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Full Length Research Paper

Genetic diversity in doubled haploids wheat based on morphological traits, gliadin protein patterns and RAPD markers

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Genetic diversity among 102 doubled haploid wheat accessions originating from CIMMYT were investigated using morphological traits, gliadin patterns and random amplified polymorphic DNA (RAPD) variation. Among the morphological traits under study, the highest amount of diversity was related to grain yield per plant, number of total tillers and number of fertile tillers. Principle components analysis and cluster analysis for morphological traits were used to effectively classify the samples. Based on these analyses, three genotypes with maximum yield and the related traits were determined. In the analysis of gliadins, 48 bands and 47 different patterns were detected. The average genetic diversity index for these proteins was calculated as H = 0.75. The mean of genetic diversity index was more for RAPDs than gliadins (H = 0.83). Although during statistical reviews one pattern in the ω area was found to have relations with the trait of spikelet per spike, no relationship was found between morphological, storage proteins and RAPDs data. As a result, it seems that applying only one of these methods is not sufficient to estimate the genetic diversity. In order to have a clearer picture of the status of genetic diversity in different populations of bread wheat, it is recommended that all the three methods be applied simultaneously.

Key words: Genetic diversity, doubled haploids wheat, morphological traits, gliadin patterns, RAPD marker.

INTRODUCTION

Genetic diversity is one of the most important factors for any crop improvement. Modern breeding process has dramatically narrowed the variation of important traits in many food crops, especially among common wheat (*Triticum aestivum* L.) cultivars which are widely used in breeding programs (Stepien et al., 2007; Carena, 2009). Resistance to biotic (diseases) and abiotic stresses (drought and salt tolerance) has become one of the most important features, as there are few known sources of them among varieties used by breeders (Stepien et al., 2007; Murphy, 2007; Hagedoorn, 2008; Stewart, 2008). Therefore, it is important to investigate genetic diversity of wheat germplasm to assess its usefulness for future breeding programs.

Principle components and cluster analyses based on agro-morphological traits have an important role in studying genetic variability, determining how agronomical plants evolve, and studying the mutual effect of environment and genotype (Richard, 1996). Several authors such as Fang et al. (1996), Beuningen et al. (1997), Waines and Murphy (2005), Naroui Rad et al. (2007) and Hettel et al. (2008) showed the application of these methods in classifying genotypes in order to select native variations, hybrid varieties, and improved populations in wheat.

Biochemical markers also are key tools in the evaluation of genetic diversity in germplasm accessions. Gliadins and glutenins constitute around 80% of the total seed proteins in wheat, of which 40% are gliadins (Lasztity, 1996). Glutenins (acid soluble) are polymeric proteins whose monomeric units are divided into high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits. Gliadins (alcohol soluble) are monomeric proteins and, based on their mobility in the acid-PAGE, are divided into four groups of α, β, γ, ω. Many of the gliadin alleles reside at six main loci on the chromosomes of the first (Gli-1) and the sixth (Gli-2)

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homological groups (Payne, 1987). There are also some minor loci as Gli-3, Gli-5, and Gli-6 that control a few minor gliadin bands (Metakovsky et al., 1997; Pogna et al., 1993). Two new gliadin alleles Gli-D4 and Gli-D5 have also been reported on the short arm of chromosome 1D (Rodriguez and Carrillo, 1996). A high degree of variety has been reported in gliadin patterns (Zillman and Bushuk, 1979; D'Ovidio et al., 1992; Branlard et al., 1993). Combination of different alleles of gliadins makes it possible to distinguish wheat genotypes. In addition, significant positive effects of certain gliadin alleles have been reported on gluten strength (Weegels et al., 1996; Metakovsky et al., 1997), agronomic traits, and environmental adaptation (Metakovsky and Branlard, 1998).

Another common method for studying genetic diversity is applying DNA markers. DNA markers have facilitated genetic studies in plant, animal and prokaryotic genomes (Kole, 2006; Lörz and Wenzel, 2007). Among the several DNA based techniques, random amplified polymorphic DNA (RAPD) has gained importance due to its simplicity, efficiency and non requirement of sequence information (Welsh and McClelland, 1990; Williams et al., 1990). RAPD provides virtually limitless set of descriptors with which to compare individual plants and among the population.

With this innovative tool, 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 (Devose and Gale, 1992; Lörz and Wenzel, 2007). RAPD analysis has been extensively used to document genetic diversity, cultivars identification and fingerprinting of genomes in wheat (Joshi and Nguyen, 1993; Cao et al., 1998; Bhutta et al., 2006; Aliyev et al., 2007).

