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Full Length Research Paper

Determination of genetic diversity among Turkish durum wheat landraces by microsatellites

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Wheat landraces represent an important source of genetic variation that can be used for future wheat breeding program. The rich wheat landraces from Turkey have not been sufficiently analyzed genetically. For this reason, genetic diversity and relationship of the landraces must be determined. In this study, genetic diversity of 20 durum wheat landraces collected from different regions of Turkey were determined using 12 highly polymorphic microsatellite (SSR) markers. Genomic DNAs were isolated from ten accessions of all durum wheat landraces and specific regions were amplified with the polymerase chain reaction by using microsatellite markers. Polymorphic bands changed 4 to 9 per each SSR locus and the most polymorphic SSR loci were Wms 18, Wms 155, Xgwm 166 and Stm 578. The dendrogram showed that durum wheat landraces can be divided into two major groups. The coefficient of similarity among all germplasm ranged from 0.35 to 0.74. According to the estimated matrices and relationship among landraces accessions, it was determined that the 46847-47341, 31738-52841 and 31634-47923 were genetically the closest. The farthest genotypes were 52835-1680. The results showed that durum wheat landraces have high genetic variability and microsatellite DNA markers could be successfully employed for revealing the variability.

Key words: Durum wheat, landraces, genetic diversity, microsatellite markers.

INTRODUCTION

Wheat is a staple food crop all over the world and is the most widely grown crop in the world and Turkey. Durum wheat (*Triticum turgidum var. durum*) is cultivated on 10% of the world wheat areas and is an important food crop in the world. The total area and production is about 20 million hectares and 30 million metric tons globally (Kahrizi et al., 2010). Since further expansion of cultivated area is not possible, the most efficient way of supplying necessary nutrition for increasing population is to increase productivity of wheat cultivation. One of the yield increases in wheat can be achieved through improvement of varieties which are resistant to disease and insect pests and also have stability across different environments and seasons. Selections toward specific targets during long breeding programs and use of common parents in crosses made the genetic base of cultivated wheat narrow and made it difficult to develop new varieties through classical plant breeding. The improvement of new varieties which have desired properties can be achieved through landraces especially for drought prone areas (Akar et al., 2009).

Breeding studies that are intended to broaden narrowed genetic base and increase yield, can benefit from landraces. Landraces undoubtedly represent an important source of genetic variation in wheat (Dreisigacker et al., 2005; Akar and Özgen, 2007). Landraces have many important traits because of long-term adaptation in their existed location. Wheat landraces were grown in different regions of Turkey, which have resistant gene to many biotic and abiotic stress factors. Moreover, some of them have many high grain quality gene or alleles.

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Table 1. The accession numbers and locations of landraces collected from different regions of Turkey.

Number	Accession number	Location	Number	Accession number	Location
1	TR 1680	İzmir	11	TR 46847	Hakkari
2	TR 31634	Mardin	12	TR 47341	Adiyaman
3	TR 31738	Siirt	13	TR 47923	Şanliurfa
4	TR 31760	Gaziantep	14	TR 47937	Adiyaman
5	TR 36935	Balikesir	15	TR 52719	Balikesir
6	TR 37094	Çorum	16	TR 52835	Burdur
7	TR 37180	Sinop	17	TR 52841	Denizli
8	TR 39321	Kastamonu	18	TR 55109	Uşak
9	TR 45305	Yozgat	19	TR 55173	Kütahya
10	TR 46555	Muğla	20	TR 57129	Antalya

Genetic diversity of the wheat landraces must be investigated for use in wheat breeding. More information about the genetic diversity within and relationships among landraces would be invaluable for the conservation and utilization of existing genetic resources (Warburton and Hoisington, 2001; Zhang et al., 2006).

Microsatellites, also known as simple sequence repeats (SSRs), have emerged as an important source of DNA markers and have been successfully applied to detect genetic diversity (Dreisigacker et al., 2005; Wei et al., 2005; Zeb et al., 2009). SSR markers have many advantages. They are locus specific and abundant. Besides, they are distributed over the genome and require only small amounts of genomic DNA for analysis. The SSRs are highly polymorphic, even among closely related cultivars. Microsatellites are one of the most promising molecular marker types that are able to identify genotypes within a species (Fahima et al., 2002; Singh et al., 2010). These properties make SSRs popular for genetic diversity studies.

