*Euphytica* **122:** 257–262, 2001.



# Genotyping of Anatolian doubled-haploid durum lines with SSR markers

Erdogan E. Hakki[1], Cigdem Savaskan[2] & Mahinur S. Akkaya[1,*]
[1]*Middle East Technical University, Department of Chemistry, Biochemistry Division, Ankara, TR-06531;* [2]*Nuclear Agriculture and Animal Research Center, Saray, Ankara, TR-06983, Turkey; (*author for correspondence; e-mail: akkayams@metu.edu.tr)*

Received 22 August 2000; accepted 25 January 2001

*Key words:* doubled-haploid durum wheat, fingerprinting, genetic relationships, SSR (microsatellites)

**Summary**

In this study, doubled haploid lines generated from the durum wheat varieties, selections from Middle Anatolian landraces, 'Çakmak-79', 'Berkmen-469' and 'Kunduru-1149' (Savaskan et al., 1997), were analyzed using ten highly polymorphic microsatellite markers for genotyping and evaluation of genetic relationships between and within the doubled-haploid (DH) lines. The average PIC value was found to be 0.531. Populations of doubled-haploid lines of landrace selected cultivars 'Çakmak-79' and 'Kunduru-1149', were the two most distant populations with $(\delta\mu)^2$ = 1.42. 'Berkmen-469' × 'Çakmak-79' and 'Berkmen-469' × 'Kunduru-1149' yielded similar genetic distances, $(\delta\mu)^2$ of 0.84 and 0.85, respectively. In addition, the genetic relationship between the progenitors of the DH lines together with other durum wheat varieties was analyzed. A meaningful relationship was obtained based on available pedigree information on the cultivars.

## Introduction

Simple sequence repeat (SSR) DNA markers or microsatellites are tandem repeats with a core unit length of 2–5 nucleotides in eukaryotic genomes. These repeat regions through locus specific PCR amplification were used as molecular markers for applications in many phylogenic and molecular genetics studies. These markers are quite randomly distributed, abundantly present, highly reproducible and polymorphic. In the last 5 years, many wheat microsatellite markers became available (Röder et al., 1995, 1998).

'Kunduru-1149' and 'Berkmen-469' are old genotypes of Middle Anatolia (Central region of Turkey), which were improved by selection from local populations based on yield increase. Turkish farmers are still using 'Kunduru-1149' for production due to adaptability and high yielding natures. 'Çakmak-79', is a cross of 'Üveyik-162' (also Middle Anatolian landrace selection) and line 61–130 (US origin, semi-dwarf durum wheat). In this study, the genetic relationship of doubled-haploid (DH) individuals of three-durum wheat cultivars, 'Çakmak-79', 'Berkmen-469' and 'Kunduru-1149' was investigated. Also, the genetic relationship of the DH lines together with their progenitor individual plants ('Berkmen-469'; 'Çakmak-79'; 'Kunduru-1149') in the background of the other Turkish durum wheat varieties were analyzed. Characterizing the genetic distances of DH lines within and between populations will be valuable for assessing the genetic diversity, thus, facilitating the use of these lines in breeding programs.

## Materials and methods

### *Plant materials*

In this study, a total of 18 individual DH lines; 3 DH seeds from 'Çakmak-79'; 3 DH seeds from 'Berkmen-469' and 12 DH seeds from 'Kunduru-1149' (Savaskan et al., 1997) together with their progenitors and some local durum wheat varieties (Dograr et al., 2000) were investigated.