This study was conducted in order to examine the genetic diversity of 102 doubled haploids of wheat (sent from CIMMYT) through studying their morphological traits, gliadin protein and RAPD markers.

MATERIALS AND METHODS

Plant materials

The materials in this study included 102 doubled haploids wheat, all of which had been provided by CIMMYT (Table 1).

Filed experiments

The genotypes were assessed along with four control groups within the augmented field design during the agricultural year 2006 - 2007 in Moghan region, Iran. Each genotype was grown in 5 rows of 3 m-long beds with spacing of 30cm between the rows. An approximate distance of 10cm was maintained between plants by hand thinning. Ten competitive random plants from the middle rows of the experimental plots were taken for recording the observation on yield per plot, number of grains per spikelets, number of fertile tillers, number of total tillers, biomass, yield per plant, harvest index, number of grains per spike, spike density, spike length, plant height, number of internodes, peduncle length, weight of 1000 grains, weight of grains per spike, internode length, seed length, seed width and number of spikelets per spike, length of lawn, weight of main spike and yield of straw. To examine interrelationships among genotypes, principle components analysis and cluster analysis were performed based on these traits.

Storage protein analysis

Separation of gliadin bands with the Acid-PAGE method were performed on all genotypes, using the Zillman and Bushuk (1979) method modified by Poperelya (1989). Seeds were individually ground and extracted overnight at room temperature with 0.2 ml solution containing 0.9% acetic acid, 18% urea and 0.01% pyronine. The samples were laid on the polyacrylamide gel, and the electrophoresis apparatus was set on 450 volts and 0.16 amperes, for 5 h. A strategy was used to identify gliadin pattern within each gliadin groups of α, β, γ and ω by comparing banding pattern of each line with all other lines and assigning specific number to each of the pattern. The first line was given pattern number 1 and subsequently compared with band pattern of all other varieties. Genotypes with similar band pattern were grouped together. This was followed by the determination of the next pattern different from the previous one(s) and identification of varieties with similar band pattern by comparing them. The strategy was followed for all the genotypes, and large numbers of different patterns were identified in each group of gliadins (α, β, γ, ω). The exercise was repeated many times to confirm the pattern of varieties within each group. Since Anza genotype was used as a check in each gel, comparison of band pattern among different varieties was easy. With this procedure, all the band patterns were identified.

The genetic diversity for each gliadin band patterns in the four areas was calculated using the following formula:

$$H = 1 - \Sigma p_i^2$$

Where, H is the genetic variation index and P_i is the frequency of the banding patterns in each area (Nei, 1973). Also, in order to find the relation between agronomical traits and protein patterns, the t-test was performed. That is, for all the traits, the average of those genotypes having a certain pattern was compared with the average of those genotypes lacking that certain pattern.

RAPD analysis

Seeds of eight to ten plants from each genotype were sown in plastic cups and germinated in the green house. The leaf samples were collected with ice box and transported to the lab. DNA was extracted from leaves (400 mg) of individual 20-day-old plants using the protocol described by Dellaporta et al. (1983). After treatment with RNase, the DNA concentration was measured by flouremeter DyNA Quant^{TM} 200. The total genomic DNA was diluted in double distilled water to a concentration of 5 ng for PCR analysis.

PCR was performed in a 50µl reaction volume containing 10 mM Tris-HCL (pH 8.3), 50 mM KCL, 3 mM $MgCl_2$, 100µM each of dNTP, 30 ng of primer, 0.001% gelatin, 30 ng of genomic DNA and 2 unit of Taq polymerase. DNA amplification reaction was performed in a thermal cycler using the following cycling program: one cycle of 94°C for 5 min; 40 cycles of 94°C for 1 min; 36°C for 1 min; 72°C for 2 min; followed by one cycle of 72°C for 10 min. A negative control PCR tube containing all components except genomic DNA was included in all thermal cycle (labnet) runs. The 18 RAPD 10-mers used (Table 2) were selected from among 80 RAPD primers in a preliminary test for oligos that amplified numerous discrete fragments. The PCR products were resolved on 1.2% agarose gels in 0.5% TAE buffer and visualized under UV light after ethidium

Table 1. Pedigree of doubled haploid wheats and α, β, γ and ω gliadin patterns.

