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STUDY OF GENETIC DIVERSITY IN WHEAT (*TRITICUM AESTIVUM*) VARITIES USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

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ABSTRACT

In this study the molecular variation of 16 bread wheat varities was assessed using RAPD markers. On the basis of RAPD-PCR analysis, out of 45 primers 17 primers were found polymorphic. A total of 142 bands were amplified with 17 primers out of which 110 were polymorphic. Fragment size ranged from 300-2800 bp and fragments produced by various primers varied from 3 to 14 with an average of 8.35 fragments per primer. Genetic similarity between the genotypes ranged from 0.316 to 0.860. Polymorphic Information Content (PIC value) calculated the informativeness of each marker and it ranged from 0.11 to 0.93, with average of 0.59. Sixteen bread wheat varieties grouped in two clusters using dendogram analysis. The result of this analysis indicated that the highest similarity was observed between Gerek-79 and Basribey-95 varieties while Sagittario and Gönen-98 varieties were the lowest similiar genotypes. As a result of Principal Component Analysis (PCA), used for detecting the genetic variation among wheat varieties, 5 groups were determined. The result of the study indicated that the registered varieties in our country, possesed relatively narrow genetic variation.

Key Words: Bread wheat, RAPD markers, genetic diversity

INTRODUCTION

Large genome size and wide range of uses have imparted wheat agronomically and nutrionally important status among the several other cereal crops. Enormously growing population and the changing of life style have posed challenges to the wheat breeders to develop newer wheat varieties with high yielding performance, high quality seed and resistance to pests and stress conditions. Therefore, during the last few decades wheat molecular breeding has gained importance. DNA marker technology is such a one important area of biotechnology which can definitely enchance the efficiency of plant breeding practices (Motawei et al., 2007).

Like any other crop species the first step in wheat improvement is full assessment of the local materials, including collection, evaluation and molecular characterization of germplasm lines. Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies (Abbas et al., 2008). The introduction of molecular markers in plant breeding has presented a valuable tool for the characterization of genetic materials. Among them, RAPD markers have been sucessfully used in wheat germplasm evaluation, because of their many advantanges. RAPD gained importance due to its simplicity, efficiency and non-requirement of sequence information. RAPD provide, virtually limitless set of descriptors to compare individiual plants and populations. With this innovative tools genetic diversity can be estimated and equally it is possible to carry out large scale screening of genetic resources held in gene banks, natural populations, ecosystems and natural reserves with this quick and rapid technique (Tahir, 2008). RAPD analysis has been extensively used to document genetic variation in Triticum (Cao et al.1998; Bedo et al. 2000;Gupta et al. 2000), cultivar identification (Malik et al., 1996) and fingerprinting genomes (Welsh and McClelland,1990).

The study aims to detect the genetic diversity of the 16 wheat cultivars under study using RAPD-marker technique.

MATERIALS AND METHOD

Plant Materials

A list of sixteen bread wheat cultivars with their known pedigrees and the origin of registration are presented in Table1.

Genomic DNA Isolation

Genomic DNA was extracted from 10-15 day old young wheat leaves using EZ-10 Spin Column Genome DNA Isolation Kit (Bio Basic, Inc.).

RAPD Analysis

Forty-five 10-mer oligonucleotides with arbitary sequence from Bio Basic were used in RAPD analysis. The PCR reaction mixture consisted of 1.25 µl 10x reaction buffer, 1.25 µl 25 mM $MgCl_2$, 1.00 µl 2.5 mM of each dNTP, 0.125 µl 5.0 units of Taq DNA polymerase (Takara Tag), 0.5 µl primer and 10 ng of genomic DNA in

Table1. List of bread wheat cultivars, their pedigrees and origin of registration.