*Table 1.* Allele sizes (bp) of DH lines using 10 microsatellite markers

| DHs | Markers | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [a]WMS2 | [b]WMS2 | WMS5 | WMS6 | WMS6 | WMS11 | WMS18 | WMS30 | WMS46 | WMS120 | WMS131 | WMS135 |
| B1 | nd | nd | 171 | 203 | 223 | 224 | 181 | 210 | 167 | 145 | 131 | 174 |
| B3 | 118 | 220 | 171 | 203 | 223 | 224 | 181 | 210 | 167 | 145 | 131 | 174 |
| B5 | 118 | 220 | 171 | 203 | 223 | 224 | 181 | 210 | nd | 145 | 131 | 174 |
| Ç1 | 122 | 224 | 171 | 201 | 247 | 228 | 183 | 210 | 169 | 162 | 159 | 174 |
| Ç2 | 122 | 224 | 171 | 201 | 247 | 228 | 183 | 210 | 169 | 162 | 159 | 174 |
| Ç3 | 122 | 224 | 171 | 201 | 247 | 228 | 183 | nd | 169 | 162 | 157 | 165 |
| K1 | 122 | 224 | 165 | 195 | 223 | 222 | 183 | nd | 169 | 148 | 155 | 174 |
| K2 | 122 | 224 | 165 | 207 | 223 | 224 | 181 | 208 | 167 | 146 | 131 | 174 |
| K3 | 122 | 224 | 165 | 195 | 223 | 222 | 183 | 208 | 169 | 148 | 131 | 174 |
| K4 | 122 | 224 | 165 | 195 | 223 | 222 | 183 | 208 | 169 | 148 | 155 | 174 |
| K5 | 122 | 224 | 165 | 207 | 223 | 224 | 181 | 208 | 169 | 148 | 131 | 174 |
| K6 | 122 | 224 | 165 | 195 | 223 | 222 | 183 | 208 | 169 | 148 | 155 | 174 |
| K7 | 122 | 224 | 165 | 195 | 223 | 222 | 183 | 208 | 169 | 148 | 155 | 174 |
| K8 | 122 | 224 | 165 | 207 | 223 | 224 | 181 | 208 | 167 | 148 | 131 | 172 |
| K9 | 122 | 224 | 165 | 199 | 207 | 224 | 181 | 208 | 167 | 146 | 131 | 174 |
| K10 | 118 | 220 | 169 | 205 | 223 | 222 | 183 | nd | 167 | 143 | 131 | 172 |
| K11 | 122 | 224 | 165 | 195 | 223 | 222 | 183 | 208 | 169 | 148 | 155 | 174 |
| K12 | 120 | 222 | 169 | 199 | 207 | 201 | 189 | 210 | 149 | 146 | 131 | 174 |
| PIC (Ave: 0.531) | 0.381 | 0.381 | 0.568 | 0.790 | 0.438 | 0.667 | 0.537 | 0.480 | 0.527 | 0.715 | 0.599 | 0.291 |

nd: not determined.
[a]: locus *Xgwm2-3A* (Röder et al., 1998).
[b]: locus *Xgwm2-3D* (Röder et al., 1998).

*DNA isolation and PCRs*

DNA was isolated from half cut seeds according to a literature procedure (Plaschke et al., 1995) with some modifications (Dograr et al., 2000). DNA samples of genotypes were amplified with 15 different microsatellite primer sets; WMS2, WMS5, WMS6, WMS10, WMS11, WMS18, WMS30, WMS43, WMS46, WMS67, WMS88, WMS95, WMS120, WMS131 and WMS135, the sequences of which and the chromosome locations were reported earlier (Röder et al., 1995, 1998). The PCR amplification conditions, electrophoresis and detection of microsatellites were performed as previously reported (Dograr et al., 2000).

*Cluster analysis*

Numerical Taxonomy and Multivariate Analysis System software (NTSYS, version 1.70) (Rohlf et al., 1992) was used to generate UPGMA dendogram by using the simple matching coefficient similarity matrix of 18 DH individuals at 10 microsatellite loci (Figure 2). The allele size data of the other durum wheat cultivars and single individuals of the progenitors of the DH lines were also incorporated into the analysis (Figure 3) at 7 of the 10 microsatellite loci used in Figure 2. The genetic distances between the populations of doubled-haploid durum wheat lines, 'Çakmak-79', 'Berkmen-469' and 'Kunduru-1149', were standardized and processed using the software RST-22 (Goodman, 1997) which calculates $(\delta\mu)^2$ as defined by Goldstein (Goldstein et al., 1995). The genetic distances were also calculated with 'Nei72' algorithm (Rohlf et al., 1992) using allele frequencies. Polymorphism information content (PIC) values (Table 1) were obtained according to the formula below (Anderson et al., 1993).

