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Assessment of genetic relationships in durum wheat cultivars using AFLP markers

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Abstract

The genetic relationships were assessed for the first time in Turkish durum wheat cultivars using AFLP markers. In the analysis, 18 AFLP primer combinations resulted in a total of 189 polymorphic loci. All of the selective primers used are Eco and Mse primers with three nucleotide extentions on the 3′ ends. The number of polymorphic markers per primer combination ranged from 4 to 24. The relationships, among nine winter and six spring type durum wheat cultivars, obtained with various algorithms are in accordance with the known pedigree information of the cultivars. Based on 'Nei72' genetic distance analysis, the most distant two cultivars are 'Berkmen-469' (winter type) and 'Diyarbakìr-88' (spring type), and the closest two are 'Selçuklu-97' and 'Sofu', with the values of 0.793 and 0.115, respectively. The closest two winter type cultivars are 'Akbasak-073-44' and 'Kunduru-414-44' (0.128).

Introduction

Various DNA fingerprinting techniques have been successfully developed and put in use for the estimation of genetic diversity in plant species. Most of the recent marker techniques are PCR-based. Such markers with no prior sequence information requirement have been significantly improved in the past decade. They include random amplified polymorphic DNA (RAPD; Williams et al. 1990), arbitrarily primed PCR (AP-PCR; Welsh and McClelland 1990), and DNA amplification fingerprinting (DAF; Caetano-Anolles et al. 1991). As an alternative to these markers, AFLP developed by Zabeau and Vos (1993, 1995) is capable of detecting non-specific but many independent loci, with reproducible amplification (Pejic et al. 1998). The AFLP bands are usually scored as presence and absence of bands among a set of genotypes.

There are many applications of AFLP markers, the genetic relationship studies being an important one (Schut et al. 1997; Aggarwal et al. 1999; Breyne et al. 1999; Singh et al. 1999). AFLP can also be used for plant improvement in breeding (Becker et al. 1995; Cervera et al. 1996; Schwarz et al. 1999; Yin et al. 1999). In this study, Turkish winter and spring type durum wheat cultivars were analyzed to assess their genetic relationships using AFLP markers.

Materials and methods

Genetic materials

Winter type durum cultivars were obtained from Ministry of Agriculture and Foresty, Central Research Institute for Field Crops, Ankara. Spring type ones were obtained from Aegean Agricultural Research Institute, Izmir. The pedigrees of the cultivars are presented in Table 1. Those with no pedigree information are selections from the local landrace populations. All the winter types are adapted cultivars based on climatic conditions of growing zones, but they are indeed facultative.

DNA isolation

Genomic DNA was isolated from 15 days old seedlings, after 2 days of dark treatment. The isolation was carried out using 200 mg of five individual plants of each line by a slight modification of CTAB method

Table 1. Pedigrees of Turkish durum wheat cultivars (Zencirci et al. 1992)

No.	Plants	Pedigree
1.	'Berkmen-469'	Selections from landraces
2.	'Çesit-1252'	61-130/Kunduru-414-44//377-2 YA 03912 1A-0A
3.	'Çakmak -79'	Üvy162/61-130
4.	'Gökgol-79'	BBal//Bye*2 TC60
5.	'Kìzìltan-91'	Üvy162/61-130//Bye*2/TE
6.	'Kunduru-1149'	Selections from landraces
7.	'Tunca-79'	185-1/61-130//Leeds
8.	'Akbaşak-073-44'	Selections from landraces
9.	'Kunduru-414-44'	Selections from landraces
10.	'Diyarbakìr-88'	$Ld393Xbell_E$-$Tehuacan^2$/Cocorit71 SE0364-1S-4S-0S
11.	'Ege-88'	Jori/Anhinga//Flamingo cm9799-126M-1M-4Y-0M
12.	'Gediz-75'	$Ld357_E$-$Tehuacan^2$xJori 27534-1M-1Y-1M-0Y
13.	'Salihli-92'	SHWA//21563/Anhinga/3/Ege88 CD27672-4AP-1AP-4AP-0AP
14.	'Selçuklu-97'	073-44*2/Ovi/3/DF21-72//61-130/Üvy 162 YA0398621A-1A-5A-6A-0A
15.	'Sofu'	Selections from landraces

(Saghai Maroof et al. 1984, the volumes were adjusted so that the purification can be performed in 2 ml microcentrifuge tubes, also the extraction buffer contained 2% CTAB instead of 1% CTAB) and pooled for each line.

