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Introduction

Wheat (*Triticum aestivum* L.) is the most important crop for Turkey, which is also one of the gene centers of wheat (Gökgürl 1939; Vavilov 1950; Harlan 1971; Özkan et al. 2002). Genetic variability is of prime importance for the improvement of many crop species, including wheat, and nearly all crop improvement programs depend on genetic diversity in the available germplasm (Graner et al. 1994; Sorrells and Wilson 1997).

Molecular markers based on polymerase chain reaction (PCR) methods, such as simple sequence repeats (SSRs) or microsatellites, have become important genetic markers in a wide range of crop species, including wheat (Ma et al. 1996). SSR markers are abundant, dispersed throughout the genome, and show higher levels of polymorphism than other genetic markers (Russell et al. 1997). These features, coupled with their ease of detection, make them ideal for identifying and distinguishing between accessions that are genetically very similar (Saker et al. 2005). Various studies have used SSR markers to investigate genetic diversity in cultivated hexaploid wheat genotypes of *T. aestivum* L. (Dreisigacker et al. 2005; Liu et al. 2005; Hao et al. 2006; Landjeva et al. 2006; Salem et al. 2008; Schuster et al. 2009). SSR markers have been successfully employed to characterize genetic diversity in seed bank collections of improved wheat germplasm (Börner et al. 2000; Huang et al. 2002) and wild relatives (Li et al. 2000; Hammer 2000).
The objectives of the present study were to evaluate the use of genomic SSRs for
determining genetic diversity among winter-type bread wheat genotypes from the
breeding program of the Anatolian Agricultural Research Institute and to compare
genetic distances based on SSRs with the polymorphic information content (PIC)
estimates of these wheat cultivars.

Materials and Methods

Plant Materials and DNA Extraction

Seven homozygous bread wheat genotypes were obtained from the Anatolian
Agricultural Research Institute in Eskisehir, Turkey; four were yellow rust-resistant
cultivars (PI178383, Izgi2001, Sonmez2001, Altay2000) and three were yellow
rust-susceptible cultivars (Harmankaya99, ES14, Aytin98). The miniprep method of
Weining and Landridge (1991), modified by Song and Henry (1995), was used to
extract total genomic DNA from leaves collected from resistant and susceptible
plants.

SSR Marker Analysis

Information on 223 genomic SSRs was obtained from a genetic map developed at
IPK Gatersleben by Röder et al. (1998). All 223 SSR primers were used for marker
analysis. The PCR volume was 25 µl, consisting of 400 nmol of forward and
reverse primers, 0.2 mM dNTP (MBI Fermentas, Germany), 2.5 mM MgCl₂,
1× PCR buffer, 0.625 U Taq DNA polymerase, and 100 ng genomic DNA as a
template. The thermal cycling consisted of 3 min at 94°C (initial denaturation); 40
cycles of 1 min at 94°C, 1 min at the annealing temperature (50, 55, or 60°C), and
1 min at 72°C; followed by a final extension at 72°C for 10 min. PCR amplification
products were separated by electrophoresis on 2.5% TBE agarose gels at 100 V for
3 or 4 h and stained with ethidium bromide for visualization under UV light.

Assessment of Genetic Diversity with Statistical Analysis

Each band amplified by each primer was scored as present (1) or absent (0) for the
seven cultivars, and the data were entered into a binary matrix as discrete variables
(1 for presence and 0 for absence of a homologous fragment). The MVSP software
(Kovach 1999) package version 3.1 was used to calculate Jaccard’s similarity
coefficients. Using these coefficients and the neighbor-joining algorithm, a
dendrogram was constructed. Genetic relationships among the seven genotypes in
this study were investigated using an unweighted pair-group method (UPGMA)
cluster analysis of Jaccard’s genetic identities for the accessions. The average PIC
was calculated for each SSR across all individuals, applying the formula given by
Powell et al. (1996), $\text{PIC} = 1 - \sum (P_i)^2$, where $P_i$ is the proportion of
the population carrying the $i^{th}$ allele, calculated for each SSR locus (Botstein et al.
1980). Average heterozygosity ($H_{av}$) is obtained by taking the average of PIC values.
for all the markers, calculated as $H_{av} = \sum [2fi (1-fi)]/N$, where $N$ is the sample size. The multiplex ratio ($MR = m + p/n$) for each assay was estimated by dividing the total number of monomorphic ($m$) and polymorphic ($p$) bands amplified by the total number of assays, or primer combinations employed ($n$). The marker index ($MI = H_{av} \times MR$) is the average heterozygosity ($H_{av}$) multiplied by the $MR$ (Powell et al. 1996).

