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Diversity analysis of genetic, agronomic, and quality characteristics of bread wheat (*Triticum aestivum* L.) cultivars grown in Turkey

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Abstract: Turkey is an important bread wheat producer. The objective of this study was to dissect the diversity of genetic, agronomic, and quality characteristics of bread wheat cultivars grown on 25% of the total wheat area in Turkey. A total of 24 wheat cultivars and 5 wild progenitors of wheat were examined using 24 simple sequence repeat (SSR) primers with a known physical locus on the A, B, and D genomes of hexaploid wheat. A total of 72 bands produced 939 alleles on the wheat cultivars and wild progenitors. Markers were efficient in discriminating the species and the highest genetic diversity information was obtained from the markers *Xgwm*312 and *Xgwm*372. Microsatellite markers clearly separated cv. Pandas from all other cultivars although it was closely related to most of them in terms of agronomic and quality traits. Four agronomic characteristics including yield component traits and eight bread quality analyses were used for the diversity analyses. A significant association between morphological and bread wheat quality traits was observed while the correlation was weak with the genetic data. Cultivars were also classified with respect to release year and origin. Molecular variance between old (released before the year 2000) and new cultivars accounted for 1% of the total variation and the variance was 3% between national and foreign cultivars. Results showed that the number of alleles was lower in national and new cultivars compared to foreign and old cultivars. Therefore, breeding sources do not appear to improve the genetic base of wheat cultivars in Turkey. Introducing new variation sources may be needed to broaden the narrowed gene pool of bread wheat.

Key words: AMOVA, bread wheat, genetic diversity, quality, SSRs

1. Introduction

Bread wheat (*Triticum aestivum* L.) is a member of the genus *Triticum* of the large family Poaceae with about 3500 species (Horvath et al., 2009). Bread wheat is an allohexaploid species (2n = 6x = 42, AABBDD) and consists of three different diploid genomes. Respectively, the progenitors of the A, B, and D genomes are *Triticum urartu* Thuman ex Gandil. (AA, 2n = 14), *Aegilops speltoides* Tausch (BB, 2n = 14), and *Aegilops tauschii* Coss. (DD, 2n = 14) (Dvorak et al., 1988, 1993, 1998; Dvorak and Zhang, 1990). Wheat is one of the most important cultivated plants in the world. It covers the largest region among cereals (approximately 218.5 million ha) worldwide (http://faostat3.fao.org). Wheat constitutes a fundamental nutrient sources for about 35% of the world population. Therefore, the gene pool of wheat, which is a highly self-pollinated plant, needs close attention since a limited gene pool could be a significant problem for wheat breeding (Afshan and Naqvi, 2011). A narrowed genetic pool increases the development of risk factors. An increase in yield and disease resistance could be provided by expanding the genetic diversity of bread wheat (Nielsen et al., 2014). A total of 173 released bread wheat cultivars exist in Turkey and have been planted on an area of 7,710,000 ha (http://www.usda.gov). Considerable numbers of those wheat cultivars have been improved by foreign sources and introduced to Turkey. Each year new genetic sources are introduced to the wheat areas; however, there is little information about how those introduced materials improved the genetic variation of the bread wheat gene pool. The genetic diversity can be estimated by different methods including DNA-based markers, pedigree records, and morphological markers. Various molecular markers (RAPD, AFLP, RFLP, SSR, DArT, and SNP) have been successfully used among wheat cultivars or progenitors (Chao et al., 2009; Nielsen et al., 2014). Simple sequence repeats or microsatellites are used in a lot of research because of their locus specificity, ease of use, codominance, and high polymorphism (Röder et

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al., 1998a; 1998b; Landjeva et al., 2007; Laidò et al., 2013). Microsatellite markers are useful for marker-assisted selection, identifying quantitative trait loci, genetic diversity, and labeling of stress-tolerant genes in wheat or wild relations (Landjeva et al., 2007; Ijaz and Khan, 2009).

The aim of this study was to identify genetic diversity among bread wheat cultivars and wheat progenitors using genetic and morphological markers. We also studied the genetic variation between foreign and nationally improved bread wheat cultivars as well as new and old ones in order to understand how such contributions have affected the genetic diversity of cultivated bread wheat planted in Turkey. The results of this study are expected to be useful to prepare strategies to enrich the gene pool of bread wheat.

2. Materials and methods

2.1. Plant material

In this study, 24 randomly selected commonly grown bread wheat cultivars, planted annually on about 2 million ha covering more than one quarter of the total wheat area (http://wheatatlas.org) of Turkey, were obtained from the Department of Field Crops in the Faculty of Agriculture at Mustafa Kemal University and are listed in Table 1. Of the 24 cultivars, 10 had been improved by foreign sources. Regardless of origin, the seven cultivars released before the year 2000 were considered old materials (Table 1). Therefore, we were able to dissect the genetic variation between the foreign and national as well as the new and old bread wheat cultivars (Table 1). In addition, wheat progenitors were obtained from the USDA (Table 2) and *Aegilops tauschii* was kindly provided by Dr Hikmet Budak of Sabancı University in İstanbul, Turkey.

2.2. Field experiment and phenotypic measurements

Wheat progenitors and 24 bread wheat cultivars were used in this experiment. The bread wheat cultivars were planted in the fields in the Reyhanlı and Antakya districts of Hatay, Turkey. The wheat progenitors were planted in pots. The field experiments were planted in different environments in the 2009–2010 (Reyhanlı; 36°15′N, 36°20′E, altitude 88 m) and 2010–2011 (Antakya; 36°17′N, 36°11′E, altitude 105 m) growing seasons. Meteorological data of the experimental areas are given in Figure 1. The experiment was conducted using a randomized complete block design with split plot arrangement, with the environments being main plot and genotypes being split plot in three replications. A 2-row plot 100 cm in length and having 20 cm between the rows was used and approximately 6 g of seeds were planted for each row. The agronomic characteristics involving yield component traits were measured. From each row, 10 representational leading spikes in the row were selected before harvest as samples to evaluate grain number per spike, spikelet number per spike, plant height (the tallest tiller from ground to the tip of the spike excluding awns in cm) and spike length (cm). After harvesting, grain quality was assessed by measuring grain volume weight (g) and 1000 kernel weight (g) (Shah et al., 1999; Campbell et al., 2003). Other bread quality characteristics such as grain protein, wet gluten, grain hardness, energy values, starch, and Zeleny index were measured using near infrared reflectance spectroscopy (FOOS Infratec 1241, Denmark).