Variety	Pedigree	Origin of registration
Basribey-95	JUPATECO-73/(sib) BLUEJAY//URES-81	Aegean Agricultural Research Institute. Menemen/İzmir
Flamura-85	RANNYAYA-12 / NADADORES-63 // LOVRIN-12	Romania
Gerek-79	MENK "S"/MY 48// 4-11/3/ YAYLA 305	Anatolian Agricultural Research Institute. Eskişehir
Golia	MANİTAL / ORSO	Italian origin and is produced by TİGEM
Gönen	II-8156-R / MARA // BLUBIRD	Aegean Agricultural Research Institute Menemen/İzmir
Harmankaya-99	FUNDULEA-29 / 2* LOVRIN-32	Anatolian Agricultural Research Institute Eskişehir
Kaşifbey-95	PFAU "S" CM38212- I- 7Y- 2M- 1Y- 3M-2Y- OM	Aegean Agricultural Research Institute Menemen / İzmir
Katea I	CHEBROS / BEZ	Bulgaria origin
Kıraç-66	YAYLA 305/ FLORANSA 71	Anatolian Agricultural Research Institute Eskişehir
Köksal-2000	KATEA-I / MOMTCHILL	Uludağ University Agriculture Faculty/Bursa
Marmara-86	BOBWHİTE "S"	-
Momtchill	-	Bulgaria origin
Pehlivan	BEZ/ TUR/5/ CFN/BEZ	Trakya Agricultural Research Institute Edirne
Sagittario	ADAM / Z-282	Produced by Tasaco Tarım AŞ.
Saraybosna	-	-
Sultan-95	AGRI / NACOZARI-76	Anatolian Agricultural Research Institute

a final volume of 12.5 µl. The amplification protocol was 94 ^{0}C for 3 min to predenature, followed by 40 cycles of 30 sec at 94^{0}C,1 min. at 38^{0}C, 72 ^{0}C for 2 min with a final extension at 72 ^{0}C for 10 min Then kept at 4^{0}C. Amplification products were analyzed by gel electrophoresis in 1.2 % agarose gel.

Data Analysis

RAPD bands were scored as 1 for presence and 0 for absence. The genetic similarity were calculated with unweighted pair group method using arithmetic average (UPGMA) as a clustering algorithm. The dendgrom was drawn using SAHN module in NTSYSpc v 2.2 software. For determination of the correlation between the similarity matrix and the dendogram drawn, the Mantel test was peformed to calculate a cophenetic value using MXCOMP module. The polymorphic information content (PIC) was computed as: PIC= 1- $\sum P_{ij}^2$ where Pij is the frequency of ith allele in the jth population, for each lokus (Botstein et al., 1980). The Principle Component Analysis (PCA) was performed to determine eigen values, percent and cumulative variance.

RESULT AND DISCUSSION

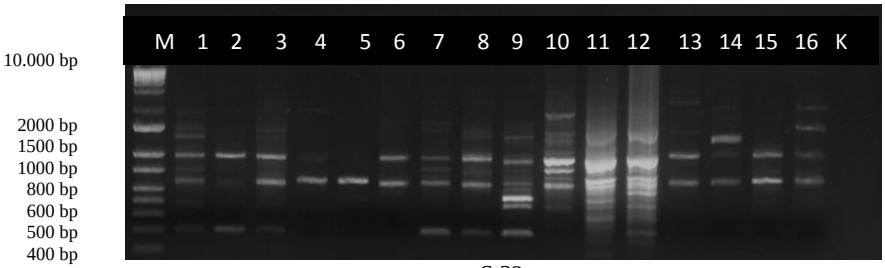
In RAPD analysis, 45 decamer primers screened for amplifiation of all the genotypes. 17 primers gave reproducible and scorable amplification product. Table 2 shows codes and sequences of 17 primers, total number of ampilification fragments from 16 wheat genotypes and the number of polymorphic fragments for each primer.

Table 2. List of RAPD primers along with their sequences, band sizes, the number of monomorphic, polymorphic and total bandsand PIC value resultant from all tested bread wheat genotypes.

