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DETERMINATION OF GENETIC VARIATION AND RELATIONSHIP IN TURKISH BARLEY CULTIVARS BY HORDEIN AND RAPD MARKERS

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ABSTRACT

Genetic variation and relationships among thirty four barley cultivars (*H. vulgare* L.) improved in Turkey were assessed by hordeins and RAPDs. Totally, 15 different hordein patterns were observed among 34 cultivars and twelve of these were cultivar specific. In comparison to hordeins, the RAPD variation observed among cultivars was higher and 80 % of these scorable bands were polymorphic. Genetic similarity (*GS*) calculated on hordein and RAPD data ranged from 0.52 to 1.00 and 0.57 to 1.00, respectively. Cluster analyses based on hordein data showed that most of the cultivars are closely related in genetical point of view. The dendrogram of hordeins were completely different from those of RAPDs and they couldn't precisely separate the barley cultivars. In addition to these, the level of polymorphism detected with hordein was lower than that of with RAPDs. Correspondence analysis by using two marker systems showed that RAPD data could distinguish almost all barley cultivars except Tokak 157/37 and Bülbül89, whereas hordein data were not able to perform to distinguish barley cultivars like RAPDs. Some polymorphic and repeatable RAPDs markers should be equipped with morphologic markers in order to identify candidate cultivars and registered cultivars before and after registration procedure.

Keywords: barley, cultivar identification, genetic relationship, hordein, RAPDs

INTRODUCTION

The correct and reliable identification of barley cultivars are of great commercial and agricultural importance due to the fact that feeding, malting properties and resistance to certain diseases are cultivar dependent. Although barley cultivars can be identified on basis of morpho-physiological and kernel characteristics, many cultivars cannot be distinguished by using these traits. Sometimes, it is necessary to use biochemical and molecular methods for complete identification. Biochemical markers such as isozymes, and proteins (hordein) could be used as an effective and exact method for barley cultivar identification. These methods were used by scientists to distinguish many European, Australian, Canadian and United States barley cultivars (Autran & Scriban 1977; Shewry *et al.* 1978; Marchylo & Laberge 1980; Kapala 1981; Cooke & Cliff 1983; Montembault *et al.* 1983; Smith & Payne 1984; Gebre *et al.* 1986; Cooke 1995; Bernardo *et al.* 1997; Perovic *et al.* 1998).

Molecular markers, such as RAPD, RFLP, AFLP, STS and microsatellites are the most powerful ways to reveal genetic diversity, identify different cultivars and undertake genetic mapping (Graner *et al.* 1991; Saghai-Maroof *et al.* 1994; Becker *et al.* 1995). These markers have made possible characterization of different barley cultivars, understanding of phylogenetic relationships, and genetic mapping (Hoffman & Bregitzer 1996; Marillia & Scoles 1996; Ordon *et al.* 1997).

The aim of this work which was the first attempt in Turkey was to reveal genetic variation and then determine genetic relationships among 34 Turkish barley cultivars by using hordein and RAPD markers.

MATERIALS AND METHODS

Thirty four barley cultivars from Turkey used in the present study are listed in Table 1.

Hordein analysis: The extraction of hordein from barley seeds and their analysis by acid PAGE was carried out according to the ISTA standard reference procedure (Cooper 1987). A meal sample extract from 20 kernels for each cultivar was analyzed for hordein composition. In each electrophoretic analysis, a meal sample extract from the reference cultivar Atem was included, adjacent to a meal sample extracts of cultivars.