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

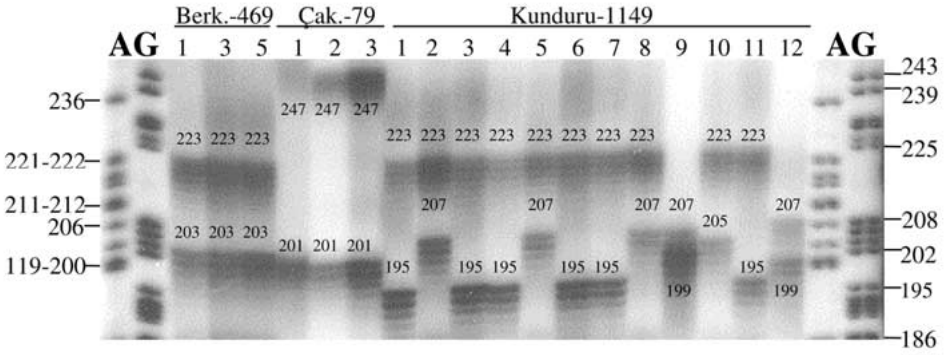


*Figure 1.* Autoradiograph of PCR products amplified with WMS6 primer set, separated on a standard DNA sequencing gel. The numbers indicate the DH lines of each cultivar. Molecular weight size marker is the 'A' and 'G' sequence products of M13mp18 ssDNA. The fragment sizes of size marker are indicated as bp on the left and the right of the autoradiograph image. The length of the PCR products are indicated on the top or the bottom of the bands.

## Results and discussion

The markers selected are from the published sequences of Röder and co-workers (Röder et al., 1995, 1998; Plaschke et al., 1995). The 15 wheat SSR markers tested are WMS2, WMS5, WMS6, WMS10, WMS11, WMS18, WMS30, WMS43, WMS46, WMS67, WMS88, WMS95, WMS120, WMS131 and WMS135. Among them, 10 markers showed high level of polymorphism with PIC values ranging from 0.291 to 0.790. The allele sizes of polymorphic markers determined precisely are shown in Table 1. The PCR amplifications of WMS10, WMS43, WMS67, WMS88 and WMS95 markers resulted monomorphic lengths, therefore, they were not included in characterizations. SSR markers of WMS2 and WMS6 (Figure 1) amplified two alleles with each individual plant. The primer set of WMS2 marker was reported amplifying two independent loci at chromosomes 3A and 3D as, *Xgwm2-3A* and *Xgwm2-3D* (Röder et al., 1998). All of the DH individual lines in this study too, resulted two PCR products. However, the larger PCR products (Table 1) obtained with WMS2 primer set cannot be amplifying a D genome locus in the durum wheat varieties, thus the bands that we observe must belong to another locus either in the A or the B genome, since the plant materials are DH lines. WMS6 marker primer set also amplified two PCR products (Figure 1 and Dograr et al., 2000), however, there is only one locus reported for the primer set at chromosome 4B, as *Xgwm6-4B* (Röder et al., 1998). Based on the observations reported here, WMS6 is indeed amplifying two independent loci, instead of heterozygous alleles at a single locus. In this study, the locus of the second WMS6 primer set PCR product has not been identified. The allele sizes ranging from 195 bp to 207 bp were considered as alleles of one locus and the ones ranging in 199 bp and 223 bp, as another locus.

The data in Table 1 shows that three of the 'Berkmen-469' DH lines (B1, B3, B5) represent the same genotype. 'Çakmak-79' DH lines represent two different genotypes, the first two (Ç1, Ç2) are the same, Ç3 is a different genotype than the others. We had four times as many 'Kunduru-1149' DH lines than that of the other two varieties. 'Kunduru-1149' DH lines were shown to represent a total of 8 different genotypes. K1, K4, K6, K7, K11 gave identical alleles. All the others represent different genotypes. These results were summarized in a dendogram (Figure 2). The highest variation within the cultivar was observed in 'Kunduru-1149'. Its DH lines show two sub-clusters (Figure 2).