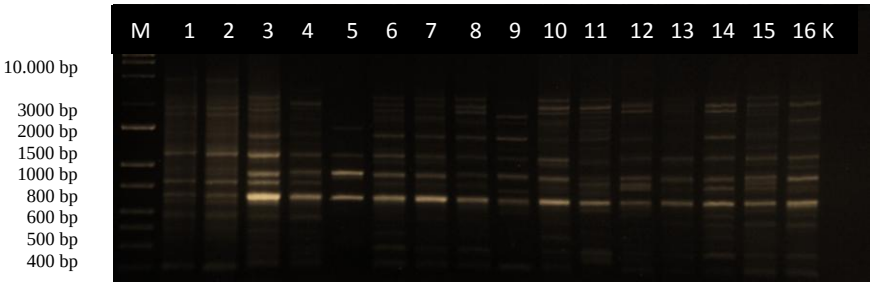
Primer	Sequence (5'→3')	Band size (bp) min-max	Polymorphic bands	Monomorphic bands	Total bands	Polymorphism ratio (%)	PIC
S 22	TGCCGAGCTG	600-2000	4	3	7	57.1 %	0.83
S 32	TCGGCGATAG	300-2000	7	1	8	87.5 %	0.11
S 34	TCTGTGCTGG	300-2600	12	1	13	92.3 %	0.92
S 98	GGCTCATGTG	500-1400	4	1	5	80 %	0.58
S 126	GGGAATTCGG	400-2000	8	1	9	88.8 %	0.88
S 127	CCGATATCCC	400-1700	13	1	14	92.8 %	0.93
S 129	CCAAGCTTCC	300-1950	9	2	11	81.8 %	0.72
S 130	GGAAGCTTGG	300-2800	6	2	8	75 %	0.32
S 132	ACGGTACCAG	300- 1800	5	-	5	100 %	0.47
S 133	GGCTGCAGAA	300-1900	8	5	13	61.5 %	0.84
S 135	CCAGTACTCC	500-2100	2	2	4	50 %	0.12
S 138	TTC CCG GGTT	500-1500	2	1	3	66.6 %	0.31
S 156	GGTGACTGTG	350-2000	8	1	9	88.8 %	0.35
S 418	CACCATCCGT	300-1000	4	4	8	50 %	0.19
S 443	CTGTTGCTAC	500-2100	8	1	9	88.8 %	0.86
S 444	AAGTCCGCTC	500-1400	2	4	6	33.3 %	0.88
S 461	GTAGCACTCC	500-1490	8	2	10	80 %	0.75
Total			110	32	142		
Mean						74.9%	0.59

A total of 142 bands were obtained among which 110 were polymorphic and 32 were monomorphic across 16 wheat genotypes. Molecular sizes of amplified fragments ranged from 300 bp to 2800 bp. The number of DNA fragments for each primer varied from 3 (S138) to 14 (S127) with an average fragments of 8.35. Furthermore, in the study polymorphic fragments per primer was determined an average of 6.47. Similiar finding reported by Cao et al. (2002) as 6.4 polymorphic fragments per primer. The ratio of number of polymorphic fragments / the total number of amplified fragments ranged between 33.3 % (S 444) and 100 % (S 132), with an average

74.9%. Various numbers of primers have been used in the study of different species of Triticum genus that revealed different degrees of polymorphism. Naghavi et al. (2004), Bhutta (2006), Shoaib and Arabi (2006) and Cenkci et al. (2007) revealed 88 %, 46.97 %, 46.67 % and 93.5 % polymorphism among wheat genotypes, respectively. The PIC values were found to be within range of 0.11 to 0.93 with the average of 0.59. The lowest and the highest PIC values obtained were for S 32 and S 127, respectively (Figure 1).