DNA isolation and RAPD analysis: Total genomic DNA was isolated from a bulk sampling of twenty individuals for each cultivars according to Anderson *et al.* (1992). RAPD reactions were performed with the following 25, 10-base primers (Operon Technologies Inc., Alameda, USA: OPA-04, OPA-12, OPA-17, OPB-01, OPB-12, OPB-14, OPC-03, OPC-13, OPE-02, OPG-10, OPG-16, OPH-19, OPI-14, OPJ-08, OPK-10, OPL-16, OPM-08, OPM-20, OPN-13, OPO-03). PCR reaction mixture consisted of 10 ng genomic DNA, 1X PCR buffer, 1.5 mM MgCl2, 200 µM of each dNTPs, 200 nM of each primer, 0.5 units of *Taq* DNA polymerase in a 25µL volume. The amplification protocol was 94 ^{0}C for 2 min to pre-denaturate, followed by 45 cycles of 94 ^{0}C for 1 min, 36 ^{0}C for 1 min and 72 ^{0}C for 1 min, with a final

Table 1. Pedigrees, growth babit and hordein banding models of 34 Turkish barley cultivars used in the study

Name of cultivars	Pedigrees	Growth habits	Hordein banding pattern
Tokak 157/37	Selection from Turkish land races	Winter	1
Zafer 160	Selection from Turkish land races	Spring	2
Yeşilköy 387	Zafer160 / land race from Kırklareli (gene bank no 3351)	Spring	3
Cumhuriyet 50	Land race from Kayseri/ Maulsholt's 2-Rijige	Winter	1
Yerçil 147	Strengs Frankengerste from Germany	Spring	4
Kaya 7794	Unknown	Spring	4
Hamidiye 85	Tokak mutant 173 TH / Tokak	Winter	5
Obruk 86	Selection from Tokak	Winter	1
Anadolu 86	Luther / BK 259-149/3 gün-82	Winter	1
Bülbül 89	13GTH / land race (Gene bank number 657)	Winter	1
Erginel 90	Escourgeon / Hop2171 (France)	Winter	6
Bilgi 91	Introduction from Mexico	Spring	7
Şahin 91	Unknown	Winter	1
Tarm 92	Tokak / land races no 4875	Winter	1
Efes 3	Unknown	Winter	1
Bornova 92	Unknown	Spring	4
Yesevi 93	Tokak / land race no 4857	Winter	1
Karatay 94	3896/I-3/Toplani/3/Rekal/1128/90 Manhaists	Winter	1
Orza 96	Tokak / land race no 4857	Winter	1
Balkan 96	Unknown	Winter	8
Kalaycı 97	Erginel 9 / Tokak	Winter/Spring	9
Kıral 97	Unknown	Winter	10
Sladoran	Introduction from Yugoslavia	Winter/Spring	11
Anadolu 98	Susuz selection / Berac (Turkey-Holland)	Winter	1
Efes 98	Tercan selection / Tipper (Turkey-England)	Winter	1
Şerifehanım 98	Unknown	Spring	12
Süleymanbey 98	Unknown	Spring	13
Angora	(Triax / line 818 no) / (Malta X Ungar) /2/ (lineno 818 / Sultan)	Winter	1
Çetin 2000	Star (İran) / 4875 no line	Winter	6
Aydanhanım	GK Omega / Tarm 92	Winter	1
Avcı 2002	Sci/3/Gi-72AB58,F1//WA1245141	Winter/Spring	14
Çumra 2001	Tokak selection / Beka	Winter	1
Çatalhöyük 2001	S 8602 / Kaya	Winter	1
Zeynelağa	(Anteres x KY63-1249) x Lignee	Winter/Spring	15

(Source: Cultivar Registration and Seed Certification Office archives)

extension at 72 ^{0}C for 10 min. Amplification products were fractionated on 2% agarose gels, stained with ethidium bromide, visualized with ultraviolet light and photographed.