Further analysis was performed by incorporating the individual progenitors of the DH lines ('Berkmen-469'; 'Çakmak-79'; 'Kunduru-1149') using a single plant as well as other 6 Turkish durum wheat varieties ('Kiziltan-91'; 'Çeşit-1252'; 'Gökgöl-79'; 'Tunca-79'; 'Kunduru-414/44'; 'Akbaşak-073/44') of which the allele sizes were previously reported (Dograr et al., 2000). The genetic relationship presented in Figure 3 is based on the amplification of 7 microsatellite primer sets (WMS5, WMS6 with two independent loci, WMS11, WMS30, WMS46, WMS120 and WMS 131, Dograr et al., 2000). The most homogeneous DH population was that of the 'Berkmen-469' lines followed by the 'Çakmak-79' lines (Figure 2). Both of these populations were also precisely clustered with their progenitors. As the 'Kunduru-1149', 'Kunduru-

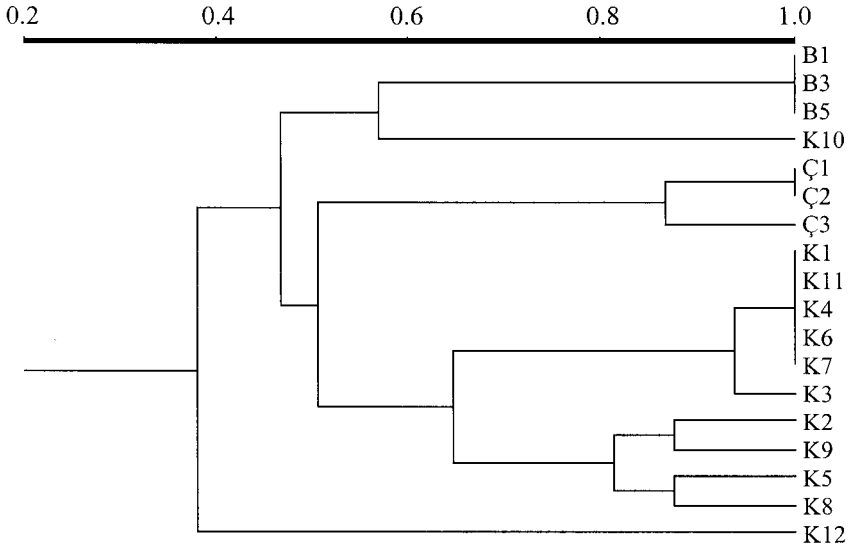


*Figure 2.* UPGMA clustering of DH individual lines based on simple matching coefficient similarity matrix of microsatellite data set of 10 markers (WMS2, WMS5, WMS6, WMS11, WMS18, WMS30, WMS46, WMS120, WMS131 and WMS135).