Microsatellites have been successfully applied in wheat for detection of genetic diversity (Huang et al., 2002; Wei et al., 2005; Zarkti et al., 2010), for genome mapping (Röder et al., 1998) and for marker assisted selection of agronomically important traits (Ateş Sönmezoglu et al., 2010). In wheat, microsatellites show a much higher level of polymorphism than other marker systems for assessment of genetic diversity. Microsatellites have been commonly used in wheat genomic research (Plaschke et al., 1995; Zhang et al., 2006; Mangini et al., 2010). Detection of genetic variation and determination of genetic relationships between wheat individuals and populations are important considerations for the efficient conservation and utilization of plant genetic resources (Mandoulakani et al., 2010).

The aim of this study is to screen some Turkish durum wheat landraces collected from highly diverse ecological regions in order to determine genetic variability and relationship.

MATERIALS and METHODS

Plant Materials

In this study, twenty durum wheat landraces were investigated. The accession numbers of the landraces were obtained from the Ege Agricultural Research Institute in İzmir, Turkey and are presented in Table 1. Twenty *Triticum turgidum durum* landraces were collected from different regions of Turkey. Additionally, a Mexican wheat variety Opata 85 was used as reference plant variety in molecular characterization. The geographic distribution of 20 durum wheat landraces is shown in Figure 1.

DNA isolation and PCRs

DNA was extracted from leaf material of each genotype according to Doyle and Doyle (1990) with some modifications. Total 25 microsatellite primers were tested and the most polymorphic set of primers were used. DNAs of durum wheat genotypes were amplified with 12 different microsatellite primers. Chromosomal location, allele number, range of allele size and PCR product size of these durum wheat landraces together with cv. Opata by microsatellite markers are shown Table 2 (Röder et al., 1998).

PCR amplifications were performed according to Röder et al. (1998) with some modifications. Polymerase chain reactions were carried out in 30 µl volume in a Thermo (Px2) thermocycler. PCR mixes contained 50 ng of wheat genomic DNA, 0.25 µM of each primer, 0.2 µM dNTP mix, 2.5 µM $MgCl_2$, 10 x PCR Buffer and 0.5 units of *Taq* DNA polymerase. The PCR cycling reactions were hot started for 5 min at 95°C. The 30 cycles were performed as follows: 94°C 1 min, 1 min at 50 to 60°C (different annealing temperature of primers), 1 min at 72°C, and a final extension step for 5 min at 72°C. PCR products were separated on 8% denatured polyacrylamide gels and visualized by ethidium bromide. Electrophoresis was applied at 300 W constant power for 7 to 8 h. 0.5 x TBE buffer was used as the running buffer during electrophoresis. The gels were stained by ethidium bromide for 15 min.

Statistical analysis

Polymorphisms among amplified bands were determined by using the Vilber Lourmat, Bio 1D, 11.04 version. Comparisons of genotypes and genetic relationships between genotypes were estimated by Numerical Taxonomy and Multivariate Analysis System software