Data analysis: The banding patterns of the hordein were scored for each cultivar and relative electrophoretic mobilities (REM) of hordein bands were calculated as described by White and Cooke 1992. The presence of a band at a given REM was designed as '1' and its absence '0'. RAPD data were scored for presence (1), absence (0) and each band was regarded as a locus. Bands of identical size amplified with the same primer were considered to be the same locus consisting of two alleles. The genetic similarities (GS) were calculated according to Nei & Li (1979). Based on the similarity matrix, a dendogram showing the genetic relationships between cultivars, was constructed using unweighted pair group method with arithmetic average (UPGMA) (Sneath & Sokal 1973) by using the software NTSYS-pc version1.80 (Rohlf 1993). Polymorphic information content (PIC) values were calculated for each RAPD primer according to the formula: PIC = 1- Σ $(P_{ij})^2$,

where Pij is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers (Botstein *et al.* 1980).

RESULTS AND DISCUSSION

Analysis of hordein from 34 cultivars yielded 15 different hordein band patterns**.** The diagrammatic representation of hordein band patterns was presented in Figure 1. The different hordein band patterns were labeled numerically (1-15) and the results are summarized in Table 1. Totally, 69 hordein bands controlled by B and C hordein loci (*Hor1 and Hor2)* were detected in 15 different hordein band patterns and their REM values ranged from 23 to 105. Because of the fact that band staining intensity may be changeable according to different staining methods, it is more reliable that only band numbers and their REM values should be taken into consideration for cultivar identification. Several studies emphasized that staining intensity of bands may be changeable and not be used for cultivar identification (Marchylo & Laberge 1981; Marchylo 1987).

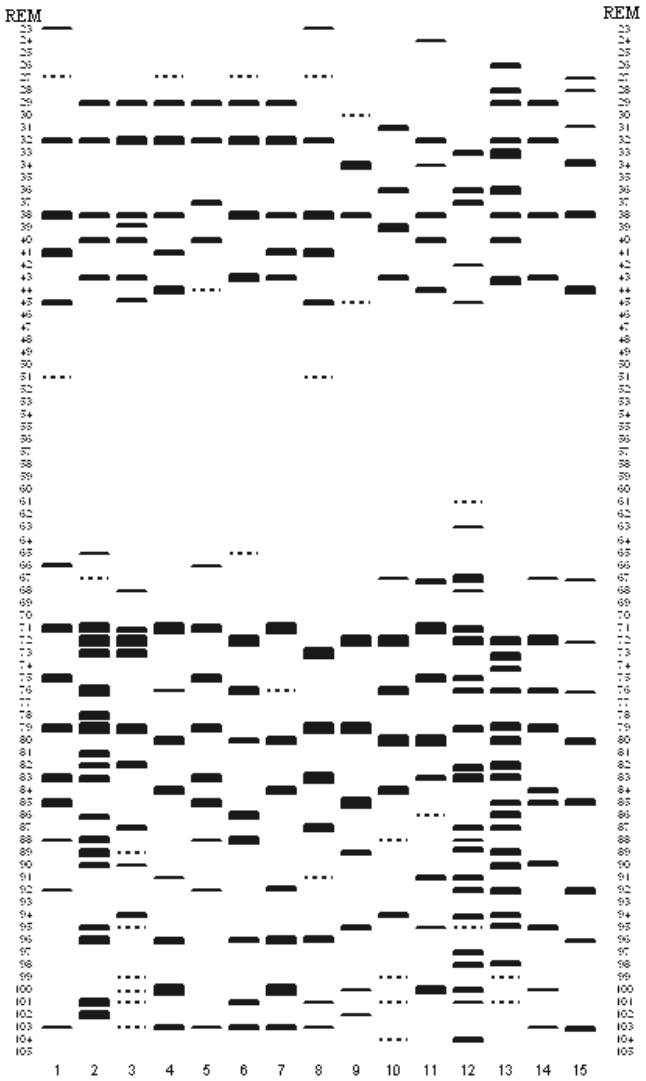

Figure 1. The diagrammatic representation of 15 hordein banding patterns found in 34 Turkish barley cultivars Numbers 1-15: hordein banding patterns, REM: relative electrophoretic mobility.