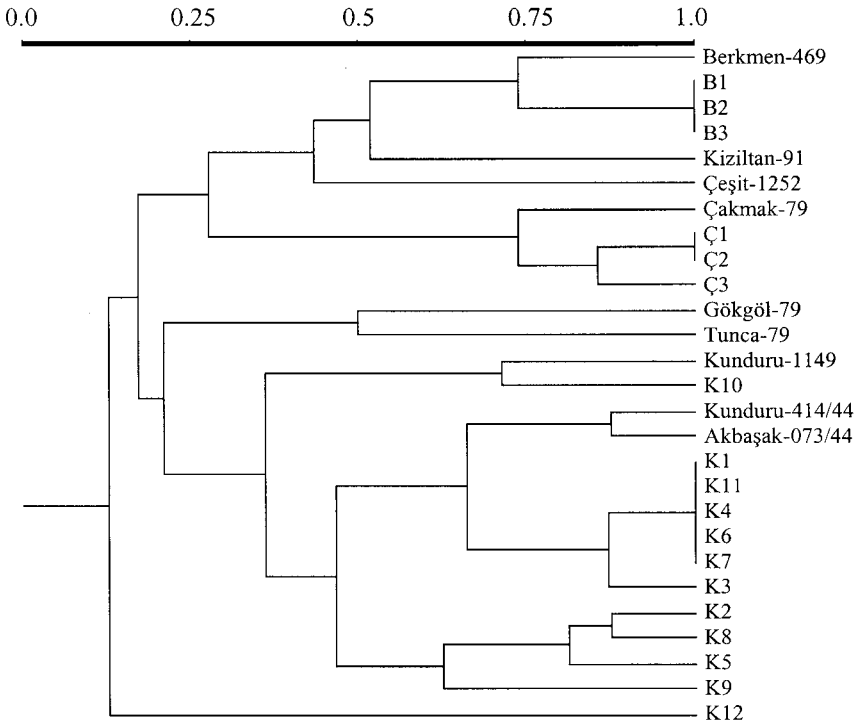


*Figure 3.* UPGMA clustering based on simple matching coefficient similarity matrix of microsatellite data set of 7 markers (WMS5, WMS6, WMS11, WMS30, WMS46, WMS120, WMS131) of DH individual lines (B1,3,5; Ç1-3; K1-12) together with their progenitors ('Berkmen-469'; 'Çakmak-79'; 'Kunduru-1149', Dograr et al., 2000) and local durum wheat varieties ('Akbaşak-073/44'; 'Çeşit-1252'; 'Gökgöl-79'; 'Kiziltan-91'; 'Kunduru-414/44'; 'Tunca-79', Dograr et al., 2000).

*Table 2.* The genetic analysis between the populations: 'Rho' values, $(\Delta\mu)^2$ and 'Nei72' genetic distances (10000 permutations and bootstraps)

| Populations | $\rho^a$ | $P^a$ | $\rho^b$ | $P^b$ | $(\delta\mu)^2$ | 'Nei72' |
|---|---|---|---|---|---|---|
| 'Berkmen-469' × 'Çakmak-79' | 0.99620 | 0.02430 | 0.76655 | 0.02100 | 0.83527 | 1.4655 |
| 'Berkmen-469' × 'Kunduru-1149' | 0.42741 | 0.04790 | 0.34975 | 0.06570 | 0.85153 | 0.9175 |
| 'Çakmak-79' × 'Kunduru-1149' | 0.55916 | 0.01180 | 0.44143 | 0.02950 | 1.41930 | 0.8872 |

$^a$ averaging over variance components; $^b$ averaging over loci.

414/44' and 'Akbaşak-073/44' were all landrace selections from Anatolia, it was not a surprise that all of them were clustered together with 'Kunduru-1149'-originated DH lines. From the DH samples, K10 has the highest similarity to a single plant of 'Kunduru-1149' while the remaining 11 lines were much similar to 'Kunduru-414/44' and 'Akbaşak-073/44' branch. K12 line remained the least related sample, in this analysis, too. From the local durum wheat varieties 'Kiziltan-91' and 'Çeşit-1252' were found to be related to the 'Berkmen-469' population while 'Gökgöl-79' and 'Tunca-79' were clustered closely to each other and related to the population of 'Kunduru-1149'. The clustering results are in accordance with the pedigree information available (Zencirci et al., 1992).