2.3. Molecular marker analysis

Genomic DNA obtained from leaves was extracted using a cetyltrimethylammonium bromide method (Saghai-Maroof et al., 1984) with minor modifications. Genomic DNA was quantified on a nanodrop (ACTGene UVS 99, NJ, USA), at $A_{260/280}$ nm. A total of 24 SSR primer pairs obtained from GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) were used in this study (Table 3). At least one SSR marker per wheat genome, except 2D, 5A, 6B, and 7D, was scored and a total of 24 scorable and polymorphic markers were used in the genetic diversity analysis. Polymerase chain reaction (PCR) analysis was performed in a 20 µL reaction volume containing 30 ng of genomic DNA, 1.5 mM of $MgCl_2$, 2 µM of each primer pair, 0.6 U of Taq polymerase, and 2 mM of dNTPs in a 10X reaction buffer. PCR amplifications were carried out in a GenePro Thermal Cycler (Bioer, China) with an initial denaturation for 5 min at 95 °C, then 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 50–60 °C, 1 min extension at 72 °C, and a final extension for 10 min at 72 °C. The amplified PCR products were electrophoresed on 3% metaphor agarose (Prona, EU) gels containing 1X TBE buffer (Tris Borate EDTA). The genomic DNA was stained with 1 µg/mL ethidium bromide solution. The gels were run at 130 V for 80 min. Gel photos were taken under UV light using a DNR MiniLumi (DNR MiniLumi, Israel) gel documentation system.

2.4. Statistical analysis

The data were scored as presence of band (1) and absence of band (0) from the SSR amplifications. To characterize the genetic variation, the observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity (he), and Shannon's information index (I) were calculated using POPGENE v1.31 (Yeh et al., 1998). A coefficient of similarity among species was calculated according to Nei (1973). A UPGMA tree was constructed using NTSYS v2.02 (Rohlf, 1998). The variance analysis of the agronomic traits data was evaluated using SAS v9.0 (SAS Institute Inc., Cary, NC, USA). The variance analysis was performed in order to analyze polymorphism information values with respect to genomes. Later, we applied a t-test for comparison of the means.

We also used a Mantel test with 10,000 permutations to correlate a distance matrix of marker data with a similarity matrix of quality and field data (Rohlf, 1998). Analysis

Table 1. Information about the pedigree and bread-making quality of the cultivars used in the present study.

Cultivar	The country where the variety was improved		Bread-making quality*	Pedigree**
Adana-99	Turkey (NS)	1999	Good	PFAU/SERI-82//(SIB)BOBWHITE[2400][2850]
Basribey-95	Turkey (NS)	1995	Good	JUPATECO-73/(SIB)BLUEJAY//URES-81[1610][2850]
Bayraktar-2000	Turkey (NS)	2000		
Bezostaja-1	Russia (FS)	1968	Good	(S)BEZOSTAYA-4[37][80][104][10][11];
Ceyhan-99	Turkey (NS)	1999		BLUEJAY(SIB)/JUPATECO-73[144]BLUEJAY(SIB)COCO-RAQUE-75[2400][2850]
Cumhuriyet-75	Turkey (NS)	1975	Moderate	SONORA-64*2//TEZANOS-PINTOS-PRECOZ/YAQUI-54/3/ANDES-64-A/4/2*FROCOR//YAQUI/KENTANA[667][114][144][2406]
Doğankent	Syria (FS)	1991	Good	4777*2//FKN/GABO/3/VEERY-5/4/BUCKBUCK/(SIB)PAVON-76[144]
Esperia	Italy (FS)	2005		
Galil	Israel (FS)	1989		HORK/YAMHILL//KALYANSONA/BLUEBIRD/3/BOBWHİTE 'S'
Golia	Italy (FS)	1991		MANITAL/ORSO[1558][1619][1624][1764][2676]
Gönen-98	Turkey (NS)	1983	Good	II-8156-R/MARA//BLUEBIRD[2850]
Guadalupe	France (FS)	1996		
Karacadağ	Turkey (NS)	1998		RED-RIVER-68/WW-15/3/BAJIO/2*OLESEN//BONANZA/4/NACOZ-ARI-76[2400];
Kaşifbey-95	Turkey (NS)	1995		HORK(SIB)/YAMHILL//KALYANSONA/BLUEBIRD[1610]
Negev	Bulgaria (FS)	2002		
Nurkent	Turkey (NS)	2001		NEELKANT
Osmaniyem	Turkey (NS)	2006		TUJ/ONELTO
Pamukova-97	Turkey (NS)	1997		VEERY/PAJONAL[706][1857]
Pandas	Italy (FS)	1984	Good	ORSO//BEZOSTAYA-1/S-1/3/GENEROSO-7/CONTO-MARZOT-TO[1558][1764]
Sagittario	Italy (FS)	2000	Good	ADAM/Z-282[1665][1764]
Seyhan-95	Turkey (NS)	1995	Good	JUPATECO-73/(SIB)BLUEJAY//URES-81[1610];
Yunak	Bulgaria (FS)	2004		
Yüreğir-89	Turkey (NS)	1989	Moderate	HD-1220/3*KALYANSONA//NACOZARI-76
Ziyabey-98	Turkey (NS)	1998	Very good	ND/VG-9144//KALYANSONA/BLUEBIRD /3/YACO/ 4/VEERY-5[2400]

* = bread-making quality information was obtained from http://www.tigem.gov.tr and http://wheatatlas.org (Accessed 15.01.2015). ** = pedigree is from the Wheat Genbank (http://genbank.vurv.cz/wheat/pedigree/default.htm. Accessed 15.01.2015). NS and FS = cultivars improved by national or foreign sources, respectively.