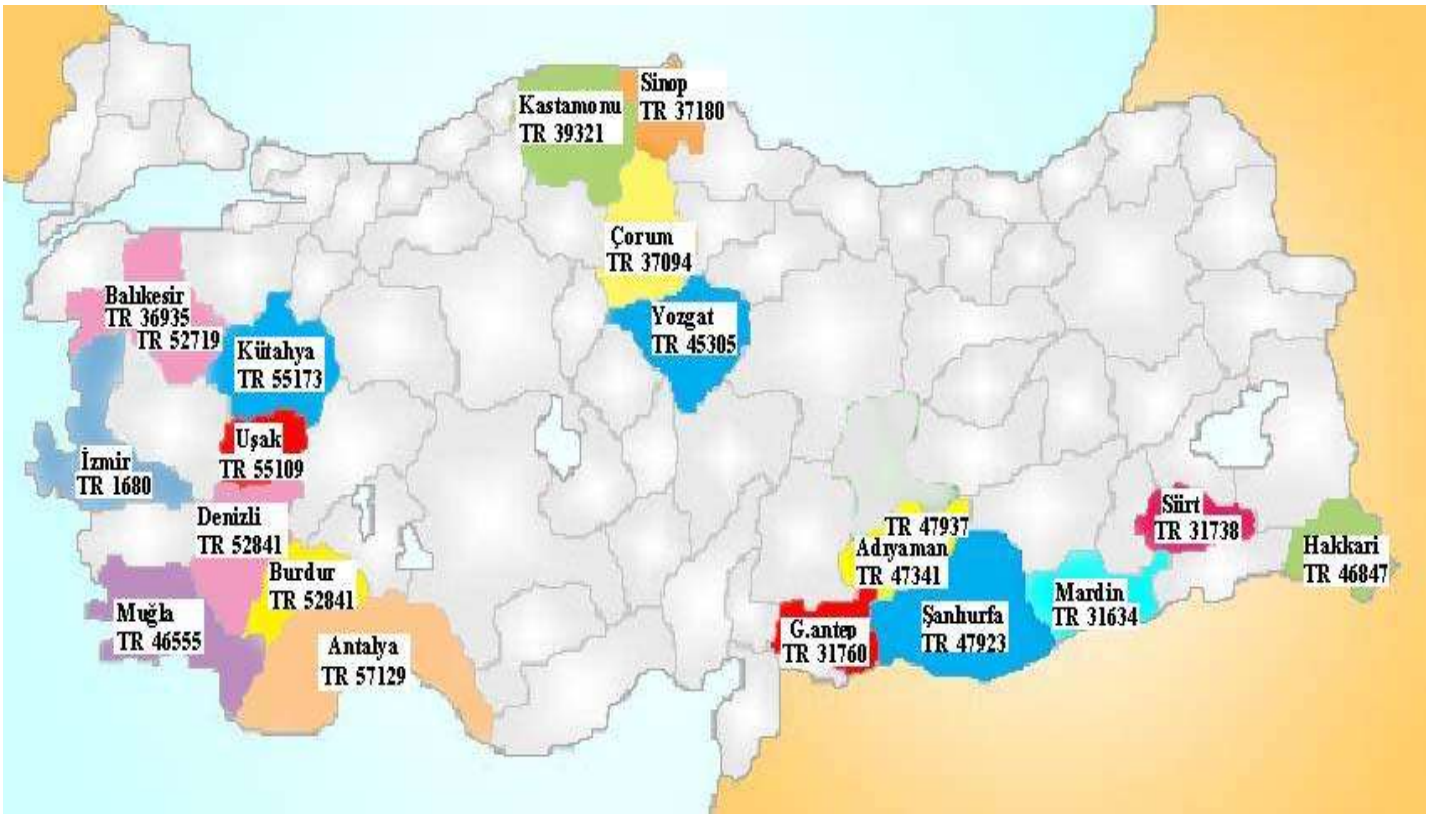

Figure 1. Geographic distribution of the landraces in Turkey (www.tarim.gov.tr).

Table 2. SSR primers and their molecular data.

Microsatellite loci	Chromosomal location	Number of allele	Range of allele size (bp)	Opata PCR product size (bp)
Wms 18	1B	9	171-201	188
Wms 155	3A	9	117-152	145
Wms 357	1A	7	110-128	121
Wms 408	5B	4	147-187	149
Xgwm 95	2A	6	117-137	130
Xgwm 130	7A	7	114-137	127
Xgwm 166	-	9	137-161	153
Hvole	Chr4	5	90-99	94
Hvm40	Chr4	7	132-155	139
Stm 560	7B	7	164-184	187
Stm 578	3B	9	147-175	176
Stm 635	3A	8	110-124	112

(NTSYSpc, version 2.11). The program was used to obtain dendrograms (Figure 3) showing the genetic distance between landrace genotypes.

