variants (alleles) across micro-satellite loci was defined as a distinct haplotype. Gene diversity (H_e) was calculated as:

$$He = 1 - \sum_{i=1}^{n} (p_i^2)$$

Where, *n* equals the number of alleles and p_i equals the frequency of the *i*th allele in the population (Weir, 1996). Haplotype diversity Hd was calculated in the same manner as gene diversity, with *n* and p_i referring to haplotypes.

Microsatellite data were scored for the presence (1) or absence (0) of alleles. 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 accessions 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).

RESULTS

The analysis of chloroplast microsatellites are summa-rized in Table 3. Size variation was observed for the five

Table 2. List of primers used in this study.

Locus	Core motif	Location	Primer sequence (5'- 3')
hvcppsbK	$(A)_{11}$	psbK/ORF174 intergenic region	TAGCCTTTGTTTGGCAAGCT TAAAACTTCTCGGCTTTTACCC
hvcptrnS1	$(A)_7$ CGC $(T)_{11}$	Downstream of *trnS*	CTTTAGCGGGCATTTCCATAA TGGTGGATTTGATAAGAACCC
hvcptrnS2	(T)10	Downstream of *trnS*	CAACTCCTTTGCGCTACACAAC CCCTTTTTTCCCATTCC
hvcprpoA	$(T)_8(CTT)_3$	Downstream of *rpoA*	CTCTCGTTTTAAATCCATTGCA TGATCCATTTCGCGAAAATA
hvcprps12	$(T)_8$	rpS12 intron I	AAGAAAGGGCTCCGGTGTAT CCACGATTTTTTATTCCACTCC

microsatellite loci. The number of alleles (size variants) per locus ranged from two to four, with gene diversity values ranging from 0.0416 (hvcprpoA) to 0.6535 (hvcptrnS2). Two alleles were found for hvcptrnS2 (108 and 109 bp), hvcptrnS1 (121 and 124 bp) and hvcprps12 (146 and 147 bp), three for hvcprpoA (118, 119 and 120 bp) and four for hvcppsbK (117, 118, 119 and 120 bp). The combination of alleles at each of five polymorphic loci generated 11 haplotypes (Table 4), of which four exist in wild barley, threein landraces and six in cultivars (Table 5).

Of the four haplotypes detected in wild barley, three (haplotypes 1, 2 and 3) were unique to wild barley accessions (Table 5) but one (haplotype 4) was shared by 13 cultivars (Table 5). Haplotype 1 was the most com-mon haplotype and found in all five provinces of South eastern Anatolia. Haplotype 3 was unique to Diyarbakır provinces. Haplotypic genetic diversity (Hd) value for wild accessions was 0.31 (Table 3). The two (hvcppsbK and hvcptrnS1) of five loci analyzed were polymorphic for wild populations. The haplotypic genetic diversity for these loci was 0.23 and 0.08 (Table 3). The level of genetic diversity observed at loci hvcptrnS1 and hvcptrnS2 was lower than the value recorded by Provan et al. (1999) who reported the value 0.11 and 0.25, respectively. Con-versely, the level of genetic diversity observed at locus hvcppsbK is higher than those recorded by Provan et al. (1999) who reported the value 0.11. The loci hvcprpoA and hvcprps12 were not found polymorphic in our study and that of Provan et al. (1999). In the previous studies, Neale et al. (1988) using chloroplast RFLPs found three haplotypes in a sample of 245 *H. vulgare* ssp. *spontaneum* accessions from Israel and Iran. Provan et al. (1999) using chloroplast microsatellites detected 11 haplotypes in the 51 *H*. *vulgare* ssp. *spontaneum* accessions which originated from Israel. We detected four haplotypes in the 23 *Hordeumvulgare* ssp. *spontaneum* accessions which originated from Southeastern Anatolia.

Three haplotypes (haplotypes 5, 6 and 7) were found in 47 landraces accessions (Table 5). One of them (haplotype 6) was also observed in a cultivar (Yesevi-93). Haplotype 5 was found in 38 landraces accessions. Hd value for landraces accessions was 0.33. The genetic diversity values at two polymorphic loci (hvcprpoA and hvcppsbK) were 0.31 and 0.12, respectively (Table 3).

Our data showed that the genetic diversity of wild accessions based on haplotype frequency (0.31) is nearly equal to that of landraces accessions (0.33).