By comparing to the hordein band patterns, it is apparent that many Turkish cultivars showed identical band patterns. However, only twelve cultivars such as Zafer160, Yeşilköy387, Hamidiye 85, Bilgi 91, Balkan 96, Kalaycı 97, Kıral 97, Sladoran, Şerifehanım 98, Süleymanbey 98, Avcı 2002 and Zeynelaga have unique band patterns.

In several studies using different electrophoresis techniques, numbers of hordein band pattern were found 70 in 353 cultivars (White & Cooke 1992), 42 in 68 cultivars (Marchylo & LaBerge 1981), 19 in 68 cultivars (Cooke *et. al.* 1983), 29 in 77 cultivars (Montembault *et.al* (1983) and 105 in 706 cultivars (Cooke *et. al.* 1995a). In this study, 15 hordein band detected in 34 Turkish cultivars showed that there was high genetic polymorphism among Turkish cultivars. Variability in numbers of hordein band patterns may be result of different level of genetic polymorphism among cultivars used and also cultivar numbers and different electrophoresis methods.

In this study, many winter barley cultivars that cannot be distinguished by PAGE, due to the fact that they have close parental relationships (Table 1). For example, Tokak 157/37 is one of the parents of Obruk 86, Tarm 92, Yesevi 93, Orza 96 and Çumra 2001. One of the parental lines of Anadolu 98 and Efes 98 was also collected Tercan and Susuz provinces where Tokak 157/37 has been predominantly cultivated. However, different pedigrees may also give identical hordein patterns as shown for Anadolu 86, Bülbül 89, Karatay 94, Anadolu 98, Efes 98, Angora and Çatalhöyük 2001. In the some previous studies conducted at various laboratories using different electrophoretic techniques have demonstrated that a number of cultivars are indistinguishable (Shewry *et al.* 1978; Marchylo & LaBerge 1981; Weiss *et al.* 1991). Weiss *et al.* (1991) could not differentiate all 55 European winter and spring barley cultivars by using SDS-PAGE and IEF methods due to the fact that there was genetically close relationship among barley cultivars used in this study and position of the genes coding for the B and C hordein is located on the same arm of chromosome 5 which limits possibilities of recombination.

Cooke (1995b) explained that certain hordein band patterns were found in high frequency due to intensive selection pressure against resistance to powdery mildew in breeding. Powdery mildew loci are located between hordein loci (*Hor1* and *Hor2*) and these loci are tightly linked by powdery mildew loci. When selection occurs in favor of some resistance alleles of powdery mildew, then some hordein alleles can be indirectly selected. Hordein band pattern 1 was found in 17 Turkish cultivars. Turkish cultivars having hordein band pattern 1 were generally selected semi-drought areas of Turkey where powdery mildew was not a serious problem. Therefore there was not any selection affect of powdery mildew on the high frequency of hordein band pattern 1.

DNAs from bulks of each of the 34 cultivars were amplified using the twenty five oligonucleotides to examine RAPD patterns. Eight out of twenty-five RAPD primers (Operon Technology Inc. Alameda CA, US) were selected according to the number and consistency of amplified fragments (Table 2). These eight primers amplified a total of

Table 2. Primer names, sequences and polymorphism degrees.

Primer names and sequences	Number of amplified bands	Number of polymorphic bands	Polymorphic / amplified bands (%)	PIC	Number of different genotypes
OPA-12 TCGGCGATAG	9	9	100	0.812	10
OPB-01 GTTTCGCTCC	15	15	100	0.851	21
OPB-12 CCTTGACGCA	13	12	92.3	0.827	15
OPE-02 GGTGCGGGAA	8	8	100	0.609	7
OPH-19 CTGACCAGCC	10	9	90	0.819	15
OPK-10 GTGCAACGTG	7	4	57	0.644	5
OPM-08 TCTGTTCCCC	12	12	100	0.848	12
OPN-13 AGCGTCACTC	11	11	100	0.487	9
Total	85	80	-	-	-
Mean	10.63	10	92.4	0.737	11.75