S/N	Pedigree	Gliadin pattern			
		α	β	γ	ω
1	CMH79A.955/CMH74A.487//CMH81A...	1	1	1	1
2	CMH79A.955/CMH74A.487//CMH81A.744/3/...	3	10	15	15
3	CMH79A.955/CMH74A.487//CMH81A.744...	1	11	16	16
4	TEG/GANFRENCH/6/CMH79A.955/4/AGA/3/4*...	2	4	4	5
5	TEG/GANFRENCH/6/CMH79A.955/4/...	3	4	7	8
6	TEG/GANFRENCH/6/CMH79A.955/4/AGA/3/...	2	4	5	5
7	TEG/GANFRENCH/6/CMH79A.955/4/...	2	4	4	5
8	CMH80.638/CMH75A.411//CMH80.638...	2	4	10	12
9	CMH80.638/CMH75A.411//CMH80.638/3/ELVIRA...	2	5	6	5
10	CMH80.638/CMH75A.411//...	2	5	4	5
11	CMH80.638/CMH75A.411//CMH80.638/3/...	3	4	6	5
12	CMH80.638/CMH75A.411//CMH80.638...	2	5	6	5
13	CMH80.638/CMH75A.411//CMH80.638/3/ELVIRA.	2	5	6	5
14	CMH82A.1294/CMH84.3621//CMH81.749…	6	1	3	1
15	TEG/GANFRENCH...	1	1	3	1
16	CMH83.2517/GANFRENCH...	2	4	11	11
17	TEG//CMH82A.1294/CMH84.3621/3/ELVIRA…	2	1	3	1
18	TEG//CMH82A.1294/CMH84.3621/3/…	2	1	3	1
19	CMH79A.955/4/AGA/3/4*SN64/CNO67//...	3	4	14	13
20	TEG//CMH82A.1294/CMH84.3621/3/ELVIRA…	1	1	3	1
21	CMH82A.1294/CMH84.3621//CMH81.749/3/...	1	1	3	1
22	CMH83.2517/GANFRENCH...	1	1	3	2
23	CMH83.2517/GANFRENCH...	3	1	7	9
24	CMH82A.1294/CMH84.3621//CMH81.749/3/ …	1	1	3	1
25	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66...	4	4	13	13
26	CMH83.2517/GANFRENCH...	3	4	11	11
27	CMH79A.955/4/AGA/3/4*SN64...	3	4	10	11
28	CMH79A.955/CMH74A.487//CMH81A...	2	5	4	5
29	CMH79A.955/CMH74A.487//CMH81A.744/3/...	7	4	14	13
30	CMH79A.955/CMH74A.487...	2	5	4	5
31	CMH79A.955/CMH74A.487//CMH81A.744...	3	7	7	10
32	CMH79A.955/CMH74A.487//CMH81A...	1	2	2	3
33	CMH80.638/CMH75A.411//CMH80.638...	3	7	7	10
34	CMH80.638/CMH75A.411//CMH80.638/3/...	3	10	17	10
35	CMH79A.955/4/AGA/3/4*SN64/CNO67//...	2	4	6	6
36	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66...	2	4	6	5
37	CMH79A.955/4/AGA/3/4*...	2	4	6	5
38	CMH79A.955/4/AGA/3/4*SN64...	2	4	6	5
39	CMH79A.955/4/AGA...	2	4	5	5
40	SIDS10/3/KAUZ//STAR/LUCO...	3	5	10	11
41	TEG/GANFRENCH...	3	4	11	11
42	CMH82A.1294/CMH84.3621//CMH81.749...	1	1	3	1
43	CMH82A.1294/NEIXIANG184//ELVIRA	2	4	4	5
44	CMH79A.955/4/AGA/3/4*SN64/CNO67//...	2	4	5	5
45	CMH80.638/CMH75A.411//CMH80.638/3/STAR...	2	4	6	5
46	CMH80.638/CMH75A.411//...	2	4	4	7
47	CMH80.638/CMH75A.411//CMH80.638...	2	4	4	5
48	CMH80.638/CMH75A.411...	3	4	10	11
49	CMH80.638/CMH75A.411//CMH80.638/3/...	3	4	11	11
50	CMH80.638/CMH75A.411//...	2	4	6	5

Table 1. Continued.