AFLP marker generation

Genomic DNA of 300 ng of each sample was digested in the presence of 3 units both of the restriction endonucleases *Eco*RI and *Tru*I (*Tru*I is isoschizomer of *Mse*I, MBI Fermentas Inc., Lithuania) in a 40 μl final reaction volume containing 1 $\times$ Universal Buffer (Stratagene, CA) and 0.1 μg Bovine Serum Albumin (BSA) for 3 hours of incubation at 37 °C. The adaptor ligation reaction mixture contained 20 μl of restriction digest aliquot, 3 μM of Eco adaptor and 30 μM of Mse adaptor, 1000 units of T4 DNA ligase (New England Biolabs, MA), 0.2 $\times$ T4 DNA ligase buffer (New England Biolabs, MA), 0.1 μg BSA, 0.2 mM of ATP. The reaction volume was completed to 25 μl by PCR grade water. The ligation was performed for 5 hours at 37 °C. Pre-selective amplification reaction mixture contained 5 μl ligation reaction products as PCR template, 75 ng of each of the pre-selective amplification primers (Eco+A and Mse+A), 0.2 mM dNTPs, PCR buffer (10 mM Tris-Cl, pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, and 1.5 units of *Taq* polymerase (MBI Fermentas Inc., Lithuania). The reaction volume was 50 μl. The cycling conditions were 20 cycles of three steps as 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min (Techne, 'Genius' thermocyler, UK). The labeling reaction contained 5 ng of EcoRI adaptor binding selective primers, 0.2 units T4 DNA kinase, 1 $\times$ T4 DNA kinase buffer, 0.05 μl of [γ^{33}P]-ATP (3000 Ci/mmol) (Institute of Isotopes Co., Ltd., Hungary) in a final volume of 0.5 μl per selective amplification PCR. The labeling reaction was performed for 10–100 sets of selective-amplification PCRs at 37 °C for 1 h and the enzyme was inactivated by 10 min of incubation at 70 °C. Selective primer combinations are presented in Table 2. All of the oligonucleotides were custom synthesized by Research Genetics, Inc., AL, USA. The reaction mixture per PCR, contained 5 μl of 1:20 diluted pre-selective amplification product as a template, 5 ng labelled Eco site primer (assuming full efficiency in end labeling), 3 ng Mse site primer, 0.2 mM dNTPs, PCR buffer (10 mM Tris-Cl, pH 8.3, 50 mM KCl), 3.0 mM $MgCl_2$, and 1 unit of *Taq* polymerase. The reaction volume was 20 μl. The cycling conditions were 11 cycles of denaturation at 94 °C for 30 s, annealing at 65 °C ($-$0.7 °C/each cycle) for 30 s, extension at 72 °C for 1 min and additional 24 cycles of denaturation, annealing and extension at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, respectively. The selective amplification reactions were stopped by the addition of formamide containing stop solution and denatured for 10 min at 94 °C prior to loading onto 4.5% of DNA denaturing polyacrylamide gel. The electrophoresis was performed until the bromophenol blue dye reached to the bottom of the

Table 2. Scores of polymorphic loci with 18 AFLP selective primer combinations.