85 products, of which 80 (94.12%) were polymorphic. For each primer, the number of polymorphic amplification products ranged from 4 to 15, with average of 10. The polymorphism degree detected by each primer, expressed as polymorphic/amplified bands ratio, was calculated. For the best evaluation, only well visible bands were considered. Primers OPA, OPB, OPE-02, OPM-08, OPN-13 led to greatest number of polymorphic bands thus having a high polymorphic/amplified bands ratio, equal to 100%. The average polymorphic information content (PIC) was 0.737 ranging from 0.487 to 0.851. The lowest and the highest PIC values were recorded for primer OPN-13 and OPB-01, respectively. DNA pattern of some cultivars obtained from OPB 12 primer is presented in Figure 2 as an example.

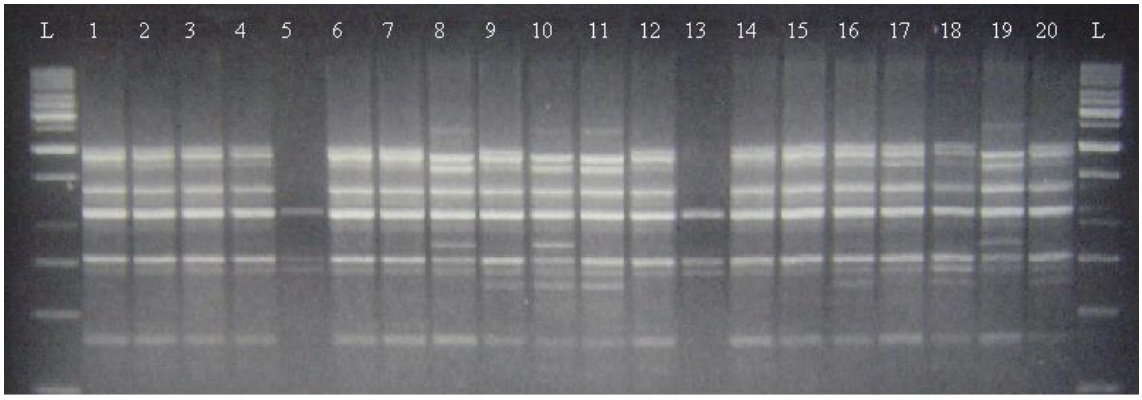

Figure 2. Example of RAPD gel with OPB-12 primer. The first twenty cultivars represented in Table 1 are displayed from left to right. Ladders were run in the outside two lines.

All hordein bands and all the 85 bands which were generated from 8 RAPD primers were subjected to calculate the genetic similarity (hordein-GS, RAPD-GS) among 34 cultivars. The hordein-GS value ranged from 0.52 to 1.00. The RAPD-GS value ranged from 0.57 to 1.00. The values of GS based on hordein are similar to those based on RAPDs.

To demonstrate the relationships among cultivars, cluster analysis (UPGMA) was performed to generate dendograms based on genetic similarity values (Figure 3 and Figure 4). The analyzed cultivars were grouped into three big clusters according to hordein-GS (Figure 3). The genetic relationship analyzed among cultivars showed that the most of the winter barley cultivars which are genetically close relationship were grouped in to the first main cluster including 19 cultivars. The second main cluster consisted of three sub groups in which the second subgroup was fully including spring ones (Kaya7794, Bornova 92, Yerçil1147 and Bilgi 91) while the first and the third sub groups were including winter (Çetin 2000 and Erginel 90) and facultative ones (Zeynelaga, Avcı2002, Kalaycı 97 and Sladoran), respectively. The most distant cultivars except for Kıral which were released as spring cultivars such as Şerifehanım 98, Süleymanbey 98, Zafer160, Yeşilköy 387 were also grouped in to the third main clusters. Some Turkish winter barley cultivars inevitably were clustered in the same or very close groups, due to fact that they are genetically very similar and share common ancestors**.** Growth habit of the cultivars determined by field data couldn't allow us to discriminate spring and winter types, so this can be resulted in some conflicts especially for the cultivars grouped in to the third and the second main clusters. In order to get concrete data for growth habit, more physiology and molecular studies equipped with field data are required.