Table 2. Wheat progenitors obtained from the USDA.

Taxon	Plant ID	Collected Area
Aegilops speltoides var. *speltoides*	84TK098-021A	Diyarbakır, Turkey
Triticum turgidum subsp. *dicoccon*		Zaragoza, Spain
Triticum monococcum subsp. *monococcum*	G2900	Manisa, Turkey
Triticum urartu	G1820	Mardin, Turkey

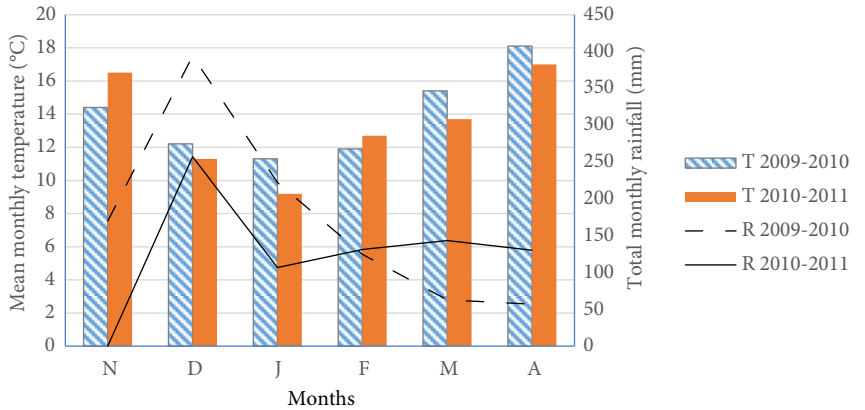

Figure 1. Meteorological data of the experimental area during the growing experiment (the 2009–2010 and 2010–2011 growing seasons in Reyhanlı and Antakya, respectively). T = temperature and R = rainfall.

of molecular variance (AMOVA) was performed using GenAlex software (Peakall and Smouse, 2006) to detect variation between and within foreign and national as well as old and new cultivars. The same software was also used for identifying the allelic frequencies and heterozygosity values of the markers used in this study. We also analyzed marker information content with respect to the genome where it is found.

3.Results

3.1. Marker analysis

From the wheat marker databases, we selected 24 SSR markers present on the A, B, and D genomes of bread wheat (Table 3). The 24 SSR markers successfully produced 72 bands resulting in 939 alleles on the genomes of 24 wheat cultivars and 5 wild progenitors. The PIC values ranged from 0.113 (*Xgwm124*) to 0.352 (*Xgwm312*) and the average was 0.205. Therefore, the greatest genetic information was obtained from *Xgwm*312 and *Xgwm*372 while the least was from *Xgwm*124 (Table 3). The highest numbers of polymorphic bands (6) were produced from *Xcfd*54 while the lowest (1 for each) were obtained from *Xcfa2292*, *Xcfd106*, and *Xcfd35*. We analyzed markers with respect to the genomes in which they were found. Three markers (*Xgwm*630, *Xcfd*5, and *Xcfd54*) belonged to more than one genome; hence, they were discarded from the analysis in which only the markers found in a single genome were analyzed. Analysis of the molecular variance results showed that marker variation among genomes was less than 1% and insignificant ($P > 0.05$) regarding the cultivars used in this study. Despite the insignificant difference, B genome markers seemed to have the least genetic diversity information compared to the markers found in A and D. We also compared single genome markers with multiple genome markers (i.e. *Xgwm*630, *Xcfd*5, and *Xcfd54*). Analysis of molecular variance also showed that variation among the markers, which were represented in single genome vs. multiple genomes was less than 1% and insignificant ($P > 0.05$). Although variations between those markers were not significant, the markers found on multiple loci rendered more allele numbers and had slightly higher genetic diversity information than those markers found in a single locus on the chromosomes.

Using 24 SSR markers, the observed number of alleles, effective number of alleles, gene diversity values, Shannon's information index, and number of total bands were also assessed for the cultivars and the progenitor plants used in this study (Table 4). According to those values, the least information was obtained from cultivars Pandas and Cumhuriyet while the greatest was obtained from Doğankent, Adana-99, Osmaniyem, Yüreğir-89, Sagittario, and Basribey-95 (Table 4). In terms of the progenitor plants, the highest genetic diversity information was obtained from *T. turgidum* subsp. *dicoccon* while the lowest was from *T. monococcum* subsp. *monococcum*.

3.2. Genetic diversity of bread wheat cultivars and progenitor plants

Using the markers and a similarity matrix, we constructed a dendrogram in which similarity coefficients ranged from 0.52 to 0.97 for all species while the coefficient range was from 0.69 to 0.97 for the cultivars. The dendrogram was composed of two main groups in which *T. turgidum* was separated from the others (Figure 2). In the second main group (B), progenitor species were in the B1 subgroup and were clearly separated from the cultivars in B2. In the second group (B2), cultivars Pandas, Kaşifbey-95, and

Table 3. Primers used in the study and their polymorphism information (including null alleles).

