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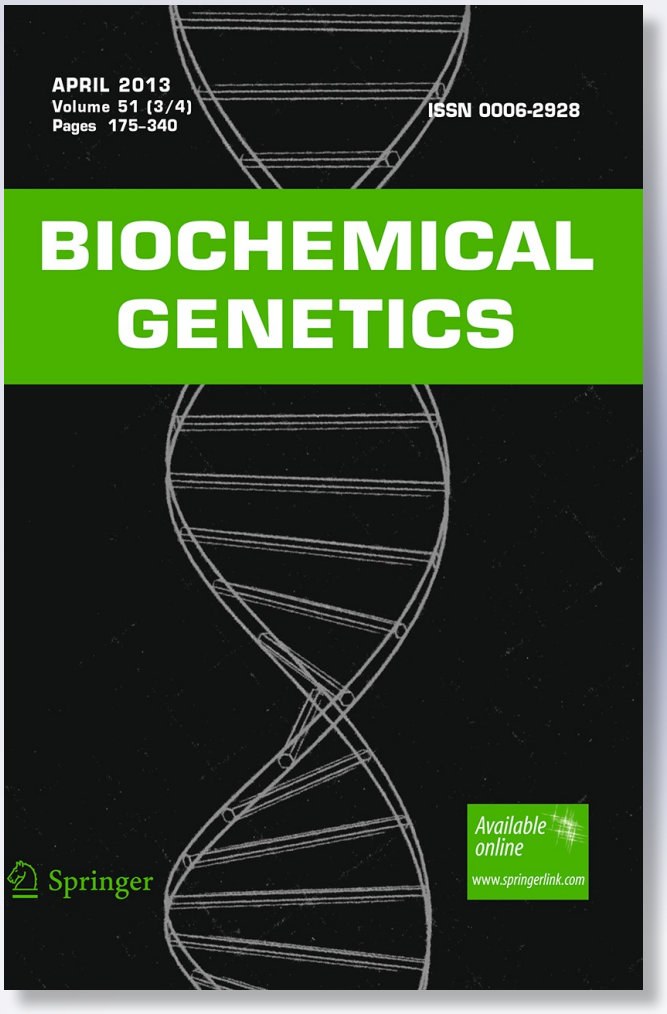

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NOTE

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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 (Gökgöl 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).

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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 $MgCl_2$, 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), $\mathrm{PIC} = 1 - \sum(P_i)^2$, 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 wheat 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

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

Parameter	Annealing temperature			Total or average
	60°C	55°C	50°C	
Primers used in the study	123	75	25	223
Primers that amplified fragments	119	74	23	216
Primers that did not amplify fragments	4	1	2	7
Primers that amplified polymorphic fragments	51	43	6	100
Primers that amplified monomorphic fragments	35	20	17	72
Primers that amplified both polymorphic and monomorphic fragments	31	11	–	42
Polymorphic fragments	310	221	21	552
Monomorphic fragments	107	56	26	189
Total fragments[a]	417	277	47	741
Percentage of polymorphism	74.88	79.78	44.68	66.45
Polymorphic fragments per primer	3.78	4.09	3.50	3.79
Multiplex ratio (total bands per primer)[b]	3.50	3.74	2.04	3.09
Average heterozygosity (H_{av})	0.6049	0.6209	0.5221	0.5829
PIC	0.5423	0.5641	0.4586	0.5217
Marker index ($H_{av} \times$ MR)	2.12	2.32	1.07	1.84

[a] Primer combinations employed

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

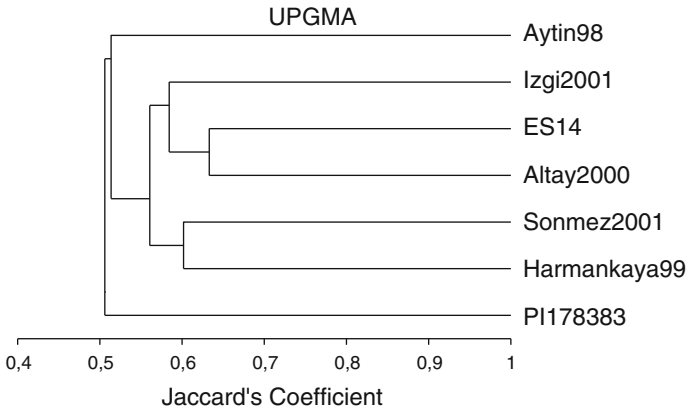

Fig. 1 Genetic similarity of seven wheat genotypes, based on Jaccard's coefficient and UPGMA cluster analysis of 741 alleles detected by 216 SSRs

one another, while those having variable coefficients of similarity are dissimilar. Under these conditions, a greater genetic distance was determined between Sonmez2001 and PI178383.