Concerning Turkish barley cultivars, the six haplotypes (haplotypes 4, 6, 8, 9, 10, 11) were found. Haplotypes 4 and 8 were the most abundant in cultivars. Haplotypes 9, 10 and 11 were unique to three cultivars; Yeşilköy, Şahin-91, Aydanhanım, respectively. Hd value for cultivars was 0.6146. All loci were polymorphic and haplotypic genetic diversity at five loci varied from 0.08 (hvcptrnS1) to 0.41 (hvcprps12) (Table 3).

The distribution of haplotypes between wild barley and landraces is different. Indeed, the haplotype 1 was most abundant in the wild accessions, whereas the haplotype 5 was majority in the accessions of landraces. Most of the cultivars shared haplotype 4 with wild accession from Gaziantep.

A dendogram was constructed (Figure 1) to indicate the genetic diversity among all 94 samples. The dendogram was divided into two distinct groups that generally correlated with the sample types. Group I was comprised of all the Turkish landraces (subgroups I, II, III), and Group II consisted of all wild barley (subgroup I and III). Turkish barley cultivars were clustered into Group I and II. Six cultivars grouped together (Group I subgroup IV), with the exception of cultivar Yesevi (Group I subgroup I), while the other 16 cultivars grouped together (group II subgroup II).

As shown in dendrogram (Figure 1), a close affinity exists among accessions of *H. spontaneum* collected from same provinces (Adıyaman Tur0725, TUR0377, TUR0290, TUR0199, TUR0405; Gaziantep TUR3433, TUR3438, TUR3434; Şanlıurfa TUR1150, TUR1273, TUR0759, TUR1144, TUR1460; Hatay TUR3427, TUR3428, TUR3429, TUR3430; Diyarbakır TUR7842, TUR7841, TUR7849) but two wild accessions were (Şanlıurfa TUR2566, Hatay TUR3431) located distance

Table 3 Allele size, allelic frequency and diversity values (*H*) for the analyzed samples.

Loci	Allele Size	Wild accessions			Landraces accessions			Cultivars			Diversity values
		Allelic frequency	Numbers of alleles	H	Allelic frequency	Numbers of alleles	H	Allelic frequency	Numbers of alleles	H	
hvcptrnS2	108	-	1	0	-	1	0	12.5	2	0.22	0.6535
	109	100			100			87.5			
hvcprpoA	118	100	1	0	-	2	0.31	87.50	2	0.22	0.0416
	119	0			19.15			12.50			
	120	0			80.85			-			
hvcppsbK	117	8.70	3	0.23	-	2	0.12	4.17	3	0.16	0.0618
	118	86.96			-			4.17			
	119	4.35			6.38			91.67			
	120	-			93.62			-			
hvcptrnS1	121	4.35	2	0.08	-	1	0	4.17	2	0.08	0.6012
	124	95.65			100			95.83			
hvcprps12	146	100	1	0	-	1	0	70.83	2	0.41	0.4889
	147	-			100			29.17			
Mean			4	0.06		6		0.09	6	0.22	
Haplotype diversity*				0.31			0.33			0.61	

* Values based on haplotype are calculated by combining data from all five loci.

Table 4. Haplotypes defined and size of five chloroplast microsatellites.

Haplotype	Loci				
	hvcptrnS2	hvcprpoA	hvcppsbK	hvcptrnS1	hvcprps12
1	109	118	118	124	146
2	109	118	117	124	146
3	109	118	118	121	146
4	109	118	119	124	146
5	109	120	120	124	147
6	109	119	119	124	147
7	109	119	120	124	147
8	109	118	119	124	147
9	109	119	117	124	147
10	108	118	119	121	146
11	109	119	119	124	146

from the subgroup of wild accessions.

DISCUSSION

Our results show that wild barley, landraces and cultivars differ significantly from each other in the haplotype composition. In similar results with previous studies (Provan et al., 1999; Neale et al., 1988), we noted intra-specific chloroplast microsatellites variation in *H. vulgare* ssp. *vulgare* and *H. vulgare* ssp. *spontaneum*. Neale et al. (1988) suggested the need for assessing intraspecific cpDNA variability prior to choosing single accessions for phylogenetic constructions at the species level and higher.