Scored loci
Eco+ACC/Mse+AAG (18)
0 0 0 0 0 0 1 1 1 1 1 1 1 1
1 0 1 1 1 1 1 1 1 0 0 0 0 0 0
1 0 0 0 0 0 1 0 0 1 0 0 1 1 0
1 1 1 1 1 1 1 1 1 0 0 0 1 1 1
1 1 1 0 1 1 1 1 1 1 1 1 1 1 1
1 0 0 0 0 0 1 0 0 - - - - - 0
1 0 0 0 0 0 1 0 0 - - - - - 0
0 1 1 1 1 1 1 1 1 1 1 1 1 1 1
0 1 1 0 1 1 1 1 1 1 1 1 1 1 1
1 0 0 0 0 0 1 0 0 0 0 0 0 0 1
1 0 0 0 0 0 1 0 0 - - - - - -
0 0 0 1 0 0 0 0 0 - - - - - -
1 0 0 0 0 0 1 0 0 - - - - - 0
1 0 0 0 0 0 0 1 0 0 0 0 0 0 0
0 0 1 0 1 1 0 1 0 0 0 0 0 0 0
0 0 0 0 0 0 1 0 0 0 0 0 0 0 0
0 1 1 1 1 1 1 0 1 0 0 0 0 0 1
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
Eco+ACC/Mse+ACT (17)
0 1 1 0 0 1 0 0 0 0 0 0 0 0 0
1 1 0 1 1 1 0 1 1 0 0 0 0 0 1
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
1 0 0 0 0 0 1 0 0 1 1 1 1 1 1
1 0 0 0 0 0 1 0 0 0 0 1 0 0 0
0 1 1 1 1 1 1 1 1 1 1 1 1 1 1
1 0 0 0 0 0 1 0 0 1 0 1 1 0 0
1 1 1 1 1 1 0 1 1 0 0 0 1 1 1
1 0 0 0 0 0 1 0 0 1 0 1 1 0 1
0 1 1 1 1 1 1 1 1 1 1 1 1 0 0
1 1 1 1 1 1 1 1 1 0 1 0 1 1 1
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 1 1 1 1 1 1 1 0 1 1 0 0 0 0
0 1 1 1 0 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 1 0 0 0 0 0
0 0 0 1 1 1 1 1 1 0 0 0 0 0 0
Eco+ACC/Mse+ATA (4)
- 0 0 0 0 0 1 0 0 0 0 0 0 0 0
- 0 0 1 1 1 1 1 1 1 0 0 0 1 1 1
- 1 1 1 1 1 0 1 1 1 1 0 1 1 1
- 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1
Eco+ACC/Mse+AAT (8)
1 0 0 0 0 0 1 0 0 1 0 1 1 1 1
1 1 0 1 1 0 0 1 1 1 1 1 1 1 1
1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1
0 1 1 1 0 1 1 1 1 0 0 1 1 1 1
0 1 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 1 1 1 1 1 0 1 0 0 0 0 1 1
0 0 0 0 0 1 0 0 0 0 0 0 0 0 0
Eco+ACT/Msc+AAG (8)
1 0 0 0 0 0 1 1 1 1 1 1 1 1 1
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
0 1 1 1 1 1 0 1 1 1 0 1 1 1 0
1 1 1 1 1 1 1 1 1 0 0 0 0 1 1
0 1 1 1 1 1 1 1 1 1 1 1 1 1 1
0 0 0 0 0 0 1 0 0 0 0 0 0 0 0
1 1 - 1 1 1 1 1 1 0 0 0 1 0 0
Eco+ACT/Msc+ACT (10)
1 1 1 1 0 1 1 1 1 1 1 1 0 0 0
1 1 1 1 1 1 1 1 1 0 1 1 0 0 0
1 1 1 1 1 1 1 1 1 0 0 0 1 1 1
0 0 0 0 0 0 0 0 0 0 0 0 1 1 1
0 1 1 1 0 1 0 1 1 0 0 0 1 1 1
1 1 1 1 1 1 0 1 1 0 0 0 1 1 1
1 0 1 0 1 1 1 0 0 0 1 0 0 0 0
1 1 1 1 1 1 1 1 1 1 1 1 0 0 0