Marker	Forward/ReversePrimer	Genome	ϕNPB	ϕNA	ϕGDV	ϕPIC	§CNA	§NCV he	§OCV he	§NAS he	§FOS he
*Xbarc*130	CGGCTAGTAGTTGGAGTGTTGG/ ACCGCCTCTAGTTATTGCTCTC	5DS	3	28	0.144	0.131	23	0.000	0.367	0.000	0.432
*Xbarc*147	GCGCCATTTATTCATGTTCCTCAT/ CCGCTTCACATGCAATCCGTTGAT	3BS	3	23	0.361	0.288	21	0.292	0.243	0.248	0.233
*Xcfa*2058	CCCATTGCCATCTCAGTCTT/ ATAGTAGGCCCAAAGCGATG	2AL	2	26	0.152	0.137	24	0.000	0.000	0.000	0.000
*Xcfa*2135	TGCCTAAATCTAAATGCCCG/ GGATAATGTGCATGTTCACCG	1AL	2	22	0.278	0.228	19	0.000	0.496	0.498	0.432
*Xcfa*2219	TCTGCCGAGTCACTTCATTG/ GACAAGGCCAGTCCAAAAGA	1AL	3	53	0.250	0.217	48	0.289	0.264	0.333	0.207
*Xcfa*2240	TGCAGCATGCATTTTAGCTT/ TGCCGCACTTATTTGTTCAC	7A	2	53	0.185	0.168	48	0.000	0.000	0.000	0.000
*Xcfa*2278	GCCTCTGCAAGTCTTTACCG/ AAGTCGGCCATCTTCTTCCT	2BL	3	53	0.304	0.254	47	0.046	0.390	0.390	0.207
*Xcfa*2292	GGACCGTTTATCCGTAAGCA/ GCCTATGCTGCTGATCCATT	1BL	1	26	0.185	0.168	24	0.000	0.000	0.000	0.000
*Xcfd*106	ACGGGTGGTTTTGCTCAGT/ ACTCCACCAGCGGAGAAATA	4DS	1	26	0.185	0.168	24	0.000	0.000	0.000	0.000
*Xcfd*211	AGAAGACTGCACGCAAGGAT/ TGCACTAAAGCATCTTCGTGTT	3DL	2	37	0.200	0.160	29	0.069	0.110	0.101	0.094
*Xcfd*35	GGGATGACACATAACGGACA/ ATCAGCGGCGCTATAGTACG	3DS	1	7	0.366	0.299	5	0.000	0.269	0.137	0.273
*Xcfd*5	TGCCCTGTCCACAGTGAAG/ TTGCCAGTTCCAAGGAGAAT	6DL, 5B*	2	51	0.220	0.183	45	0.000	0.244	0.235	0.216
*Xcfd*54	TCGTTCCAAAATGCATGAAA/ AAGGGCCAGAAATCTGTGTG	4BL, 4DL*	6	105	0.250	0.218	94	0.000	0.217	0.065	0.249
*Xcfd*60	TGACCGGCATTCAGTATCAA/ TGGTCACTTTGATGAGCAGG	6DL	2	53	0.211	0.183	47	0.000	0.184	0.000	0.216
*Xcfd*61	ATTCAAATGCAACGCAAACA/ GTTAGCCAAGGACCCCTTTC	1D	3	44	0.277	0.228	38	0.235	0.485	0.476	0.463
*Xcfd*88	TAGGCATAGTTTTGGGCCTG/ GGTAGAAGGAAGCTTCGGGA	4AL	6	74	0.158	0.136	67	0.166	0.273	0.287	0.288
*Xcfd*9	TTGCACGCACCTAAACTCTG/ CAAGTGTGAGCGTCGG	3DL	5	28	0.201	0.162	24	0.380	0.382	0.349	0.407
*Xgwm*124	GCCATGGCTATCACCCAG/ ACTGTTCGGTGCAATTTGAG	1B	3	24	0.132	0.113	22	0.470	0.367	0.470	0.000
*Xgwm*312	ATCGCATGATGCACGTAGAG/ ACATGCATGCCTACCTAATGG	2A	2	28	0.455	0.352	25	0.366	0.409	0.411	0.384
*Xgwm*372	AATAGAGCCCTGGGACTGGG/ GAAGGACGACATTCCACCTG	2A	3	26	0.411	0.325	24	0.284	0.299	0.296	0.287
*Xgwm*480	TGCTGCTACTTGTACAGAGGAC/ CCGAATTGTCCGCCATAG	3A	5	50	0.183	0.159	44	0.359	0.162	0.320	0.144
*Xgwm*493	TTCCCATAACTAAAACCGCG/ GCAACATCATTTCTGGACTTTG	3BS	4	26	0.294	0.232	25	0.362	0.265	0.258	0.344
*Xgwm*630	GTGCCTGTGCCATCGTC/ CGAAAGTAACAGCGCAGTGA	2A, 2BS*	4	50	0.157	0.132	42	0.000	0.241	0.249	0.247
*Xwmc*10	GATCCGTTCTGAGGTGAGTT/ GGCAGCACCCTCTATTGTCT	7B	4	26	0.327	0.268	25	0.250	0.221	0.214	0.233
Mean			3	38.9	0.245	0.205	34.8	0.149	0.245	0.222	0.223

NPB = number of polymorphic bands, NA = total number of alleles including null alleles, GDV = genetic diversity values, PIC = polymorphism information content, CNA = number of alleles including null alleles for cultivars only, NCV = new cultivars, OCV = old Cultivars, NAS = cultivars from national sources, FOS = cultivars from foreign sources, he = expected heterozygosity values, ϕ = progenitor species were included in the analysis, and § = progenitor species were not included in the analysis.

Table 4. Cultivars and progenitor species used in this study and their genetic diversity information.

Cultivars	na*	ne*	h*	I*	Number of total bands
Karacadağ	2	1.986	0.497	0.690	33
Yunak	2	1.986	0.497	0.690	39
Nurkent	2	1.999	0.500	0.693	35
Doğankent	2	2.000	0.500	0.693	36
Galil	2	1.999	0.500	0.693	35
Adana-99	2	2.000	0.500	0.693	36
Osmaniyem	2	2.000	0.500	0.693	36
Yüreğir-89	2	2.000	0.500	0.693	36
Negev	2	1.994	0.499	0.692	38
Seyhan-95	2	1.994	0.499	0.692	38
Sagittario	2	2.000	0.500	0.693	35
Ceyhan 99	2	1.994	0.499	0.692	34
Golia	2	1.999	0.500	0.693	37
Pamukova-97	2	1.994	0.499	0.692	34
Esperia	2	1.999	0.500	0.693	37
Pandas	2	1.927	0.481	0.674	29
Basribey-95	2	2.000	0.500	0.693	36
Gönen	2	1.962	0.490	0.684	31
Bayraktar-2000	2	1.986	0.497	0.690	33
Kaşifbey-95	2	1.976	0.494	0.687	32
Cumhuriyet-75	2	1.943	0.485	0.678	29
Guadalupe	2	1.994	0.499	0.692	34
Ziyabey-98	2	1.994	0.499	0.692	34
Bezostaja-1	2	1.993	0.498	0.691	31
Ae. speltoides	2	1.790	0.441	0.633	23
T. dicoccon	2	1.946	0.486	0.679	30
Ae. tauschii	2	1.737	0.424	0.616	22
Ae. urartu	2	1.564	0.361	0.547	17
T. monococcum	2	1.420	0.296	0.472	13
Mean	2	1.937	0.481	0.673	32.17

na = the observed number of alleles, ne = the effective number of alleles [Kimura and Crow (1964)], h = Nei's (1973) gene diversity, and I = Shannon's information index [Lewontin (1972)].