51	CMH80.638/CMH75A.411//CMH80.638/3/...	2	4	4	5
52	CMH80.638/CMH75A...	4	5	13	14
53	CMH80.638/CMH75A.411//CMH80.638/3/WBLL1...	2	4	4	5
54	CMH79A.955/4/AGA/3/4*SN64/CNO67...	2	4	4	5
55	CMH79A.955/4/AGA/3/4*SN64/...	2	4	6	5
56	CMH79A.955/4/AGA/3/4*SN64/CNO67//...	2	4	6	5
57	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66...	2	4	4	5
58	CMH79A.955/4/AGA...	2	4	5	5
59	CMH76.1084/2*CMH72A.429//ELVIRA/6/...	2	5	11	17
60	CMH79A.955/4/AGA/3/4*SN64/CNO67...	2	5	6	5
61	CMH79A.955/4/AGA/3/4*SN64/CNO67//…	2	5	6	5
62	CMH74A.630/SX/CNO79/3/SW895124*2/FASAN...	1	3	1	1
63	CMH83.2517/GANFRENCH...	1	1	1	1
64	CMH79A.955/4/AGA/3/4*SN64/CNO67//…	2	5	4	5
65	CMH79A.955/4/AGA/3/...	2	5	5	5
66	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66...	2	5	4	5
67	CMH74A.630/SX/CNO79/3/SW89-5124*2/...	5	3	1	1
68	CMH79A.955/4/AGA/3/4*SN64/CNO67...	2	5	6	5
69	SIDS10/3/KAUZ//STAR/LUCO-M/6/...	3	5	10	11
70	CMH79A.955/4/AGA/3/4*SN64...	2	5	5	5
71	CMH79A.955/4/AGA/3/4...	2	4	5	5
72	CMH79A.955/4/AGA/3/4*SN64/CNO67//…	2	5	6	5
73	CMH79A.955/CMH74A.487//CMH81A.744/3/...	1	5	18	18
74	CMH79A.1380/GANFRENCH/6/CMH9A.955/4/...	3	10	12	11
75	CMH76.1084/2*CMH72A.429//ELVIRA/6/...	1	1	1	1
76	CMH83.2578/GANFRENCH/6/...	3	9	8	8
77	CMH76.1084/2*CMH72A.429//...	3	4	12	11
78	CMH83.2517/ELVIRA/6/CMH79A.955/4/AGA...	1	1	1	1
79	CMH81.794/4/CHEN/AEGILOPS (TAUS)...	2	4	5	5
80	CMH76.1084/2*CMH72A.429//ELVIRA/6/...	1	3	3	1
81	VEE/CMH77A.917//VEE/3/ELVIRA/6/...	2	5	4	7
82	CMH83.2517/ELVIRA/6/CMH79A.955...	2	4	4	5
83	CMH79A.955/CMH74A.487//CMH81A.744/3/...	1	1	1	1
84	CMH76.1084/2*CMH72...	1	1	3	4
85	VEE/CMH77A.917//VEE/3/...	3	8	9	8
86	CMH76.1084/2*CMH72A.429//ELVIRA...	2	5	5	5
87	CMH83.2517/ELVIRA/6/CMH79A.955/4/AGA...	3	4	11	11
88	CMH80.638/CMH75A.411//CMH80.638/3/...	3	4	12	12
89	CMH80.638/CMH75A.411//CMH80.638/3/CHIBIA...	2	5	6	5
90	CMH80.638/CMH75A.411//CMH80.638/3/...	1	2	1	1
91	CMH80.638/CMH75A.411//CMH80.638/3/STAR/...	4	4	13	13
92	CMH80.638/CMH75A.411//CMH80.638/3/STAR/...	4	5	14	13
93	CMH80.638/CMH75A.411//CMH80.638/3/...	4	4	13	13
94	CMH80.638/CMH75A.411//CMH80.638...	2	5	4	5
95	CMH80.638/CMH75A.411//CMH80.638/3/ELVIRA..	2	12	19	19
96	CMH80.638/CMH75A.411//CMH80.638/3/ELVIRA.	2	4	4	5
97	CMH80.638/CMH75A.411//...	3	4	10	11
98	CMH80.638/CMH75A.411//CMH80.638/3/...	2	6	6	7
99	CMH80.638/CMH75A.411//CMH80.638/3/ELVIRA..	3	4	11	11
100	CMH80.638/CMH75A.411//CMH80.638/3/...	2	4	6	5
101	CMH80.638/CMH75A.411//CMH80.638/3/ELVIRA	2	5	4	5
102	CMH80.638/CMH75A.411//...	2	5	4	5

Table 2. Primer sequences, total number of bands amplified and analyzed, polymorphic bands, genetic diversity index, size range of amplified products, the total and mean primer