Unique haplotypes were presented in most wild (haplotypes 1, 2, 3), landraces (haplotypes 5 and 7) and cultivars (haplotypes 8, 9, 10 and 11). Wild accessions and landraces do not share the same haplotypes. Only one haplotype (haplotype 6) detected in landraces is found in a cultivar (Yesevi-93), while 13 cultivars shared a haplotype (haplotype 4) with wild accession from Gaziantep. Molina-Cano et al. (2005) detected that 15 haplotypes were found, 11 in 34 *H. vulgare ssp. spontaneum* accessions and seven in 132 landraces and 20 cultivars. Landraces have four unique haplotypes and shared three haplotypes with *H. vulgare ssp. spontaneum* accessions. According to Provan et al. (1999) only two haplotypes were found in 125 landraces. These two

Table 5. Chloroplasts haplotypes in the wild, landraces and cultivars.

Accessions type, locations and cultivar names		Number of accessions	hvcptrnS2	hvcprpoA	hvcppsbK	hvcptrnS1	hvcprps12	Haplotype
Wild barley	Adıyaman	5	109	118	118	124	146	1
	Gaziantep	3	109	118	118	124	146	1
		1	109	118	119	124	146	4
	Şanlıurfa	5	109	118	118	124	146	1
		1	109	118	117	124	146	2
	Hatay	1	109	118	117	124	146	2
		4	109	118	118	124	146	1
	Diyarbakır	1	109	118	118	121	146	3
		2	109	118	118	124	146	1
Landraces	Turkey	38	109	120	120	124	147	5
		3	109	119	119	124	147	6
		6	109	119	120	124	147	7
Cultivars	Tarm-92	1	109	118	119	124	146	4
	Çetin-2000	1	109	118	119	124	146	4
	Yerçil	1	109	118	119	124	146	4
	Karatay-94	1	109	118	119	124	146	4
	Obruk-86	1	109	118	119	124	146	4
	Angora	1	109	118	119	124	146	4
	Çumra-2001	1	109	118	119	124	146	4
	Anadolu-98	1	109	118	119	124	146	4
	Tokak 157/37	1	109	118	119	124	146	4
	Bülbül-89	1	109	118	119	124	146	4
	Kral-97	1	109	118	119	124	146	4
	Bilgi	1	109	118	119	124	146	4
	Sladoran	1	109	118	119	124	146	4
	Yesevi-93	1	109	119	119	124	147	6
	Barbaros	1	109	118	119	124	147	8
	Zeynelağa	1	109	118	119	124	147	8
	Çatalhöyük	1	109	118	119	124	147	8
	Orza-96	1	109	118	119	124	147	8
	Erginel	1	109	118	119	124	147	8
	Balkan-96	1	109	118	119	124	146	8
	Anadolu-86	1	109	118	119	124	146	8
	Yeşilköy	1	109	119	117	124	147	9
	Şahin-91	1	108	118	119	121	146	10
	Aydanhanım	1	109	119	119	124	146	11

haplotypes were a subset of the 11 haplotypes found in ssp. *spontaneum* and one of these was found in all the European cultivars. These authors stated that this is a bottleneck effect resulting from the domestication of a species from its wild progenitor. Tanksley and McCouch (1997) denoted that genetic diversity is gradually reduced during the domestication process and further depleted in the breeding of varieties due to strong selection for agriculturally desirable traits. However, in contrast to the results of these authors who found much lower levels of diversity in cultivated barley, the levels of haplotypic diversity obtained for Turkish cultivars (0.61) are higher than that of landraces (0.33) and the wild barley accessions (0.31) (Table 3). Because of the fact that diverse foreign germplasm have hitherto been used as a parent in the breeding of some Turkish barley cultivars, the level of genetic diversity for cultivars was found higher than that of landraces and wild accessions. However, this study emphasized that barley domestication events in Turkey have not reduced genetic variations; there is still great potential in germplasm collection of CRIFC seed genebank to be evaluated in breeding. Domestic germplasm should be taken into consideration for cultivar breeding and incorporated into the crossing program