Bayraktar-2000 were separated from the other cultivars, which were placed in two sub-subgroups. In B2.1, there were cultivars Galil, Golia, Pamukova-97, Gönen, and Cumhuriyet-75, while B2.2 was composed of two separate groups B2.2.1 and B2.2.2. B2.2.1 contained cultivars Karacadağ, Nurkent, Negev, and Seyhan-95 with similarity coefficients that ranged from 0.86 to 0.90 while B2.2.2 branched into two subgroups (Figure 2). In B2.2.2.1, Osmaniyem and Yüreğir-89 seemed very similar with a similarity coefficient of 0.97. In B2.2.2.2, the similarity coefficients among the cultivars ranged from 0.87 to 0.91 and Yunak, Bezostaja-1, and Adana-99 were separated from the others. In B2.2.2.2, the winter cultivars Yunak, Bezostaja-1, and Esperia appeared to be closer to each other than did Pandas and Bayraktar-2000 in B1 and B2 (Figure 2).

3.3. Agronomic and quality data analysis

For the field and agronomic analyses, we only used registered wheat cultivars since the field data of the progenitor species were not recorded. Analysis of variance

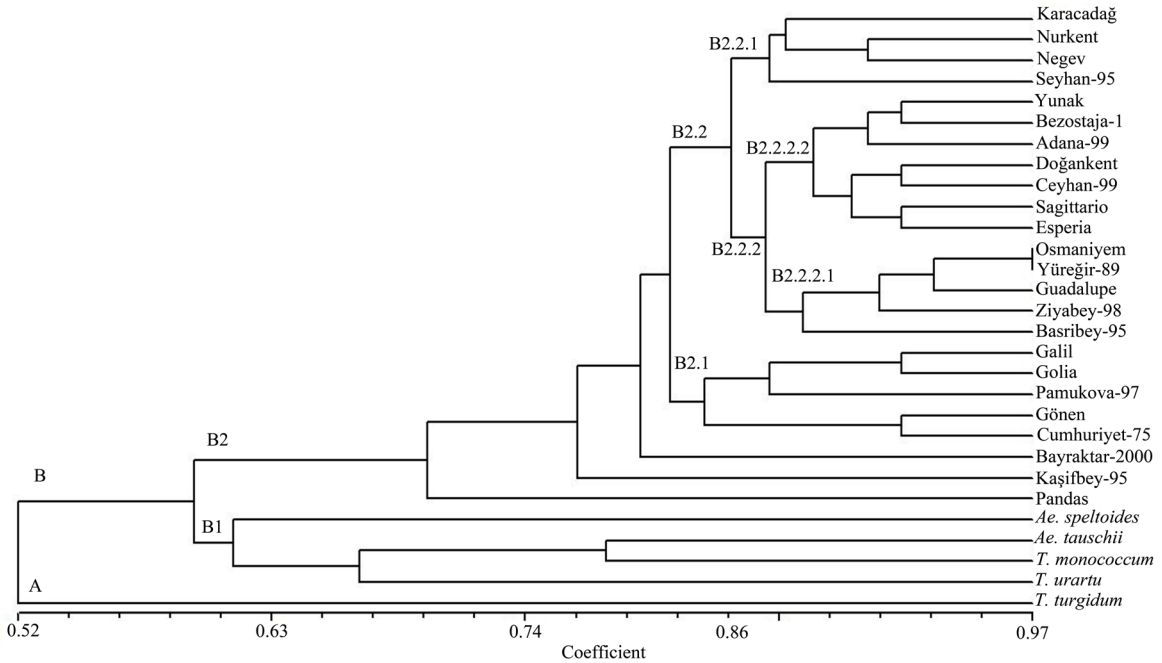

Figure 2. Genetic diversity between wheat cultivars and progenitor plants.

results showed that there were significant differences among the genotypes in terms of spikelet length, plant height, spikelet number, and kernel number ($P < 0.01$). We used multivariate approaches especially for the analysis of agronomic and quality data since, except for kernel number, there was no significant difference between variances of the two growing seasons (Brown and Forsythe's test, $P > 0.05$). Mean differences in agronomic traits regarding the cultivars are shown in Table 5. The highest plant height was identified in Bezostaja-1 and Bayraktar-2000, while the lowest was found in Golia. The longest spike belonged to Cumhuriyet-75, while the shortest one was from Golia and Esperia. The number of grains was the highest in Kaşifbey-95, while the lowest grain number was obtained from Bayraktar-2000. The number of spikelets was highest in Bezostaja-1, while it was lowest in Cumhuriyet-75 (Table 5).

3.4. Agronomic traits among cultivars

In order to correlate genetic similarity values with those of agronomic traits, we constructed a dendrogram using the agronomic measurements mentioned in the Materials and methods section (Figure 3). The similarity coefficients using Euclidian distance ranged from 0.81 to 24.20. According to the dendrogram, the cultivars were separated into two main groups, named A and B (Figure 3). In group A, cultivars Bezostaja-1 and Bayraktar-2000 were highly separated from the others. In group B, Golia and Kaşifbey-95 were clearly separated from the other cultivars and did not form a group. The rest of the cultivars were placed within 2 subgroups. In B1, cultivars Adana-99 and Yüreğir-89 were more similar compared to Nurkent. In B2, there were two sub-subgroups in which B2.1 also had two sub-sub-subgroups. In sub-sub-subgroup B2.1.1, cultivars Basribey-95 and Doğankent were very similar to each other and separated from cultivars Guadalupe and Negev. In sub-sub-subgroup B2.1.2, Esperia and Sagittario had very close agronomic characteristics compared to the others. In the other subgroup B2.2, cultivars Karacadağ and Seyhan-95 seemed to be very similar to each other and separated from cultivars Cumhuriyet-75, Yunak, and Osmaniyem. We compared the agronomic trait data with the genetic distance matrix using the Mantel test with 10,000 permutations and found that there was no correlation ($P > 0.05$).