Scored loci (continued)
1 1 1 1 1 1 1 1 1 0 0 0 1 1 1
1 1 1 1 1 1 1 1 1 1 1 1 0 0 0
Eco+ACT/Mse+ATA (5)
1 1 1 1 1 0 1 1 1 0 1 0 1 1 1
1 1 1 1 1 0 1 1 1 0 1 0 1 1 1
1 1 1 1 1 1 0 1 1 1 1 1 1 1 1
0 0 0 0 0 0 0 0 0 1 1 1 0 0 0
0 0 0 0 0 0 1 1 1 0 1 0 0 1 0
Eco+ACT/Mse+AAT (9)
1 1 1 1 1 1 1 1 0 0 0 1 0 1 1
1 0 0 0 0 0 1 1 1 1 1 1 1 1 1
0 0 0 0 0 0 1 1 1 1 1 1 1 1 1
0 0 0 0 0 0 1 1 1 1 1 1 1 1 1
0 1 1 1 1 1 0 1 1 1 1 0 1 1 1
1 1 0 1 0 1 1 1 1 1 1 1 1 1 1
0 1 0 1 1 1 0 1 1 1 1 1 1 1 1
1 0 0 1 1 0 1 1 1 1 1 0 1 1 1
0 1 0 1 1 1 1 1 1 1 1 1 1 1 1
Eco+AGC/Mse+AAG (19)
0 0 1 0 1 1 1 1 1 0 0 0 1 1 1
1 1 1 1 1 1 1 1 1 0 1 0 1 0 0
0 1 1 1 1 1 1 1 1 1 1 1 0 0 0
0 0 1 1 1 1 1 0 0 0 0 0 1 1 0
1 1 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 0 0 0 0 1 0 0 0 0 0 1 0 0
1 0 1 1 1 0 1 1 1 0 1 1 1 1 1
0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
1 0 0 0 0 0 0 0 0 0 0 0 0 0 1
0 0 0 0 0 0 0 0 0 0 0 0 1 1 1
1 1 1 1 1 1 1 1 1 1 0 1 1 1 1
1 1 1 1 1 1 1 1 1 0 1 0 1 1 1
0 0 1 0 0 0 0 0 1 1 1 1 1 1 1
1 1 1 1 1 1 1 1 1 0 0 0 1 1 1
1 0 0 0 0 0 0 0 0 1 1 1 0 1 1 1
0 0 1 1 1 0 0 0 0 0 1 1 0 0 0 0
1 1 1 1 1 1 1 1 1 1 1 0 1 1 1
1 1 1 1 1 1 1 1 1 0 1 0 0 0 0
1 0 0 0 0 0 0 0 1 1 1 1 1 1 1
Eco+AGC/Mse+ACT (8)
1 1 1 1 1 1 1 0 1 1 0 1 1 1 1
1 1 1 1 0 0 1 1 1 1 1 1 1 1 1
0 0 0 1 0 0 1 1 1 1 1 0 1 1 1
1 0 0 1 0 1 1 1 1 1 1 1 1 1 1
1 1 1 1 1 1 0 0 0 0 0 0 0 1 0
1 1 1 1 1 1 0 0 1 0 1 1 1 1 0
0 1 0 1 0 0 1 1 0 1 1 1 1 1 1
1 0 1 1 0 0 1 1 0 0 1 1 1 1 1
Eco+AGC/Msc+ATA (24)
0 1 0 0 0 0 1 0 1 1 1 0 1 1 1
1 0 1 0 1 1 1 1 1 1 0 0 0 0 1 1
1 0 1 1 1 1 1 1 1 1 0 1 0 1 1 1
1 1 1 0 0 1 1 0 0 1 1 1 1 1 0 0
0 0 0 1 0 0 0 0 0 1 1 1 1 0 0
1 0 1 1 0 0 1 0 1 0 1 0 1 1 1
1 1 1 1 0 0 1 0 1 1 1 1 1 0 0
0 0 0 0 1 0 1 0 0 0 0 0 1 1
1 1 0 1 1 0 1 0 0 0 0 0 0 0 0
0 0 1 0 1 1 1 1 1 0 0 0 0 1 1
1 0 0 0 0 0 1 0 0 0 1 0 0 0 0
1 0 1 0 1 1 1 1 1 1 0 0 0 0 1 1
0 0 1 1 0 0 1 1 0 1 1 1 1 1 1
1 0 0 0 0 0 1 1 1 1 1 1 1 1 1
0 1 0 0 0 0 0 0 0 0 0 0 1 1 1
1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
0 1 0 0 0 1 0 1 1 1 1 1 1 1 1
1 0 0 0 0 0 1 0 0 1 0 0 0 0 0
1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
0 1 0 0 1 0 0 0 1 1 0 0 0 1 1
1 0 0 0 0 1 1 1 1 1 1 1 1 1 1