The highest RADP-GS (1.00) was found between Tokak 157/37 and Bülbül 89, while the lowest RADP-GS was observed between Angora and Avcı 2002 (0.57). It indicated that all 34 barley cultivars except Tokak 157/37 and Bülbül 89 could be distinguished by RAPD analysis (Figure 4).

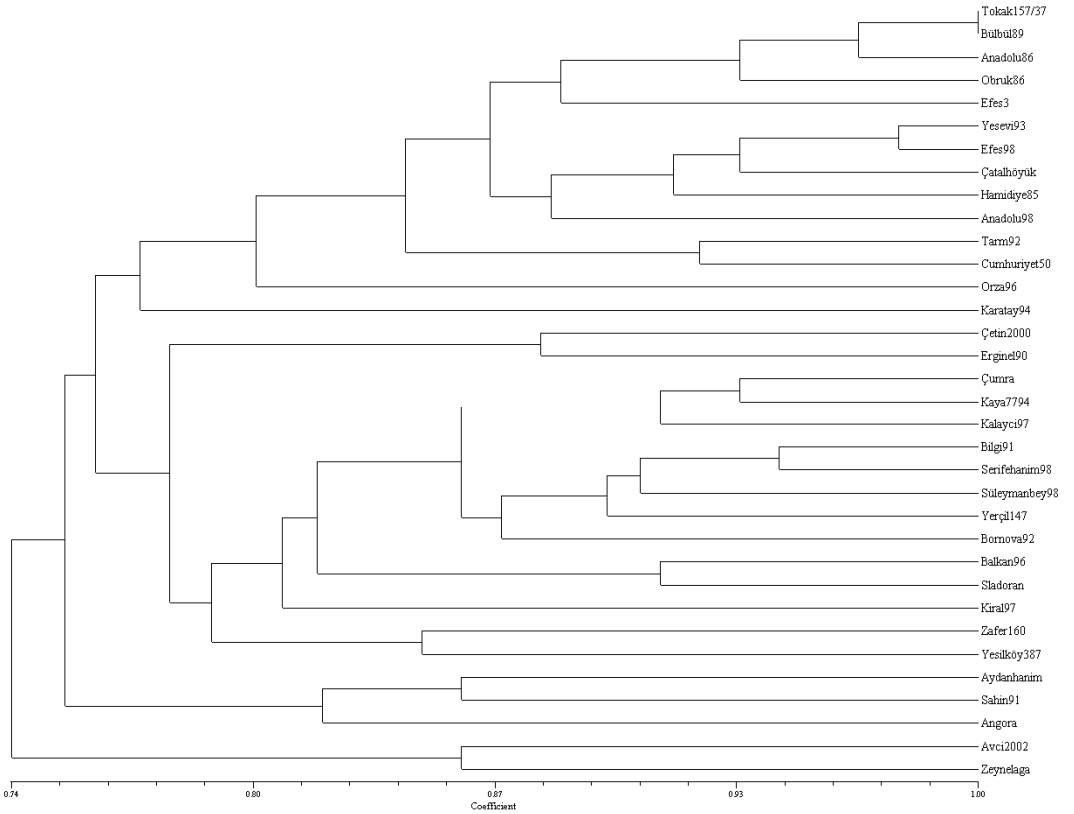

Figure 3. Phenogram showing genetic diversity among 34 Turkish barley cultivars using hordein data.