Primer	Sequence ($5'$-$3'$)	Total bands	Bands analyzed	Polymorphic bands	Genetic diversity index	Fragment size
GLE-01	CAGGCCCTTC	18	17	14	0.852	400-2800
GLE0-2	TGCCGAGCTG	32	28	26	0.907	200-2700
GLE-03	GATGACCGCC	10	8	5	0.795	350-1500
GLE-04	AATCGGGCTG	19	17	9	0.747	450-1900
GLE-05	AGGGGTCTTG	20	14	10	0.775	500-2400
GLE-06	GGTCCCTGAC	14	13	10	0.789	250-1900
GLE-07	GAAACGGGTG	16	13	10	0.774	280-2100
GLE-08	GTGACGTAGG	23	19	17	0.872	280-2200
GLE-09	GGGTAACGCC	18	15	13	0.857	500-2500
GLE-10	GTCCCGACGA	19	18	16	0.902	450-1200
GLE-11	CAATCGCCGT	24	21	17	0.884	200-1860
GLE-12	TCGGCGATAG	28	26	23	0.895	380-2200
GLE-13	TGGACCGGTG	21	15	12	0.728	200-1900
GLE-14	TGCGTGCTTG	17	17	14	0.764	580-1400
GLE-15	TTCCGAACCC	16	10	5	0.685	430-1100
GLE-16	CTCACCGTCC	20	20	20	0.921	280-2900
GLE-17	GACCGCTTGT	19	19	18	0.914	450-3000
GLE-18	AGGTGACCGT	12	12	10	0.885	250-2300
Total	-	346	302	273	-	200-3000
Mean/primer	-	19.22	16.77	15.16	0.83	-

bromide staining (Sambrook et al., 1989). Reproducibility of the RAPD analytical procedure was investigated with repeated analysis of samples. Only those bands which showed consistent amplification were chosen for use in this study. These RAPD amplification products reproducible across all plants were assigned a number (1, 2, 3, ...n) and each one was treated as a unit character coded as 1 (present) or 0 (absent). Then other calculations, including similarity coefficient matrix, genetic diversity index, and cluster analysis, were performed according to the methods mentioned in the analysis of the storage proteins section. Finally, through comparing similarity matrices by means of Mantel test, the dendrogram obtained from storage proteins, RAPD markers and agromorphological data were investigated.

Principle components analysis and clustering of genotypes by morphological traits were done by SPSS12 (Spss, 2003). For clustering of genotypes by protein and RAPD markers, NTSYS 2.01 programs (Rohlf, 1998) were used.

RESULTS AND DISCUSSION

Morphological traits analysis

The highest coefficient of variation, that is, the highest genetic diversity in the genotypes under study were grain yield per plant (45.56%), total number of tillers (42.35%), and number of fertile tillers (40.24%). Correlation between traits was used to determine the linear relationship between morphologic traits. There is a significant correlation between yield per plant (0.662), number of grains per spike (0.587), spike density (0.506), number of spikelet per spike (0.506), biomass (0.502), harvest index (0.435), total number of tillers (0.333) and number of fertile tillers (0.286) with grain yield ($p<0.01$). According to the fact that yield is a polygene trait, and it is difficult to improve it directly, traits which have high correlation with the yield might prove helpful and indirectly improve the yield.

Principle components analysis was performed for all the traits under study, through which the first six components could justify 71% of the total variance among genotypes. In the first component, which accounted for more than 24% of variance among genotypes, the traits of biomass, grains weight per spike, yield per plot, number of grains per spike, number of fertile tillers, spike density, and number of total tillers had the highest values. Since the trait of yield and those correlated to that had the greatest share in creating this component, it was named the yield component. Genotypes which were selected by this component included genotypes 56, 91, and 48. Genotype 56 with the yield per plot of 12.4 t / h, genotype 91 with the yield of 11.98 t / h and genotype 48 with the yield per plot of 10.2 t / h had the highest yield among the genotypes under study. Using correlation matrix, Shahid et al. (2005) analyzed principle components and selected the first three principal components. He reported that they were relevant to plant height, number of grains per spike, and length of spike, respectively.

Cluster analysis, according to the morphological traits using the Ward method, was used to classify all the genotypes into four groups. In this classification,

genotypes with high yield were placed in one group (Figure 1). Beuningen et al. (1997) studied 289 varieties of American, Canadian, and Mexican spring bread wheat in three different environments and reported that 16 principle components justified 80% of the total variance, and cluster analysis classified all the variations into 16 main groups. In another study based on the traits days to maturity, plant height, spike length, number of grains per spike, 1000-grain weight, and grain yield per spike, Hettel et al. (2008) divided 137 varieties of bread wheat into nine groups through cluster analysis.