S 32



S 127

Figure 1. RAPD profiles of 16 Bread Wheat genotypes with primers S 32, S 126 and S 129. **M**: Marker **1:** Sultan, **2:** Sagittario, **3:** Katea-I, **4:** Momtchill, **5:** Gönen-98, **6:** Kıraç-66, **7:**Gerek-79, **8:** Basribey-95, **9:** Kaşifbey-95, **10:** Marmara-86, **11:** Flamura, **12**: Pehlivan, **13:** Köksal-2000, **14:** Saraybosna, **15:** Golia, **16:** Harmankaya-99, **K:** Control

The genetic similarity among the varities ranged from 31.6 % to 86 %. Maximum genetic similarity estimate was observed between varities Gerek-79 and Basribey, closely followed by comparison between varities Golia and Harmankaya (GS= 85%). On the contrary,some varities displayed low genetic similarity such as Sagittario and Gönen-98 by 31.6 % and Gönen-98 and Marmara-86 by 34 % (Table 3).

Table 3. The similarity matrix of bread wheat genotypes.

G	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.00															
2	0.763	1.00														
3	0.659	0.709	1.00													
4	0.659	0.635	0.787	1.00												
5	0.439	**0.316**	0.353	0.418	1.00											
6	0.644	0.691	0.711	0.667	0.483	1.00										
7	0.717	0.651	0.695	0.752	0.457	0.742	1.00									
8	0.689	0.619	0.667	0.747	0.400	0.674	**0.860**	1.00								
9	0.512	0.500	0.584	0.526	0.372	0.549	0.583	0.638	1.00							
10	0.591	0.634	0.615	0.660	0.341	0.710	0.755	0.688	0.587	1.00						
11	0.494	0.507	0.525	0.512	0.312	0.634	0.552	0.558	0.593	0.627	1.00					
12	0.512	0.450	0.629	0.611	0.372	0.571	0.583	0.638	0.644	0.587	0.642	1.00				
13	0.614	0.561	0.615	0.680	0.455	0.667	0.776	0.729	0.609	0.702	0.554	0.630	1.00			
14	0.594	0.547	0615	0.691	0.396	0.642	0.685	0.661	0.610	0.654	0.604	0.590	0.729	1.00		
15	0.511	0.619	0.645	0.626	0.333	0.674	0.620	0.612	0.553	0.708	0.612	0.574	0.667	0.716	1.00	
16	0.500	0.585	0.571	0.577	0.318	0.624	0.571	0.583	0.522	0.702	0.602	0.565	0.596	0.636	0.854	1.00

G: Genotye, **1:** Sultan, **2:**Sagittario, **3:**Katea-I, **4:**Momtchill, **5:** Gönen-98, **6:** Kıraç-66, **7:** Gerek-79, **8:**Basribey, **9:** Kaşifbey, **10:** Marmara-86, **11:**Flamura, **12**: Pehlivan, **13:** Köksal-2000, **14:** Saraybosna, **15:**Golia, **16:** Harmankaya-99

The dendogram resulting from the UPGMA cluster analysis showed that the studied varieties could be divided into two main clusters. The first cluster contained only one variety called Gönen-98, on the other hand the second cluster was divided into two sub-cluster and the first sub-cluster contained three varities of Flamura, Pehlivan and Kaşifbey, while the second cluster contained the other varities (Figure 2).

As can be seen in Figure 3, the varieties gathered in 5 groups. The variety of Gönen-98 and the variety of Sultan were located in alone in the first and second