Scored loci (continued)
1 0 1 1 1 1 1 0 0 0 1 1 1 1 1
1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
Eco+AGC/Mse+AAT (5)
1 1 - 1 1 0 1 1 1 0 1 1 1 1 1
0 1 - 1 1 1 1 1 1 1 1 1 1 1 1
1 1 - 1 1 0 1 1 1 1 1 1 1 1 1
0 1 - 1 1 0 1 1 0 0 0 1 1 1 1
- 1 - 1 1 1 1 1 1 1 0 0 0 1
- 1 - 1 1 - 1 1 0 1 1 1 1 1 1
Eco+AGG/Mse+AAG (8)
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
1 0 0 1 0 0 0 1 1 1 1 1 1 0 1
0 0 1 0 0 1 0 0 1 1 1 1 1 1 1
0 1 1 1 1 1 0 1 1 0 1 1 0 0 0
1 1 1 1 1 1 0 0 1 1 1 1 0 0 1
0 1 1 1 1 1 1 0 0 1 1 1 1 0 1
0 1 1 1 1 1 1 1 1 1 1 1 0 0 1
Eco+AGG/Mse+ATA (7)
1 0 0 1 0 0 1 1 1 0 0 1 1 0 1
1 0 1 1 1 1 1 0 0 0 0 0 - - -
0 1 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 1 0 0 0 0 0 0 0 0 1 1 1 1
- 1 1 1 1 1 0 0 1 0 0 1 1 1 1
- - 0 0 0 0 1 0 1 0 0 0 0 0 0
- - 0 - 0 0 0 0 0 0 1 1 0 0 0
Eco+ATC/Mse+ATA (15)
1 0 0 0 0 0 0 0 0 0 1 1 1 0 0
0 1 0 1 1 0 0 1 1 1 1 1 1 1 1
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1 1 0 1 1 0 0 1 1 1 1 1 1 1 1
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 1 0 0 1 0 0 0 1 1 1 1 1 0
0 1 1 1 1 1 1 1 1 1 1 1 1 1 1
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 1 1 0 0 0 0 1 1 1 1 1 1 1 1
0 0 1 1 0 0 0 0 0 0 0 0 1 0
1 0 1 0 1 1 0 1 1 1 1 1 1 1 1
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1 1 1 1 1 0 0 0 0 1 0 0 0 0 0
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 1 1 0 1 1 0 1 1 1 1 1 1 1
Eco+ATG/Mse+AAG (6)
- - 1 - 1 - 0 1 1 0 0 0 1 1
- - - - 1 - 0 1 1 0 0 0 0 0 1
- - - - 1 - 0 1 1 0 0 0 0 0 1
- - - - 1 - 0 1 1 1 0 1 1 1 1
- - - - 0 - 1 0 0 0 0 0 0 1 1
- - - - - - 0 0 0 1 1 1 1 1 0
Eco+ATG/Mse+ATA (6)
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
1 1 0 0 1 1 1 1 1 0 0 1 1 1 1
1 0 0 1 0 1 1 1 1 1 1 1 1 1 1
0 1 0 1 1 1 1 1 1 1 1 1 1 1 1
1 0 0 0 0 0 1 0 0 0 0 0 0 0 0
0 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Eco+ATT/Mse+ATA (11)
1 - 0 - 1 1 1 1 1 1 1 1 1 1 1
1 - 0 - 1 1 0 1 1 0 0 0 0 1 0
0 - 0 - 0 0 1 0 0 0 0 0 0 0 0
1 - 0 - 0 0 1 0 0 1 1 - - - -
1 - 0 - 0 0 1 0 0 0 0 0 0 0 0
0 - 0 - 0 0 0 0 0 1 1 1 1 1 0
0 - 0 - 1 1 0 0 0 1 - - - - -
0 - 1 - 1 1 0 1 1 1 1 1 1 1 1
1 - 0 - 0 0 1 0 1 1 - - - - -
1 - 0 - 0 0 1 0 0 0 - - - - -
0 - 0 - 0 0 1 0 0 0 0 0 0 0 0