When assessing genetic relationships using microsatellite markers, especially for molecular ecology and evolutionary studies, using an appropriate distance or similarity algorithm specifically developed for the mutation models of microsatellite markers would be more proper. The algorithm used in this study (Goodman, 1997) is based on the stepwise mutation model applied to microsatellites (Slatkin, 1995) for 'rho' calculations and genetic distance is denoted as $(\delta\mu)^2$ (Goldstein et al., 1995). Thus, we believe a better estimation of genetic distance is obtained between the populations of DH lines in this study. 'Berkmen-469' × 'Çakmak-79' and 'Berkmen-469' × 'Kunduru-1149' pair-wise comparisons show a closer genetic relationship, $(\delta\mu)^2$ of 0.84 and 0.85, respectively, than the other pair-wise comparisons. This might be due to a possible close relationship between 'Üveyik-162', the parent of 'Çakmak-79', and 'Berkmen-469', since they are both landrace selections. When K10 and K12, which are clustered very differently than the other 'Kunduru-1149' DH lines, as can be seen in Figure 2, were omitted in the calculations of $(\delta\mu)^2$ based genetic distance, the 'Berkmen-469' and 'Kunduru-1149' became closer with $(\delta\mu)^2$ value of 0.65. This distance is probably meaningful, if K10 and K12 genotypes are represented with much lower frequencies in the real population than the one observed in this study. It is shown that the differentiation between the populations is the highest between 'Berkmen-469' and 'Çakmak-79' with 'rho' values averaging over variance components and over loci were 0.996 and 0.767, respectively. On the other hand, 'Nei72' genetic distance calculations yielded different results, an opposite trend in relationships between populations were found (Table 2). The genetic relationship found between 'Berkmen-469' and 'Çakmak-79' using $(\delta\mu)^2$ is better reflecting the distances between these populations based on the accepted historical view and other studies (Zencirci et al., 1992).

## Conclusion

'Berkmen-469', 'Kunduru-1149' and one of the parents of 'Çakmak-79' were developed as selections from the local varieties, it is likely that they possess the features of multiple lines or exist as populations. Because they are adapted to the land and the climate, they can also offer a potential for the discovery of hidden gene and/or gene families of agronomic importance. Thus, DH lines can be exploited as pure sources of the best agronomic characters in breeding.

DH lines evaluated in this study are also being studied as potential sources in wheat breeding studies. 'Kunduru-1149' DH lines which showed different morphological characters, such as dark color coleoptile or different spike and seed shapes, were confirmed as different genotypes according to the results obtained in this study. These lines are also being studied in terms of selecting best agronomic characters of plant height, spike length, ear heading date and 50-grain weight. There are correlations with these features and the genetic similarities obtained within the DH lines. The completion of the evaluation of agronomic characters, together with complete correlation with DH genotypes discriminated as a result of microsatellite marker analysis will allow breeding

of new cultivars with desired agro-ecological properties. It will be possible to speed up the selection process by using the microsatellite markers presenting polymorphism correlated with the characters of importance.

## Acknowledgements

The supports from Scientific and Technical Council of Turkey 'TÜBİTAK' (TBAG-1720), State Planning Organization 'DPT', METU and TWAS (RGA No. 96-052 RG/BIO/AS) research funds are gratefully acknowledged. Authors thank to Prof A. Kence for reviewing the manuscript.

## References

Anderson, J.A., G.A. Churchill, J.E. Autrique, S.D. Tanksley & M.E. Sorrells, 1993. Optimizing parental selection for genetic linkage maps. Genome 36: 181–186.

Dograr, N., S. Akin-Yalin & M.S. Akkaya, 2000. Discriminating durum wheat cultivars using highly polymorphic simple sequence repeat DNA markers. Plant Breed 119: 360–362.

Goldstein, D.B., A. Ruiz Linares, L.L. Cavalli-Sforza & M.W. Feldman, 1995. Genetic absolute dating based on microsatellites and the origin of modern humans. Proc Natl Acad Sci USA 92: 6623–6727.

Goodman, J.S., 1997. R-St CALC-A collection of computer-programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Mol Ecol 6: 881–885.

Plaschke J., M.W. Ganal & M.S. Röder, 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91: 1001–1007.

Rohlf, F.J., 1992. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, version 1.70. Exeter Publications, New York.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M-H. Tixier, P. Leroy & M. Ganal, 1998. A microsatellite map of wheat. Genetics 149: 2007–2023.

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

Savaskan, C., C. Ellerbrook, L.J. Fish & J.W. Snape, 1997. Doubled haploid production in Turkish durum wheat using crosses with maize. Plant Breed 116: 299–301.

Slatkin, M., 1995. A measure of population subdivision on microsatellite allele frequencies. Genetics 139: 457–462.

Zencirci, N., B. Aktan & A. Atli, 1992. Genetic relationships of Turkish durum wheat cultivars. Tr J Agric Forest 18: 187–192.