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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References

Ahmad M (2002) Assessment of genomic diversity among wheat genotypes as determined by simple sequence repeats. Genome 45:646–651

Akfirat FS, Aydin Y, Ertugrul F, Hasancebi S, Kazan K, Budak H, Akan K, Mert Z, Bolat N, Yorgancılar O, Uncuoglu AA (2010) A microsatelite marker for yellow rust resistance in wheat. Cereal Res Commun 38:203–210

Almanza-Pinzon MI, Khairallah M, Fox PN, Warburton ML (2003) Comparison of molecular markers and coefficients of parentage for the analysis of genetic diversity among spring bread wheat accessions. Euphytica 130:77–86

Bohn M, Utz HF, Melchinger AE (1999) Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs and SSRs and their use for predicting progeny variance. Crop Sci 39:228–237

Börner A, Chebotar S, Korzun V (2000) Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long-term maintenance. Theor Appl Genet 100:494–497

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Dreisigacker S, Zhang P, Warburton ML, Skovmand B, Hoisington D, Melchinger AE (2005) Genetic diversity among and within CIMMYT wheat landrace accessions investigated with SSRs and implications for plant genetic resources management. Crop Sci 45:653–661

Ercan S, Ertugrul F, Aydın Y, Senturk-Akfırat F, Hasancebi S, Cetin L, Akan K, Mert Z, Bolat N, Cakmak M, Altınkut-Uncuoglu A (2010) An EST-SSR marker linked with yellow rust resistance in wheat (*Triticum aestivum* L.). Biol Plantarum 54:691–696

Fufa H, Baenziger PS, Beecher BS, Dweikat I, Graybosch RA, Eskridge KM (2005) Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. Euphytica 145:133–146

Gökgöl M (1939) Turkish wheats. Yeşilköy Seed Breeding Institute Publications 2(14). Tan Press, Istanbul (in Turkish)

Graner A, Ludwig WF, Melchinger AE (1994) Relationship among European barley germplasm, II: comparison of RFLP and pedigree data. Crop Sci 34:1199–1205

Hammer K (2000) Microsatellite markers: a new tool for distinguishing diploid wheat species. Genet Resour Crop Evol 47:497–505

Hao C, Wang L, Zhang X, You G, Dong Y, Jia J, Liu X, Shang X, Liu S, Cao Y (2006) Genetic diversity in Chinese modern wheat varieties revealed by microsatellite markers. Sci China, Ser C Life Sci 49:218–226

Harlan JR (1971) Agricultural origins: centers and noncenters. Science 174:468–473

Huang XQ, Börner A, Röder MS, Ganal MW (2002) Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor Appl Genet 105:699–707

Kovach WL (1999) MVSP: A multivariate statistical package for Windows, version 3.1. Kovach Computing Services, Pentraeth, p 133

Landjeva S, Korzun V, Ganeva G (2006) Evaluation of genetic diversity among Bulgarian winter wheat (Triticum aestivum L.) varieties during the period 1925–2003 using microsatellites. Genet Res Crop Evol 53:1605–1614

Li YC, Fahima T, Peng JH, Röder MS, Kirzhner VM, Beiles A, Korol AB, Nevo E (2000) Edaphitic microsatellite DNA divergence in wild emmer wheat, *Triticum dicoccoides*, at a microsite: Tabigha, Israel. Theor Appl Genet 101:1029–1038

Liu ZH, Anderson JA, Hu J, Friesen TL, Rasmussen JB, Faris JD (2005) A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor Appl Genet 111:782–794

Ma ZQ, Röder MS, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123–130

Özkan H, Brandolini A, Schafer-Pregl R, Salamini F (2002) AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. Mol Biol Evol 19:1797–1801

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK (2000) The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet 100:584–592

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Russell JR, Fuller JD, Macaulay M, Hatz BG, Jahoor A, Powell W, Waugh R (1997) Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor Appl Genet 95:714–722

Saker M, Nagchtigall M, Kuehne TA (2005) Comparative assessment of DNA fingerprinting by RAPD, SSR and AFLP in genetic analysis of some barley genotypes. Egypt J Genet Cytol 34:81–97

Salem KFM, El-Zanaty AM, Esmail RM (2008) Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatallite markers. World J Agric Sci 4:538–544

Schuster I, Vieira ESN, da Silva GJ, Franco FA, Marchioro VS (2009) Genetic variability in Brazilian wheat cultivars assessed by microsatellite markers. Genet Molec Biol 32:557–563

Song W, Henry RJ (1995) Molecular analysis of the DNA polymorphism of wild barley (*Hordeum spontaneum*) germplasm using the polymerase chain reaction. Genet Resourc Crop Evol 42:273–280

Sorrells ME, Wilson WA (1997) Direct classification and selection of superior alleles for crop improvement. Crop Sci 37:691–697

Tams SH, Bauer E, Oettler G, Melchinger AE (2004) Genetic diversity in European winter triticale determined with SSR markers and coancestry coefficient. Theor Appl Genet 108:1385–1391

Vavilov NI (1950) The phytogeographic basis of plant breeding. In: The origin, variation, immunity and breeding of cultivated plants (Trans. K Starr Chester). Chronica Botanica, Waltham, MA, USA

Weining S, Langridge P (1991) Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. Theor Appl Genet 82:209–216

You GX, Zhang XY, Wang LF (2004) An estimation of the minimum number of SSR loci needed to reveal genetic relationships in wheat varieties: information from 96 random samples with maximized genetic diversity. Mol Breed 14:397–406

Zhang XY, Li CW, Wang LF, Wang HM, You GX, Dong YS (2002) An estimation of the minimum number of SSR alleles needed to reveal genetic relationships in wheat varieties I: information from large-scale planted varieties and cornerstone breeding parents in Chinese wheat improvement and production. Theor Appl Genet 106:112–117