Storage proteins analysis

Among the studied genotypes, 48 different bands and 47 different patterns were detected in most of which polymorphism were observed (Table 1). In the ω area, 18 bands and 19 different patterns were observed. Among the patterns in this area, ω5 with the frequency of 43.13% in 44 genotypes, ω1 pattern in 17 genotypes, and ω4 in 13 genotypes were observed. In the γ area, 19 patterns were observed, which patterns γ4 and γ6 with frequencies of 19.6 and 18.62%, respectively, had the most frequencies. Here each of the patterns γ1, γ8, γ9, γ15, γ16, γ17, γ18, and γ19 were observed in one genotype only. In the area β, 9 bands were observed. Two bands, β2 and β5, each with a frequency of 0.99% and after them two bands β6 and β7 each with a frequency of 97.05% had the highest frequencies, whereas the lowest frequency was that of β4 and β9. In β area, 12 different patterns were observed, and the highest frequency was that of pattern β4 (44.1%) in 45 genotypes followed by β5 in 24 genotypes. The lowest frequency was that of patterns β8, β16, β11, and β12.

However, a total of 7 patterns were observed in α area among which the highest frequencies were that of α2, α3, and α1. In this area, 9 bands were observed. Figure 2 shows representative examples of variation detected by the A-PAGE gel system. Figure 3 shows an ideogram of different gliadin patterns in the α,β, γ and ω gliadin region observed in the genotypes studied.

Using Nei formula, the genetic diversity based on the patterns was calculated for each of the four areas, where area γ proved to have the most diversity (H=0.872), followed by ω (H = 0.767) and β (H = 0.714), and the least diversity being that of α (H=0.646).

Caballero et al. (2004) studied the genetic diversity of Spanish common wheat through pattern variety of gliadin. Among 403 studied genotypes, they observed 61 patterns in ω, 44 patterns in γ, 19 pattens in β, and 15 patterns in α. The study conducted by Xu et al. (2008) showed that greater levels of gliadin variation existed in *T.turanicum*, and a total of 72 gliadin patterns were found among 87 accessions. In this study, it was observed that areasω,γ, and β had high variety patterns but the least pattern variety was observed in area α. In our study area α had the least pattern variety. This may be either due to greater staining intensity of α gliadin, and separation of these proteins may not be complete in a one-dimensional electrophoresis system. Although enough care was taken to get all the bands separated, more than one protein may be present in a band in the region. The second reason for the least protein variety in the area α is that only chromosome 6A alleles took part in the formation of the bands, while chromosomes 6B and 6D did not get involved (Sozinov and Poperelya, 1980). Tanaka et al. (2003) have also reported larger variation in γ and ω gliadins than α and β gliadins in Japanese cultivars.

Cluster analysis according to gliadin bands were used to classify all the genotypes into 9 main groups (Figure 4). The highest similarity index was observed between genotypes 37 and 45, with similarity coefficient of 0.765 and then between genotypes 7 and 54 with similarity coefficient of 0.758. The least similarity index was observed between genotypes 1 and 95, with similarity coefficient of 0.23. Similarity mean for the total matrix for all genotypes was calculated as 0.484. Although many of the genotypes under study had similar parents, gliadin electrophoresis showed considerable diversity among them. However, recently, Dreisigacker et al. (2004) reported no significant differences among wheat lines from CIMMYT (based on SSR and pedigree analyses). Metakovsky and Branlard (1998) reported that the genetic diversity in breeding materials could be monitored by means of the analysis of polymorphic markers. The gliadin pattern of wheat genotypes is not affected by the environment (Zillman and Bushuk 1979), and the gliadin markers are an easy, cheap and powerful tool (Metakovsky and Branlard, 1998). It is recommended that gliadin pattern should be used in assessing the genetic resources of wheat. Wang et al. (2006) reported that the gliadin pattern could reflect the genetic diversity in durum wheat. Zhang et al. (1995), Hou et al. (2004), and Lan et al. (1999) also suggested that A-PAGE method should be used to introduce and assess wheat germplasm resources.

In examining the relation between the observed patterns and the agronomic traits, one pattern in the area ω proved to be significantly related to the number of grains per spikelet, and therefore, this can be used as a marker for increasing the yield.