Scoring corresponds to plant line numbers, 1–15, from left to right. The absence and the presence of the bands, and unscorable data were represented by '0', '1' and '-', respectively. Number of polymorphic loci at every combination (bold characters) is shown in parenthesis.

gel of 40 cm in length. The dried gels were exposed to Kodak Bio-Max/MR film for several days.

Analysis of polymorphisms

The banding patterns were analyzed directly on the autoradiograph and reanalyzed on the enlarged scanned image by eye. The polymorphism was scored as the absence or the presence of the bands for every selective primer combination.

Genetic relationship analysis

Genetic similarity and diversity analysis among fifteen-durum wheat varieties were performed using the data analysis sofware, NTSYS-version 1.7 (Numerical Taxonomy and Multivariate Analysis System, Rohlf, 1992). The similarity index coefficients were calculated according to simple matching coefficient (SM), 'DICE' and 'O' methods (Rohlf, 1992). The genetic distance matrix was obtained using 'Nei72' algorithm. Unweighted pair-group method arithmetic average (UPGMA) clustering was used for tree constructions.

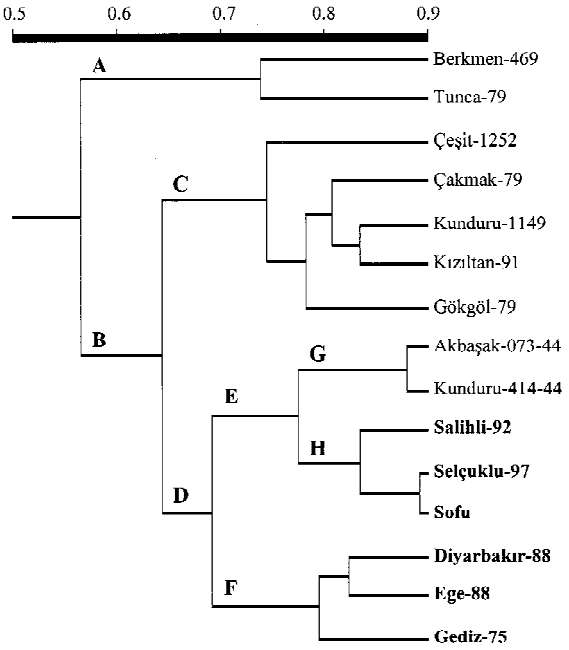

Figure 1. DICE based UPGMA cluster tree of both winter and spring type (bold characters) durum wheat cultivars.

Results and discussions

The selective amplification primers were chosen among the ones of Heun (Heun et al. 1997) shown to be useful specifically for wheat. During the optimization of the several stepwise reaction conditions, 100 ng DNA per unit of each enzyme in a 40 μl restriction volume resulted in better digestion.

In our hands, the modified CTAB isolation method worked better, when compared to a short isolation method (Röder et al. 1995) successfully used for microsatellite markers. It was shown that, when different plant organs are used as source of genomic DNA, AFLP markers display different banding profiles (Donino et al. 1997). Thus, all the DNA isolations were performed on the leaf materials. The degree of possible polymorphism within lines was not investigated, in this study.

There can be complications arising from the PCR artifacts, such as various mismatches and the amplification of the same size products belonging to different loci. In our study, there were 121 ambiguous appearances in some samples where, it was not decided, if there was a band or not. However, the cluster trees obtained with 2714 scored bands, are in accordance with the known pedigree information.