RESULTS AND DISCUSSION

A total of 25 microsatellite primers (Wms 3, 11, 18, 46, 155, 325, 357, 408, 513, Xgwm 95, 130, 166, 190, 295, 389, 458, 577, Hvole, Hvm 40, Stm 18, 527, 560, 578, 626 and 635) were tested and selected, and the most polymorphic primers (Wms 18, 155, 357, 408, Xgwm 95, 130, 166, Hvole, Hvm 40, Stm 560, 578, 635) were used in this study (Figure 2). The allele number and allele size of the primers are presented in Table 2. The number of alleles detected by a primer ranged from 4 to 9 among

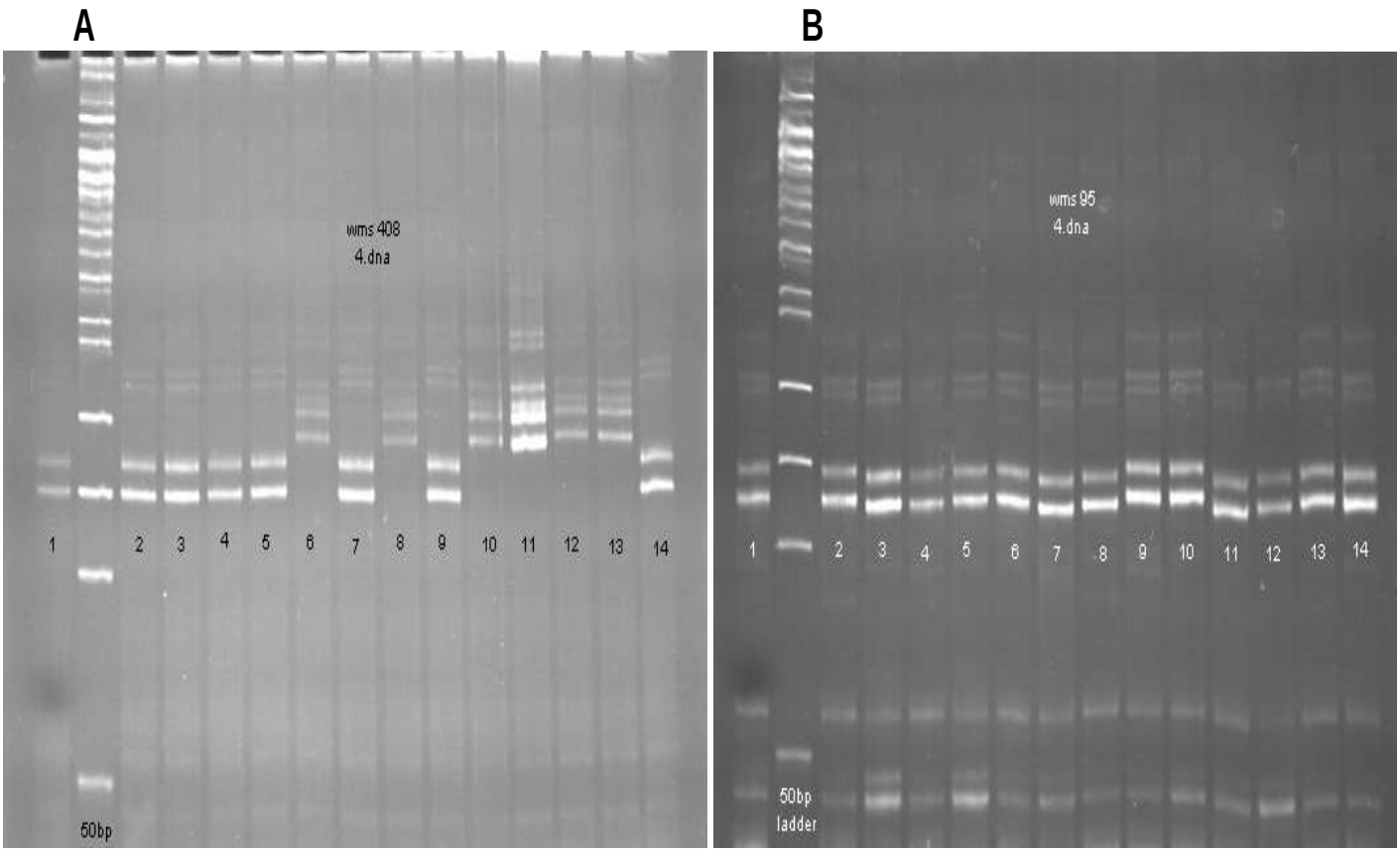

Figure 2. The SSR marker profiles of landraces using Wms 408 (A) and Xgwm 95 (B).

the landraces. The total allele number in the constructed dendrogram was 45. The most polymorphic microsatellite markers were Stm 578, Wms 18, Wms 155 and Xgwm 166 with 9 alleles. Dreisigacker et al. (2005) observed that higher diversity within the Turkish than within the Mexican landraces. This can be explained by a much longer evolutionary history of wheat landraces in Turkey.

Similarity and diversity between and within durum wheat landraces included in this study were estimated. The coefficient of similarity ranged from 0.35 to 0.74. The landraces were clustered into different groups and subgroups. The 20 durum wheat landraces formed two major groups. Group 1 consisted of 52835, while group 2 included all the remaining landraces. Group 2 was separated into several different subgroups. According to the estimated relationships among landrace accessions, it was found that the 46847-47341, 31738-52841 and 31634-47923 were genetically the closest ones. The most distinctive landraces were 52835-1680. The landraces 46847 collected from Hakkari and the 47341 collected from Adiyaman were not distinguishable. It was determined that the genetic relationship between 31738 (from Siirt) and 52841 (from Denizli) was very close at molecular level. However, Siirt and Denizli were geographically 1473 km far away each other. These results indicated that the genetic relationships between these durum wheat landraces did not overlappwith their geographic origin. This clear evident which was also mentioned by Fahima et al. (2002) show us that geographic distance alone may not explain genetic diversity between landraces .

The results showed that Turkish durum wheat landraces have high genetic variability. The genetic variability of landraces has been affected by various factors throughout their evolutionary history. Genetic variability between landraces is an indicator that selection criteria of farmers are different. This also shows that genetic structures of landraces grown region are more different than varieties in other regions. This is an important genetic treasury and an indicator that landraces are still quite important as germplasm for plant breeders. In fact, this result is an expected and desirable situation and similar findings have been determined from different materials by many scientists (Strelchenko et al., 2004; Dreisigacker et al., 2005; Wei et al., 2005).

In our study, variations between accessions of landraces were also established. Ten different samples of each landraces clustered together, and genetic differences within the landrace genotypes were significant. This condition is an expected and desirable property in