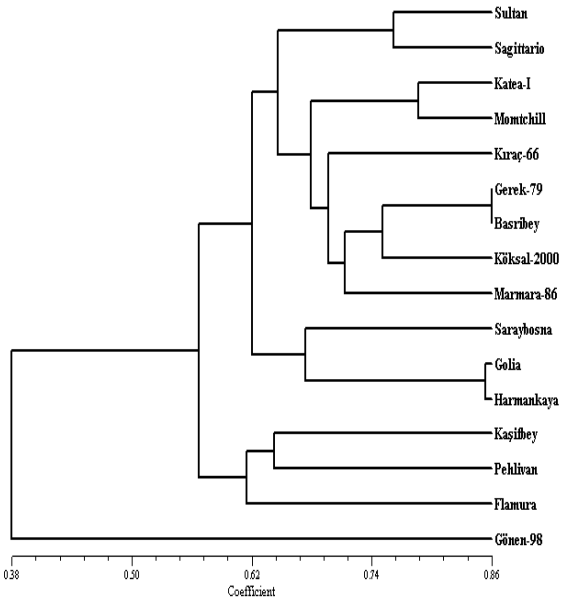


Figure 2. UPGMA dendogram showing the relationships among bread wheat genotypes.

group,respectively. In the third group varieties of Momtchill, Sagittario, Katea-I, Kıraç-66 and Koksal-2000, while Saraybosna, Kaşifbey, Marmara-86 and Pehlivan are in fourth group and finally, in group 5 varieties of Flamura, Golia and Harmankaya took place. We have previously obtained similar results with this distribution at the dendogram. Indeed dendogram separated into 2 groups, and Group 2 in itself divided into sub-groups formed in different groups (Figure 3). However, although the varieties of Sultan and Sagittario take part in the same group, in terms of similarity in dendogram, the end of principal components analysis were found in different places. As stated before, the reason for this, such as the introduction of varieties of different geographical regions, or disclose the use of parents with different genetic content. As seen in the Table 1 variety of Sultan-95 is recommended for Central Anatolia and Transitional Zone. In addition, Sagittario variety suitable for coastal areas and a variety of Italian origin. Parents also formed with a different genetic content.

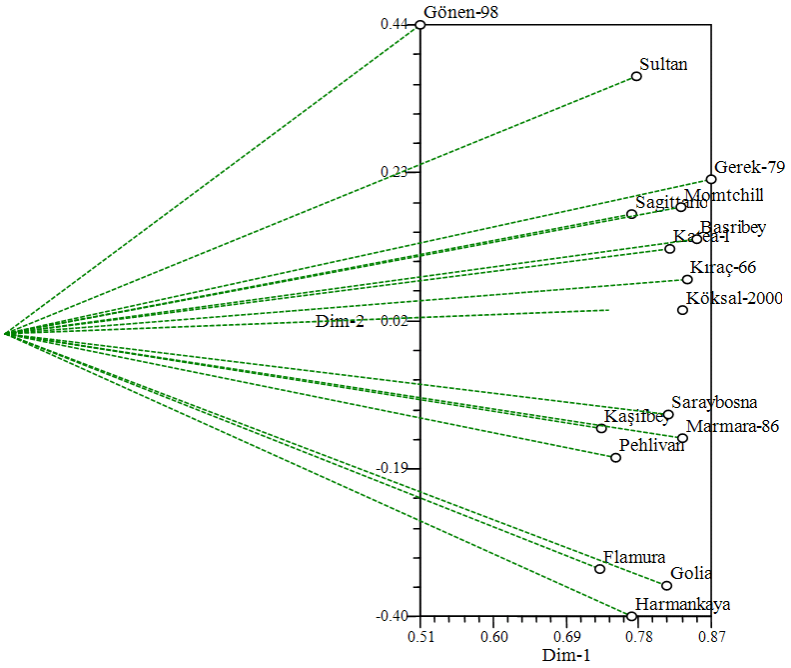


Figure 3. Principles Component Analysis of RAPDs by 17 primers for 16 bread wheat genotypes.

CONCLUSION

The present study was aimed to determine the genetic variation among bread wheat cultivars, grown in Turkey using RAPD markers. Our result showed that the breeding pool for hexaploid wheat is narrow relative to levels of diversity among and within closses in hexaploid wheat as reported by Chen et al. (1994), and Altıntaş et al. (2008) in the previous researches. However, the result shows of PCA showed that, the formation of 5 group, of bread wheat varieties has not narrow the genetic basis of variation yet.

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