Numbers of polymorphic bands, totaling 189, obtained using 18 AFLP primer combinations are presented in Table 2. The most polymorphic primer combinations were Eco+AGC/Mse+ATA; Eco+AGC/Mse+AAG; Eco+ACC/Mse+AAG; and Eco+AGC/Mse+ACT with 24, 19, 18, and 17, respectively.

Relative distances, which are presented in Table 3, show two very closely related pairs 'Selçuklu-97' with 'Sofu' and 'Akbaşak-073-44' with 'Kunduru-414-44'. These relationships are also reflected in Figure 1.

According to the constructed trees (only the tree obtained with similarity index coefficient based on 'DICE' is shown in Figure 1) durum wheat varieties fell into two major categories, A and B. A was observed with two winter type samples, 'Berkmen-469' and 'Tunca-79'. Cluster B, with remaining other winter types, was divided into two subclusters, one with all the winter types, Çesit-1252', 'Çakmak-79', 'Kìzìltan-91', 'Kunduru-1149' and 'Gökgöl-79' and the other with spring types, 'Salihli-92', 'Selçuklu-97', 'Sofu', 'Diyarbakìr-88', 'Ege-88', 'Gediz-75' and two winter types 'Akbasak-073-44' and 'Kunduru- 414-44'. The observation of two winter type samples, 'Akbasak-073-44' and 'Kunduru-414-44', in the spring type cluster, D, can be expected, since winter types are also considered as facultative

Table 3. Genetic distance diagonal matrix, based on Nei 72

Lines	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.758													
3	0.662	0.335												
4	0.564	0.258	0.254											
5	0.619	0.269	0.229	0.200										
6	0.694	0.317	0.199	0.285	0.181									
7	0.302	0.638	0.584	0.445	0.537	0.545								
8	0.595	0.336	0.371	0.250	0.243	0.281	0.401							
9	0.529	0.320	0.343	0.271	0.254	0.269	0.426	0.128						
10	0.793	0.556	0.687	0.551	0.638	0.630	0.601	0.457	0.385					
11	0.684	0.500	0.519	0.394	0.560	0.560	0.501	0.344	0.306	0.194				
12	0.676	0.532	0.566	0.490	0.611	0.545	0.552	0.408	0.395	0.217	0.243			
13	0.542	0.495	0.510	0.394	0.525	0.517	0.433	0.321	0.288	0.307	0.291	0.268		
14	0.598	0.471	0.396	0.389	0.373	0.413	0.442	0.278	0.235	0.393	0.372	0.380	0.156	
15	0.551	0.446	0.443	0.368	0.353	0.430	0.432	0.220	0.193	0.398	0.392	0.385	0.206	0.115

The line numbers are the same as in Table 1.

types, in addition, 'Akbaşak-073-44' is present in the pedigree of 'Selçuklu-97'.

The clustering of winter type cultivars is in a very good agreement with the pedigrees of the cultivars (Table 1). For example, 'Çesit-1252', 'Çakmak-79' and 'Kìzìltan-91' have '61-130' as one of their ancestors. In addition to '61-130', 'Üvy-162' is also part of the pedigrees of 'Çakmak-79' and 'Kìzìltan-91'. Including 'Tunca-79' to the above three winter type durum wheat cultivars, '61-130' is a common ancestor of all the four types. Both 'Gökgöl-79' and 'Kìzìltan-91' have 'Bye∗2' as one of the parents. In all the trees, 'Gökgöl-79' linked to the cluster in which 'Kìzìltan-91' is present. 'Çakmak-79' and 'Kìzìltan-91' share two common ancestors ('Üvy162' and '61-130'). This was reflected by AFLP markers.

Based on the available pedigree information, all the spring type cultivars sub-clustered together in all the trees obtained using different algorithms. Both 'Diyarbakìr-88' and 'Gediz-75' have 'Tehuacan$^{2'}$ as one of the parents. 'Jori' is the part of the pedigrees of two cultivars, 'Ege-88' and 'Gediz-75'. In all the trees, the link between, 'Ege-88' and 'Salihli-92' may be because of the presence of 'Anhinga' in their pedigrees. In addition to 'Anhinga', 'Ege-88' is also an ancestor of 'Salihli-92'. The presence of both ancestors, '61-130' and 'Üvy162', in 'Selçuklu-97' links this cultivar to the winter type cultivars in cluster B.