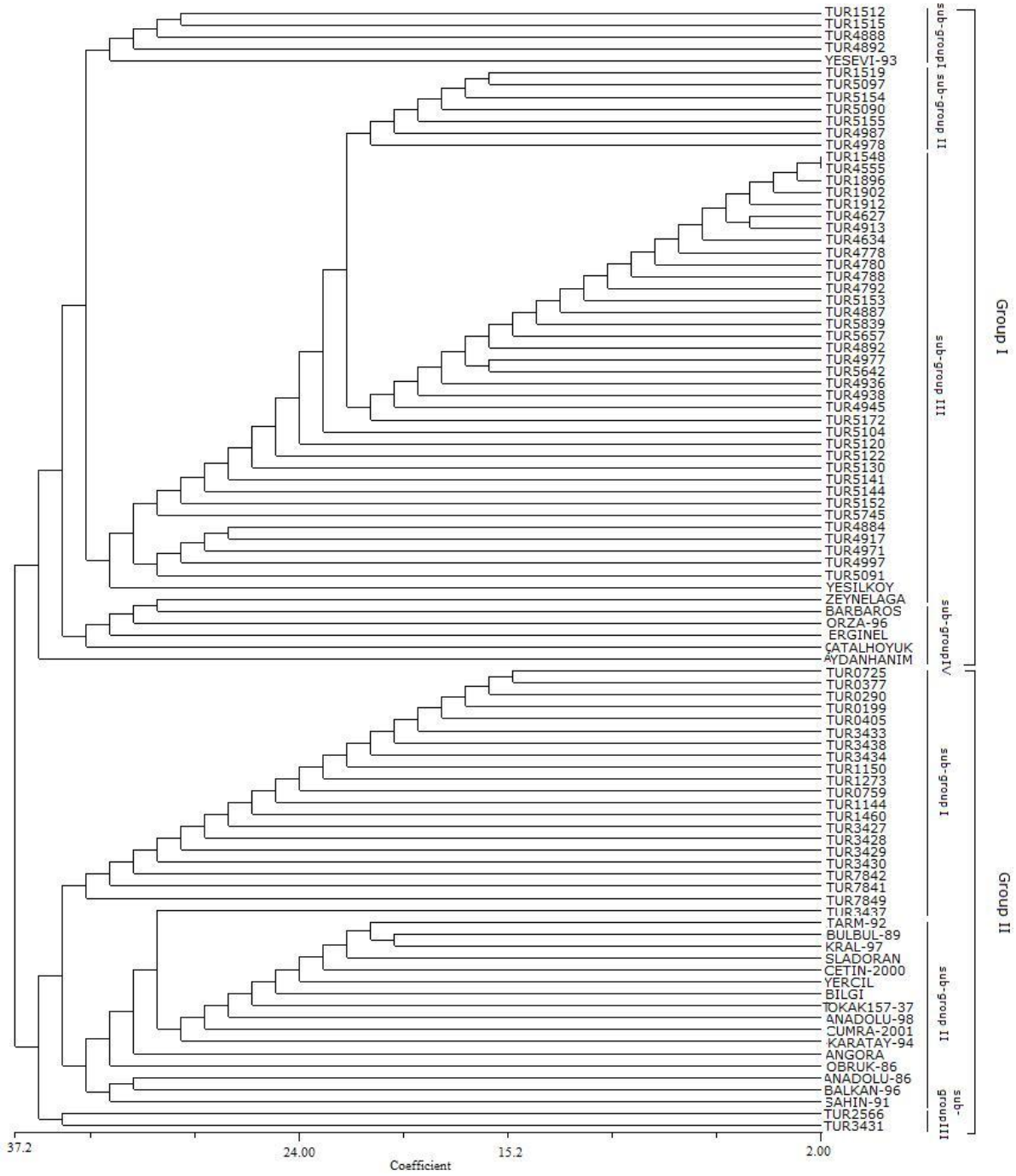

Figure 1. Dendrogram constituted by the UPGMA method.

in order to overcome genetic bottleneck.

The cluster analysis indicated that a close proximity exists between accessions of *H. spontaneum* collected from same provinces of Southern Anatolia (Group II subgroup I). However no very clear proximities exist between landraces and wild accessions. The landraces populations were found genetically different from natural populations of wild *H. spontaneum* in Southern Anatolia. This could possibly account for some of the cases where landraces have undergone important change since domestication and accumulated mutations for years or were artificially introduced from other areas.

Consequently, this study represents that the level of diversity in the barley collection of CRFIC seed genebank is considerably high in Turkey and also the result of this research recommend that chloroplast microsatellite markers offer a powerful tool to detect the genetic diversity in barley accessions.

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