Results

SSR Marker Data

Of the 223 SSR primers, 216 produced reproducible bands, and 142 of those primers can be used for genetic diversity assessment of the seven wheat varieties because of polymorphism displayed among the wheat cultivars. The markers covered all 21 chromosomes of the hexaploid wheat genome (with 1–4 SSR markers per chromosome) and characterized the genetic diversity of the seven genotypes. One hundred of the 223 SSR primers examined produced polymorphic fragments, whereas 42 primers amplified both polymorphic and monomorphic fragments. The present study shows a relatively high level of polymorphism (66.45%) among the seven genotypes. The SSR analysis produced 741 fragments, with molecular weights of 30–1100 bp. Amplification produced 407 fragments in cultivar PI178383, 416 in Harmankaya99, 397 in Altay2000, 404 in Izgi2001, 395 in ES14, 417 in Sonmez2001, and 398 in Aytin98.

Banding patterns for each genotype were distinguished with 87 primers (41% of all SSR primers tested). There were 152 unique fragments (20.51%) that could distinguish genotypes: 39 in PI178383, 12 in Harmankaya99, 17 in Altay2000, 18 in Izgi2001, 13 in ES14, 23 in Sonmez2001, and 36 in Aytin98. Primer XGWM72 gave 7 distinguishable fragments (3 for Aytin98, 2 for ES14, 1 each for PI178383, and Izgi2001), XGWM296 gave 6 (3 for Harmankaya99, 1 each for PI178383, Harmankaya99, and Altay2000), XGWM234 gave 5 (2 each for PI178383 and Aytin98, 1 for ES14), XGWM408 gave 5 (2 each for Sonmez2001 and Aytin98, 1 for Izgi2001), and XGWM296 gave 4 (3 for Sonmez2001, 1 for PI178383).

The loci used in this study ranged from one fragment (for 49 primers) to 18 fragments (for Xgwm642), with an average of 3.09 alleles per locus.

The PIC values per locus ranged from 0.2149 for the Xgwm182 locus to 0.9272 for Xgwm642, with an average of 0.5217 for all loci (Table 1).

Phylogenetic Data

In the dendrogram, the range of genetic distance or coefficient of similarity among wheat cultivars was 0.479–1.000 (Fig. 1). Cultivar PI178383 constitutes a more peripheral branch of the dendrogram, being the most differentiated of the seven genotypes. The analysis of these data revealed greater similarity between Altay2000 and ES14 (0.633) and less similarity between PI178383 and Sonmez2001 (0.479). Those cultivars that display similar coefficients of similarity are genetically close to
one another, while those having variable coefficients of similarity are dissimilar. Under these conditions, a greater genetic distance was determined between Sonmez2001 and PI178383.

Table 1 Genetic diversity of seven *Triticum aestivum* genotypes based on SSR marker analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Annealing temperature</th>
<th>Total or average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60°C</td>
<td>55°C</td>
</tr>
<tr>
<td>Primers used in the study</td>
<td>123</td>
<td>75</td>
</tr>
<tr>
<td>Primers that amplified fragments</td>
<td>119</td>
<td>74</td>
</tr>
<tr>
<td>Primers that did not amplify fragments</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Primers that amplified polymorphic fragments</td>
<td>51</td>
<td>43</td>
</tr>
<tr>
<td>Primers that amplified monomorphic fragments</td>
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<td>20</td>
</tr>
<tr>
<td>Primers that amplified both polymorphic and monomorphic fragments</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>Polymorphic fragments</td>
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<td>221</td>
</tr>
<tr>
<td>Monomorphic fragments</td>
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<td>56</td>
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<td>Total fragmentsa</td>
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<td>277</td>
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<tr>
<td>Percentage of polymorphism</td>
<td>74.88</td>
<td>79.78</td>
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<tr>
<td>Polymorphic fragments per primer</td>
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<tr>
<td>Multiplex ratio (total bands per primer)b</td>
<td>3.50</td>
<td>3.74</td>
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<td>Average heterozygosity ((H_{av}))</td>
<td>0.6049</td>
<td>0.6209</td>
</tr>
<tr>
<td>PIC</td>
<td>0.5423</td>
<td>0.5641</td>
</tr>
<tr>
<td>Marker index ((H_{av} \times MR))</td>
<td>2.12</td>
<td>2.32</td>
</tr>
</tbody>
</table>

a Primer combinations employed

b MR estimated by dividing the total number of bands (monomorphic and polymorphic) amplified by the total number of assays