3.5. Diversity of quality traits among cultivars

The cultivars were clustered into two groups in terms of quality traits using Euclidian distances (Figure 4). In group A, cultivar Bezostaja-1 was farther from cultivars Esperia, Gönen, and Pamukova-97, which was in subgroup A1. Group B was clustered under two subgroups, B1 and B2. The B1 subgroup included two sub-subgroups, of which B1.1, with Doğankent and Yunak, was separated from Adana-99, Golia, Kaşifbey-95, Ceyhan-99, and Ziyabey-98. Sub-subgroup B1.2 included cultivars Nurkent, Pandas, Galil, and Guadalupe. In subgroup B2, cultivar Osmaniyem was separated from the other cultivars

Table 5. Agronomic trait means and their differences (Duncan test at P < 0.05). Cultivars having the same letters are not significantly different.

Cultivar	Plant height (cm)		Spike length (cm)		Grain number		Spikelet number	
Adana-99	100.4	b	10.87	bac	60.50	fcebdg	40.03	bac
Basribey-95	87.32	fhegkij	10.07	fgdec	62.37	cebd	40.85	ba
Bayraktar-2000	108.4	a	9.35	fgeh	45.33	k	37.20	fdec
Bezostaja-1	110.3	a	10.92	bac	46.93	kj	42.33	a
Ceyhan-99	92.80	fcebd	10.12	fgdec	53.83	fikhjg	37.57	fbdec
Cumhuriyet-75	89.62	fhegi	11.92	a	48.80	ikj	33.92	g
Doğankent	90.27	fhegd	10.55	bdec	62.23	fcebd	40.32	bac
Esperia	81.82	kj	8.62	h	50.47	ikhj	39.10	bdac
Galil	88.07	fhegkij	10.10	fgdec	65.07	cbd	38.08	fbdec
Golia	73.43	l	8.43	h	49.95	ikhj	37.02	fgdec
Gönen	85.52	fhgkij	9.52	fgdeh	58.43	fcehdg	38.72	bdec
Guadalupe	84.20	hgkij	9.90	fgdec	65.92	cb	39.43	bac
Karacadağ	91.85	fcegd	10.00	fgdec	53.11	ikhjg	35.32	fg
Kaşifbey-95	89.33	fhegij	10.68	bdc	74.94	a	40.40	bac
Negev	80.52	k	11.53	ba	62.88	cbd	37.60	fbdec
Nurkent	99.33	cb	10.80	bac	67.63	b	39.78	bac
Osmaniyem	97.55	cbd	9.50	fgdeh	50.98	ikhj	35.87	fgde
Pamukova-97	94.48	cebd	8.92	gh	54.08	fiehjg	35.73	fge
Pandas	92.95	fcebd	10.98	bac	58.08	fcehdg	40.32	bac
Sagittario	83.70	hkij	9.17	fgh	51.77	ikhj	40.05	bac
Seyhan-95	91.35	fhegd	10.38	fbdec	52.77	ikhjg	34.95	fg
Yunak	94.13	cebd	10.27	fdec	51.35	ikhj	40.83	ba
Yüreğir-89	100.4	b	11.58	ba	56.58	fiehdg	39.65	bac
Ziyabey-98	82.07	kij	10.72	bdac	56.63	fiehdg	38.78	bdec

clustered under the B2.1 sub-subgroup, which contained the B2.1.1 and B2.1.2 sub-sub-subgroups. The B2.1.2 group had cultivars Bayraktar-2000, Cumhuriyet-75, and Seyhan-95 separated from the other cultivars in B2.1.1. We compared the quality trait data with the genetic distance matrix using the Mantel test with 10,000 permutations and found that there was no correlation (P > 0.05). However, there was significant correlation between the data of the quality traits and those of the agronomic traits (r = 0.264, P = 0.017).

3.6. Variation between national and foreign cultivars

We examined the genetic heterogeneity of the cultivars according to their release year (Table 3). AMOVA indicated that the molecular variance between the national and foreign cultivars accounted for only 3% of the total variation, while 97% resided within the sets (P = 0.118). The diversity values of the national (0.222) and foreign (0.223) populations were also similar. Using 24 SSR markers on 24 cultivars, we determined the expected heterozygosity (he) between the national and foreign cultivars and found no significant difference (P = 0.975). On the other hand, the number of private alleles was one for the population of national cultivars while it was three for the foreign ones.

3.7. Variation between old and new cultivars

The AMOVA results showed that the molecular variance between the old and new cultivars accounted for only 1%