These preliminary results suggest that AFLP markers used for this study are applicable for DNA fingerprinting of Turkish durum wheat cultivars for genotype identification and genetic relationship studies. As it is observed with this study, the durum wheat cultivars are very similar genetically, thus, AFLP markers can be used in marker assisted parental selection for breeding to widen the genetic diversity in cultivars while having desirable characters introgressed.

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References

Aggarwal R.K., Brar D.S., Nandi S., Huang N. and Khush G.S. 1999. Phylogenetic relationships among *Oryza* species revealed by AFLP markers. Theor. Appl. Genet. 98: 1320–1328.

Becker J., Vos P., Kuiper M., Salamini F. and Heun M. 1995. Combined mapping of AFLP and RFLP markers in barley. Mol. Gen. Genet. 249: 65–73.

Breyne P., Rombaut D., Van Gysel A., Van Montagu M. and Gerats M. 1999. AFLP analysis of genetic diversity within and between *Arabidopsis thaliana* ecotypes. Mol. Gen. Genet. 261: 627–634.

Caetano-Anolles G., Bassam B.J. and Gresshoff P.M. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technology 9: 553–557.

Cervera M.T., Gusmao J., Steenackers M., Van Gysel A., Van Montagu M. and Boerjan W. 1996. Application of AFLP™-based molecular markers to breeding of *Populus* spp. Plant Growth Reg. 20: 47–52.

Donino P., Elias M.L., Bougourd S.M. and Koebner R.M.D. 1997. AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. Genome 40: 521–526.

Heun M., Schafer-Pregl R., Klawan D., Castagna R., Accerbi M., Borghi B. and Salamini F. 1997. Site of einkorn wheat domestication identified by DNA fingerprinting. Science 278: 1312–1314.

Nei M. 1972. Genetic distance between populations. Am. Nat. 106: 283–292.

Pejic I., Ajmone-Marsan P., Morgante M., Kozumplick V., Castiglioni P., Taramino G. and Motto M. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theor. Appl. Genet. 97: 1248–1255.

Röder M.S., Plaschke J., König S.U., Börner A., Sorrells M.E., Tanksley S.D. and Ganal M.W. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246: 327–333.

Rohlf F.J. 1992. NTSYS-pc numerical taxonomy and multivariate analysis system, version 1.70. Exeter Publications, New York.

Saghai Maroof M.A., Soliman K.M., Jorgensen R.A. and Allard R.W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 81: 8014–8018.

Schut J.W., Qi X. and Stam P. 1997. Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. Theor. Appl. Genet. 95: 1161–1168.

Schwarz G., Michalek W., Mohler V., Wenzel G. and Jahoor A. 1999. Chromosome landing at *Mla* locus in barley (*Hordeum vulgare* L.) by means of high-resolution mapping with AFLP markers. Theor. Appl. Genet. 98: 521–530.

Singh A., Negi M.S., Rajagopal J., Bhatia S., Tomar U.K., Srivastava P.S. and Lakshmikumaran M. 1999. Assessment of genetic diversity in *Azadirachta indica* using AFLP markers. Theor. Appl. Genet. 99: 272–279.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. and Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407–4414.

Welsh J. and McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18: 7213–7218.

Williams J.G.K., Kubelik A.K., Livak K.J., Rafalski J.A. and Tingey S.V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res.18: 6531–6535.

Yin X., Stam P., Johan Dourlejin C. and Kropff M.J. 1999. AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley. Theor. Appl. Genet. 99: 244–253.

Zabeau M. and Vos P. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application EP 534858A1.

Zencirci N., Aktan B. and Atli A. 1992. Genetic relationships of Turkish durum wheat cultivars. Tr. J. Agricul. Forestry 18: 187–192.