Fig. 1 Genetic similarity of seven wheat genotypes, based on Jaccard’s coefficient and UPGMA cluster analysis of 741 alleles detected by 216 SSRs
Discussion

The SSR markers in this study yielded reproducible polymorphic bands in seven genotypes of *T. aestivum*, providing a powerful and reliable molecular tool for analyzing genetic diversity and relationships in wheat. In a study by Ahmad (2002), 13 wheat cultivars of diverse origins were evaluated using 43 SSR markers, selected on the basis of their known genetic locations to give uniform coverage for all three wheat genomes (A, B, and D). The study detected 156 polymorphic alleles at 43 loci, with a wide range of allelic variants for each locus; the range of alleles per locus was 2–8 (average 3.6). Another study (Prasad et al. 2000) examined the utility of a set of 20 wheat SSR markers to detect DNA polymorphism, identify genotypes, and estimate genetic diversity among 55 elite wheat genotypes. The range of alleles per locus was 1-13, averaging 7.4, and the PIC range was 0.21–0.90, averaging 0.71. Our study found a similar PIC range (0.21–0.93), and the average PIC for these markers was estimated to be 0.52; however, our range for the number of alleles per locus was 1–18, with an average of 3.05.

In estimating the minimum number of SSR loci needed to reveal the genetic relationship among wheat varieties, it has been suggested that scanning 71–73 SSR loci with higher diversity could reflect the genetic relationship among wheat germplasms objectively and build a stable dendrogram (Zhang et al. 2002; You et al. 2004). In our study, 216 SSR primers reported by Röder et al. (1998) were used to assess genetic diversity among wheat genotypes. Fufa et al. (2005) have reported that 68 wheat SSR markers screened for amplification products and polymorphism information produced 141 bands (monomorphic and polymorphic) across 30 hard red winter wheat cultivars, with a range of 1–5 and average of 3 bands per locus. Genetic diversity per locus was 0.289–0.958, and the average genetic distance across all loci in 30 cultivars was 0.623. The average genetic distance from all 68 markers was 0.427. Fufa et al. (2005) suggest that the higher SSR-based distance could be due to more complete coverage of the genomes by the markers or to the diversity of the lines used in their study. Using a more diverse set of cultivars, Almanza-Pinzon et al. (2003) found higher levels of diversity; their SSR markers were more polymorphic than those in previous studies (Plaschke et al. 1995; Bohn et al. 1999). In our study, the percentage of polymorphism was calculated as 66.45%, the range of genetic similarity was 0.479–0.633, and the average genetic distance across all loci in seven cultivars was 0.556. In this regard, the genetic diversity of seven winter hexaploid wheat genotypes was assessed by 223 SSR markers covering all 21 chromosomes with 1–4 SSR markers per chromosome.

Knowledge of the genetic diversity of a species is important for the choice of crossing parents in an accession and hybrid breeding (Tams et al. 2004). Using SSRs, our study found considerable diversity among wheat accessions at the DNA level and identified diverse genotypes (e.g., PI178383) for use in breeding programs for wheat improvement. The F$_2$ individuals derived from these cultivar crosses were screened for resistance to yellow rust at the seedling stage in greenhouses and at the adult stage in the field to identify DNA markers genetically linked to resistance (Akfirat et al. 2010; Ercan et al. 2010). Analyzing higher numbers of genotypes may
not add much practical value to a general plant improvement program, unless a specific crossing program is aimed toward the improvement of specific traits (Ahmad 2002). It is therefore important that a focused breeding approach should be adopted while analyzing genetic diversity estimates for suitable parent selection to gain high value and practical impact on a breeding program.

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