RAPD marker analysis

In order to examine genetic diversity among the samples, 80 random primers were studied. Some of these random primers produced no band, very weak amplified products, or repetitive bands and they showed very low DNA polymorphism. Among the primer used, only 18 primers, which showed clear polymorphism and reproducible banding patterns, were used in investigating genetic diversity (Table 2). The profiles obtained with GLE-12

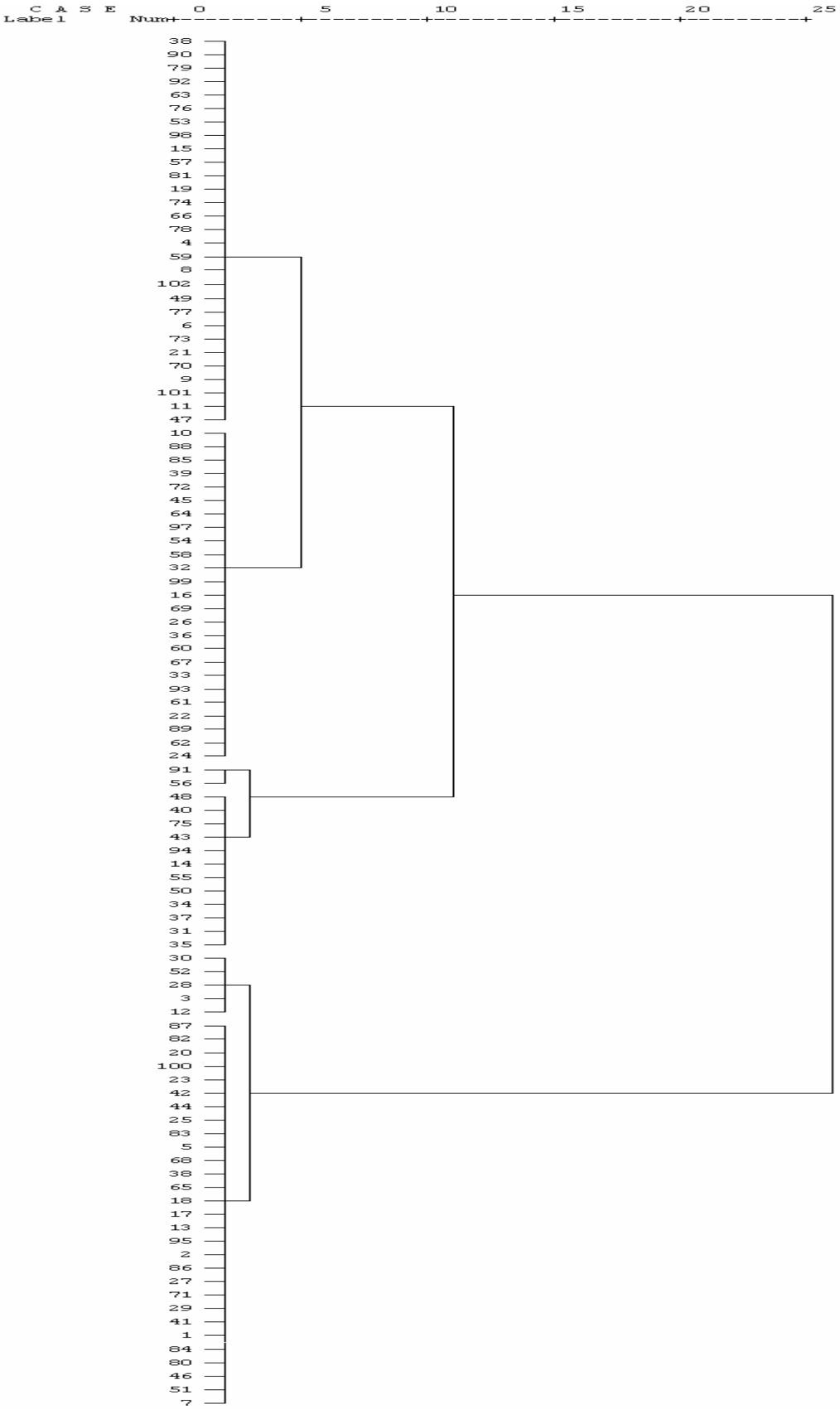

Figure 1. Dendrogram showing the genetic relationships among 102 doubled haploid wheats based on morphological traits

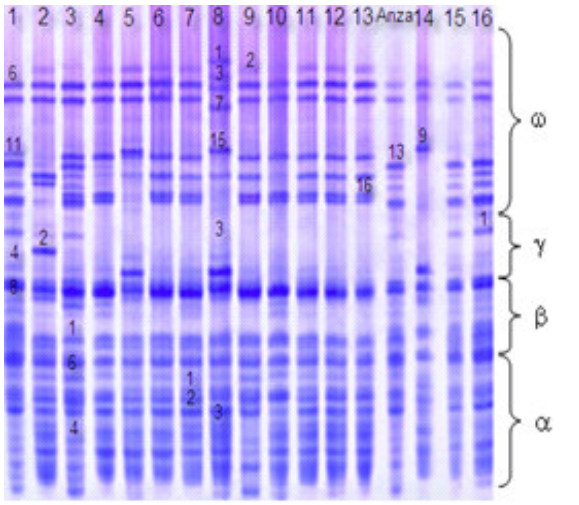

Figure 2. Gliadin profiles of representative accessions of doubled haploid wheat.