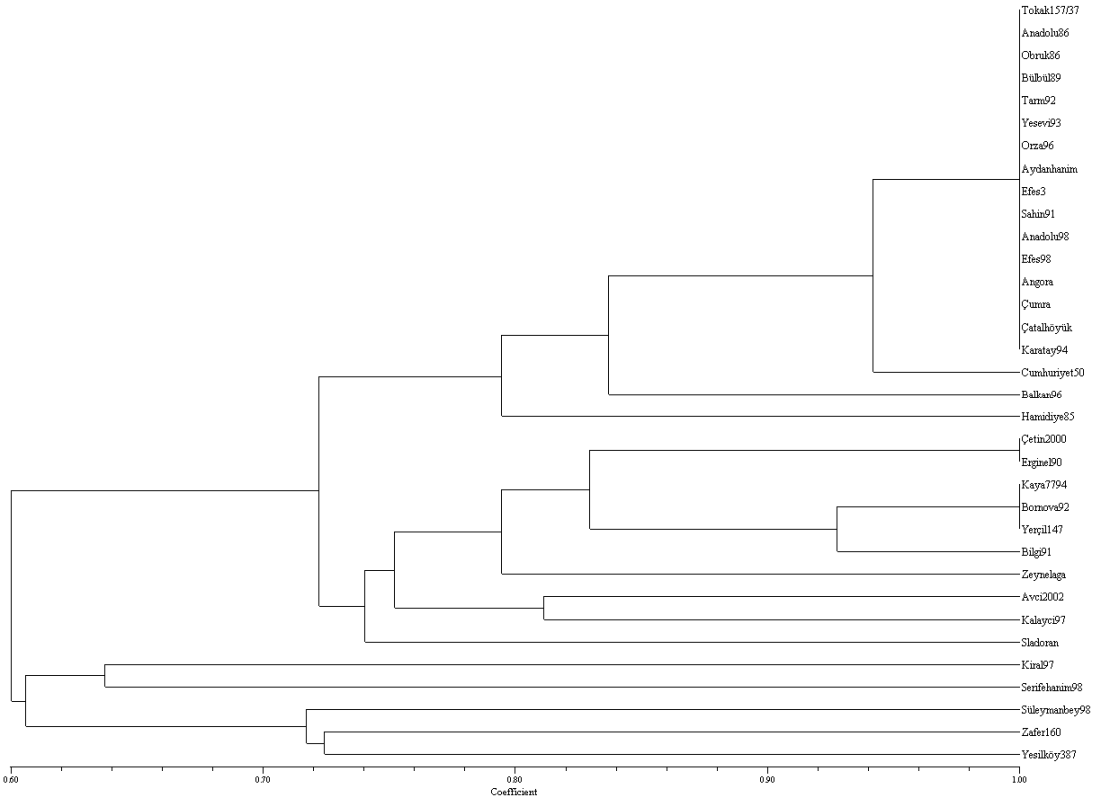

Figure 4. Phenogram showing genetic diversity among 34 Turkish barley cultivars using RAPD data.

These results clearly indicate that RAPDs are polymorphic and especially more informative for estimating genetic relationships. In addition, RAPD markers are more easily handled and thus are becoming more desirable to estimate genetic relationships among related cultivars.

CONCLUSIONS

Spring and winter barley cultivars were able to be roughly differentiated by using hordein data but not RAPDs. However, further studies are required including very diverse barley germplasm in order to verify this idea. Similar genetic background especially among the winter barley cultivars can result in genetic bottleneck so that reason very diverse germplasm should be incorporated in to the crossing program in order to overcome this problem. According to the result of this study, combination of hordein and RAPD markers can be used for discrimination of genetically distance barley cultivars. However, some barley cultivars used in this study which were clustered within the same group by hordein and RAPD should also be identified by using different DNA based techniques such as SSR and STS, two dimensional electrophoretic analyses of hordein and isoenyzme and combination of two methods with morpho- physiological traits before and after cultivar registration.

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