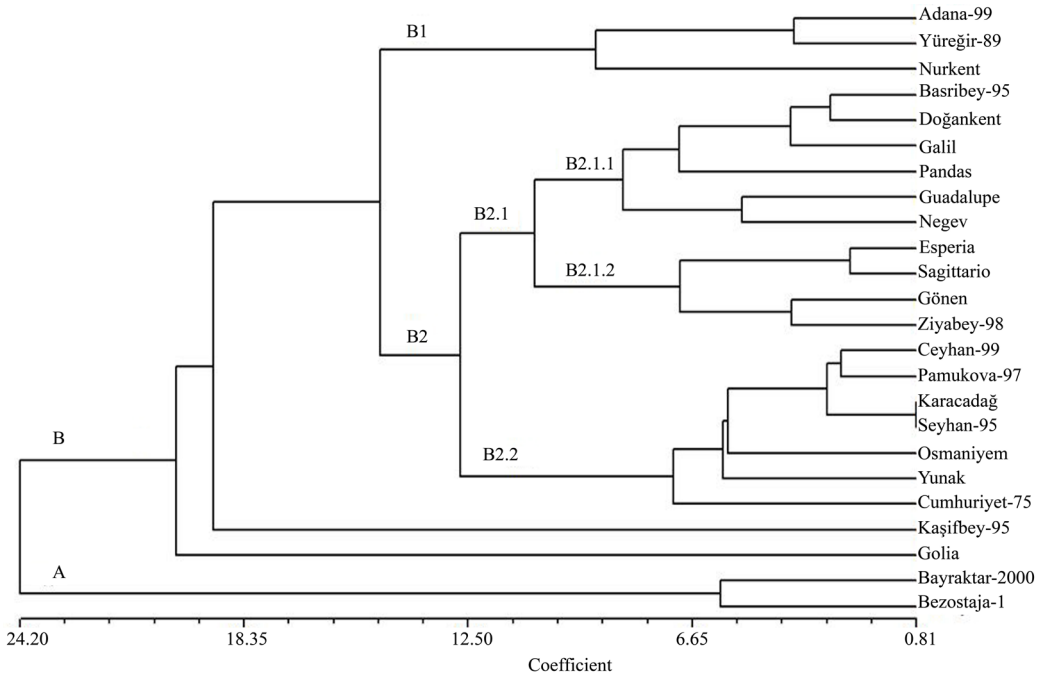

Figure 3. Dendrogram involving diversity of agronomic traits in wheat cultivars.

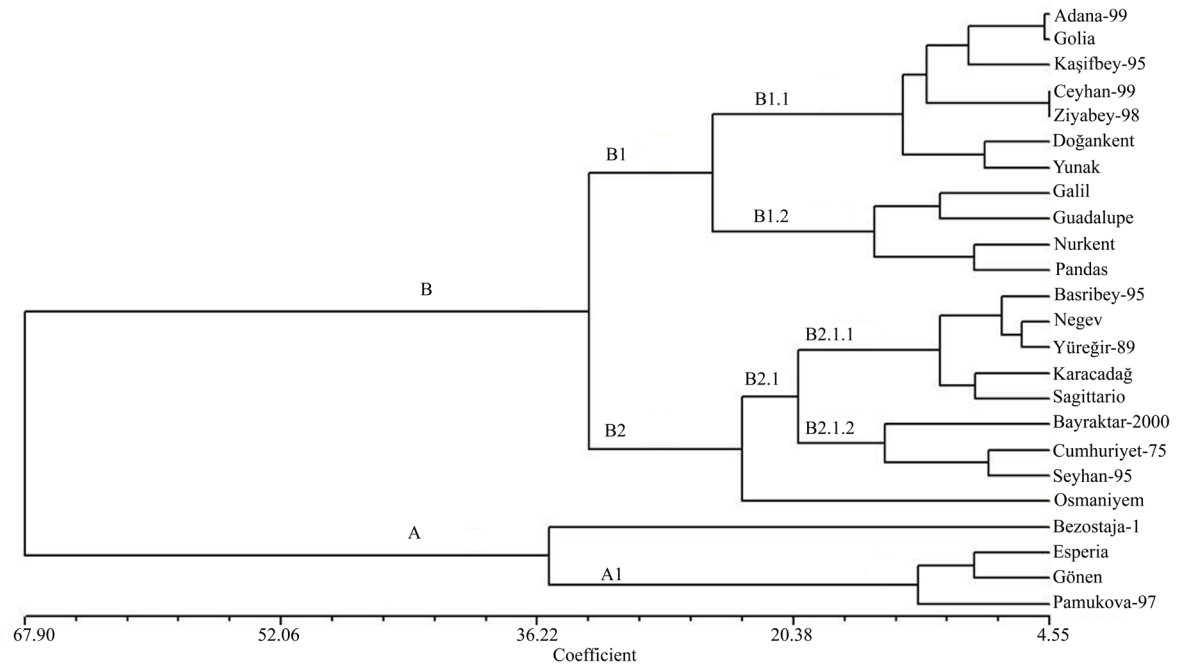

Figure 4. Dendrogram involving diversity of quality traits in wheat cultivars.

of the total variation, while 99% resided within the sets (P = 0.343). The number of private alleles was four for the old cultivars while it was two in the new cultivars. The old and new cultivars were significantly different in terms of he values ($P < 0.001$) and the old cultivars have significantly larger he values (mean = 0.245) than those of new cultivars (mean = 0.149) (Table 4).

3.8. Variation between progenitors and modern breeding cultivars

We determined the genetic variation between the progenitors and modern wheat cultivars using AMOVA. The results showed that the molecular variance between the progenitors and the modern breeding cultivars accounted for 41% of the total variation, while 59% resided within the sets ($P < 0.001$). A total of 10 private alleles were found for the modern cultivars while the number of those was 14 for the progenitors.

4. Discussion

A total of 24 SSR markers representing three genomes of bread wheat discriminated the progenitor species from the cultivars. We found no significant molecular variation among the genomes in terms of allele numbers produced by the markers used in our study, although the B genome markers had previously produced more allele numbers (Huang et al., 2002, 2007). The PIC values of the markers seemed relatively low since we treated the markers as dominant markers; hence the PIC and he values show a maximum of 0.5 (e.g., 50% (0) and 50% (1)). In fact, when adjusted to proportion of the maximum obtainable values, the dominant markers actually show higher mean PIC values than do the codominant markers (Stodart et al., 2007). A mean PIC value of 0.205 may be considered low, implying a narrow genetic diversity (Arabbeigi et al., 2014). Using seven Turkish wheat cultivars and 223 genomic SSR markers, the mean PIC value was found to be 0.522 (Akfırat and Uncuoğlu, 2013). Five Turkish durum wheat varieties were differentiated with seven SSR markers with a relatively high range of PIC values (0.227–0.887) (Hakkı et al., 2014).