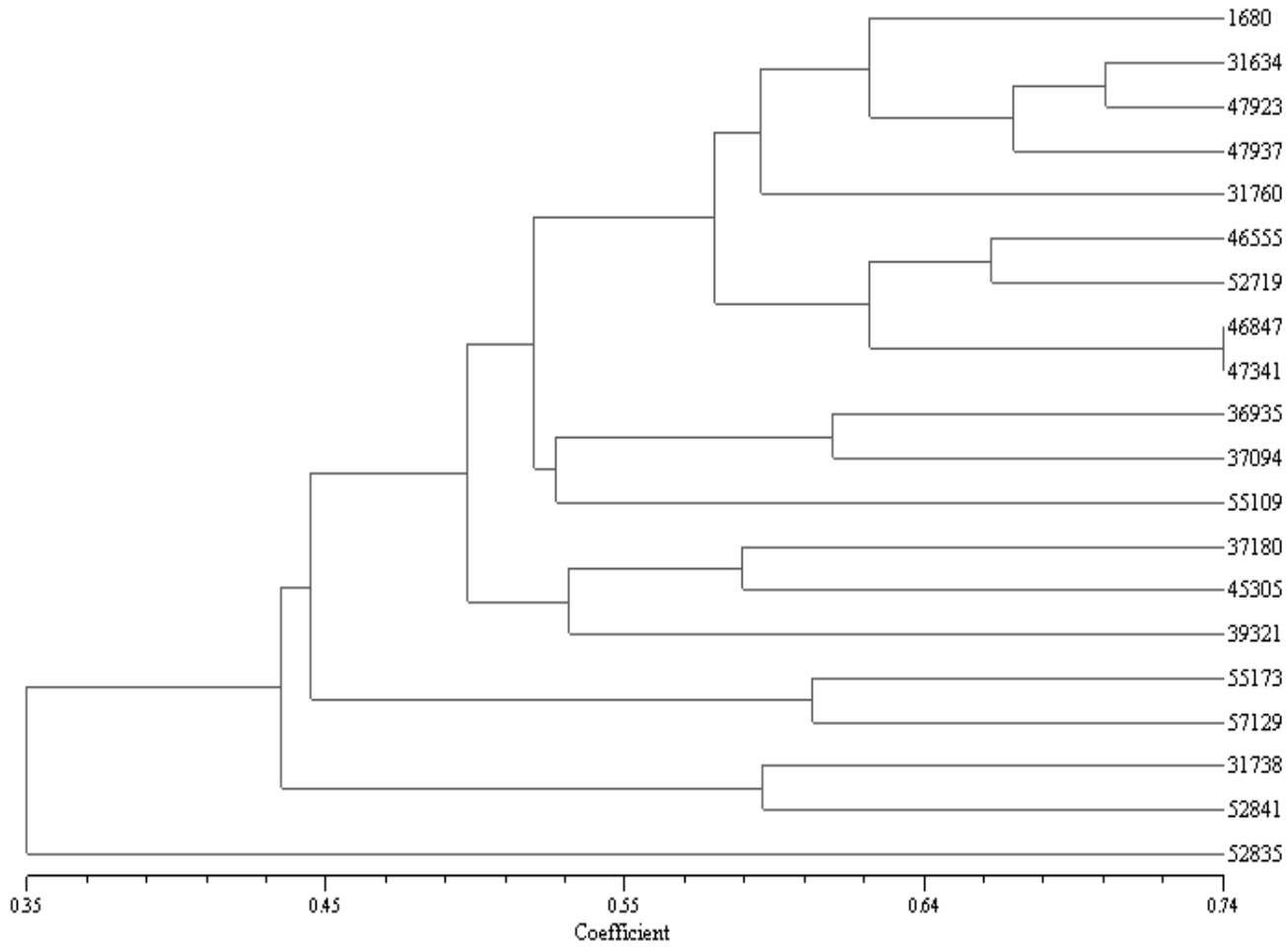

Figure 3. Dendrogram based on SSR markers of the durum wheat landraces.

landraces, because they are genetically heterogeneous. This was observed in many landraces used in this study. Similar results have also been found by other researchers (Eujayl et al., 2001; Huang et al., 2002). Eujayl et al. (2001) reported that many durum wheat genotypes showed compound SSR profiles (2 or 3 alleles); this is either due to their heterozygosity or different genetic structure. This is expected because the genotypes are not pure lines.

The results of this study indicate that a relatively small number of microsatellite primers could be used to distinguish genetic variation among wheat genotypes (Wei et al., 2005; Zarkti et al., 2010). Simple sequence repeats (SSRs) provide important information about the genetic variation of wheat landraces. Plaschke et al. (1995), Fahima et al. (1998) and Dreisigacker et al. (2005) obtained similar results in tetraploid and hexaploid wheat. The results obtained in this study indicates that simple sequence repeats are useful for the estimation of the genetic relationship between and within wheat landraces and SSRs are highly polymorphic in durum wheat germplasm (Prasad et al., 2000; Zeb et al., 2009; Mangini et al., 2010).

In conclusion, microsatellite markers demonstrated genetic differentiation and similarity between and within twenty Turkish durum wheat landraces and the valuable genetic distances between landraces facilitate the use of these lines in durum wheat breeding programs.

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