In our study, the markers derived from different genomes resulted in similar allele numbers, although the B genome markers had the least informative alleles. This is probably due to the fact that our markers were not equally represented in each genome and chromosome arm. Initially, we used markers to represent each chromosome arm; however, those were not polymorphic or scorable enough. We also used three markers found in more than one locus in different chromosomes to compare them with the single locus markers. In the present study, the number of scorable and polymorphic multilocus markers was limited since it was thought that their genome coverage might have been intriguing. Interestingly, multilocus markers seemed substantially more informative for detecting genetic diversity. The present study also determined which cultivars had the highest and lowest genetic diversity information. Most of the cultivars had relatively high genetic diversity information while Pandas showed the lowest genetic diversity information of all cultivars. This finding could be significant in terms of considering them for molecular breeding and marker-assisted selection systems.

When all of the cultivars and progenitor plants were considered, there seemed to be a remarkable variation. Variation within the cultivars was less compared to variation within the progenitor plants, as expected. Genetic diversity was also higher within the wild progenitors compared to the hexaploid wheat, using RFLP and RAPD markers (Nagaoka and Ogihara, 1997). It should also be noted that the transferability of the SSR markers used in this study seemed to be convenient and may be utilized in evolutionary studies for grass species. In a previous study, some EST–SSR markers were also found to be useful for dissecting grass genomes (Bandopadhyay et al., 2004).

Some of the wheat cultivars had a similar pedigree background although genetically they did not seem similar. For example, Pandas had Bezostaja-1 in its pedigree but was far apart from it in the dendrogram. Similarly, Kaşifbey-95 had almost the same pedigree background as Galil; however, they are also far from each other. Some cultivars from the same sources remained close, such as Esperia and Sagittario. Such discrepancy between genetic data and pedigree information could be attributed to selection pressure, unequal parental contribution, and the relatedness of ancestors without a known pedigree (Soleimani et al., 2002; Marić et al., 2004). Similar patterns were also present in the dendrogram constructed using agronomic traits. In terms of the agronomic data, the variation among cultivars was also relatively small. The agronomic traits pattern was also like that of the quality traits, in which more than 80% of the cultivars were very closely related. This similarity was probably caused by the fact that most of the parents of the cultivars in Turkey were improved by just a few centers, especially ones connected to the International Maize and Wheat Improvement Center (CIMMYT). A loss of genetic diversity was also reported for CIMMYT and CIMMYT-related modern wheat cultivars in comparison to landrace cultivars, *Aegilops tauschii*, and traditional landrace cultivars (Reif et al., 2005). In our study, it also seems that wild relatives have significant sources for broadening the gene pool of bread wheat.

The genetic similarity coefficients among the wheat cultivars were around 0.20, implying that most of the cultivars are within less than 20% of the genetic variation. It should be also noted that we combined spring and winter wheat cultivars in the analyses. This becomes more apparent when cultivars are classified in terms of origin and release ages. The results of the AMOVA suggested that there is little genetic variation between the old and new wheat cultivars as well as between the national and foreign-originated ones. It seems that the new cultivars did not add greatly to the genetic variation since their genetic

diversity values were lower than those of the old cultivars. It should be noted that there were four private alleles in the old cultivars, while there were two in the new ones. The modern breeding cultivars also did not seem to increase the variation of European cultivars. A clear separation of European wheat cultivars before and after 1970 revealed that the more recent the European cultivars were, the more similar they were to each other (Roussel et al., 2005). In a more recent study, comprising mostly modern breeding cultivars and 7000 molecular markers, the fraction of polymorphic markers was found to be very low, suggesting a relatively narrow wheat gene pool in Europe (Nielsen et al., 2014). Similarly, the foreign materials had almost the same genetic diversity values as the national ones. The number of cultivars used in this study is about 10% of the total nationally listed bread wheat cultivars; however, these cultivars are planted on more than 25% of the total wheat areas. Using AFLP and selective amplification of microsatellite polymorphic loci (SAMPL) markers to differentiate wheat cultivars in Turkey released from 1936 to 2000, one study found that the genetic diversity between old and recent wheat cultivars was limited (Altıntaş et al., 2008). These results were also in corroboration with ours. Therefore, such low variation among the national and foreign populations may pose risks in terms of narrowing gene pool of bread wheat in Turkey. Although there was no significant variation between the national and foreign cultivars, the number of private alleles was even lower in the national cultivars. It should also be noted that, in our study, the number of national cultivars (14) was higher than the number of foreign ones (10), indicating the need for the introduction of new alleles from sources such as landraces and wild relatives. Significant molecular variation between the progenitors and the modern wheat cultivars as well as the larger number of private alleles in the progenitor genomes suggested that progenitors are useful sources for increasing the narrowed gene pool.

We found no significant correlation between the genetic data and the agronomic or quality trait data, probably because the changes in environmental conditions affected the traits. Such results have been also reported for the comparison of DNA markers and morphological data (Marić et al., 2004; Salem et al., 2008). In one study using SSR markers to differentiate 30 red winter wheat cultivars, a significant but very low correlation was found between morphological traits with genetic distance values (Fufa et al., 2005). A low correlation between DNA markers and morphological traits could be attributed to a large portion of genomes involving noncoding regions as well as coding regions while morphological characters largely undergo artificial selection (Semagn, 2002). However, the agronomic data seemed to be a relatively good predictor of the quality data due to the small but significant correlation between them.

In conclusion, SSR markers are useful tools to differentiate wheat cultivars and could be used for further genetic analysis and marker-assisted selection. Our study is one of the recent studies involving the genetic, morphologic, and quality characteristics of the winter and facultative-type bread wheat varieties grown in Turkey. The genetic maintenance of wheat varieties in comparison with their progenitors was also discussed and significant findings were revealed. To better understand the genetic and phenotypic diversity of the wheat varieties in Turkey, which is one of the large producers, it could be suggested that the number of cultivars or polymorphic markers be increased for future studies. Therefore, more useful markers and cultivars may be discovered for use in breeding programs. Progenitor species also seem to be a potential source of variation to broaden the genetic pool of bread wheat. Breeding institutes should use more diverse parental materials, including but not limited to landraces and wild relatives.

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