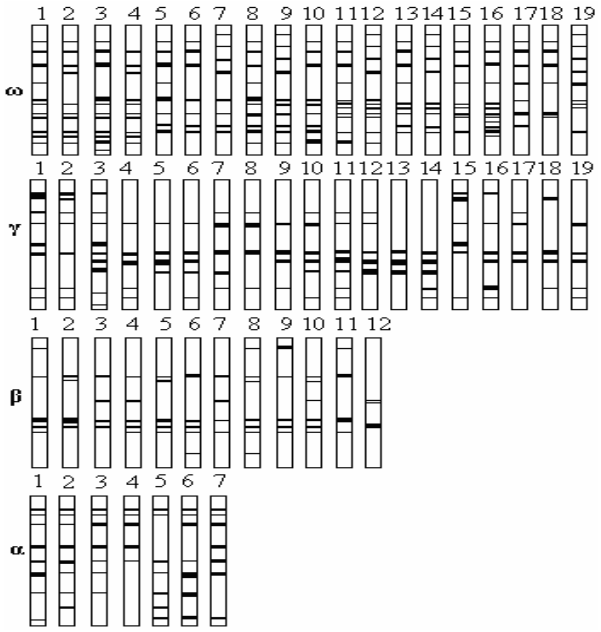

Figure 3. Ideogram of different gliadin patterns in the regions of α,β, γ and ω gliadins observed in doubled haploid wheat lines. The numbers shown on top of ideograms denote the of electrophoretic banding patterns identified among all genotypes.

primer are shown in Figure 5. The polymorphism percentage, based on the bands produced by each primer, ranged from 50 to 100%, and the polymorphism mean, based on all the bands, was 90.39%. The sizes of the fragments that were amplified with different primers were different, however, for the statistical analysis purposes, bands within 200-3000 base pairs having high repeatability were selected and calculated; among these, 302 strong bands were used in the analysis. The mean of the number of produced DNA fragments for each primer was 19.22. The highest and the lowest number of produced DNA fragments were those of GLE-2 and GLE-3 primers, respectively. Also the lowest number of polymorphic band was that of GLE-3 primer. The highest genetic diversity index among the primers used in this study and among all the genotypes was that of primer GLE-16, and the lowest genetic diversity was that of primer GLE-15. The mean of total genetic diversity among all the primers and for all the samples was calculated as 0.83, which indicates that the population under study has had a considerable diversity at DNA level. Cluster analysis was carried out on the basis of RAPD markers with UPGMA method and with the help of Nei and Lees' (1979) similarity coefficients. Figure 6 illustrates the dendrogram resulting from the analysis of RAPD markers for the lines under study. If the cutting is done from the distance of 0.58, the genotypes are classified into 8 groups. The highest genetic similarity was found between lines 8 and 10 with similarity coefficient of 0.784, and after that were lines 34 and 43 with similarity coefficient of 0.772, while the lowest similarity was that of lines 67 and 88 with the similarity of 0.253. Average similarity in the whole matrix for all the lines was calculated as 0.493.

One of the main applications of these clusters is the estimation of the genetic distance between genotypes, that is, there is a greater genetic distance between genotypes which are in different groups. Therefore, these results can be applied in identifying parents, to performing appropriate crosses, and reaching maximum heterosis in hybridization programs.

Comparison between morphological traits, RAPD polymorphisms and storage proteins

Comparing the different dendrogram obtained from morphological traits with that obtained from the analysis of gliadins and from RAPD markers showed that when different methods are used, genetic diversity patterns are different. The correlation coefficient between distance matrix of the lines based on morphological traits and that obtained from gliadins was 0.183. This correlation was calculated as 0.088 for the morphological traits and the matrix obtained from RAPD marker, and as 0.106 for the matrix obtained from gliadin analysis and from RAPD markers. None of the above correlations were statistically significant. Although in statistical investigations, one pattern in the area ω was proved to be statistically related to the trait of number of spikelet per spike, the matrix obtained from all the morphological traits showed no relation with the matrix obtained from gliadins.

Through examining agronomical traits, isozyme markers and RAPD markers, Kaufman and Rousseeuw (1990) studied the genetic diversity of 70 wheat genotypes. In

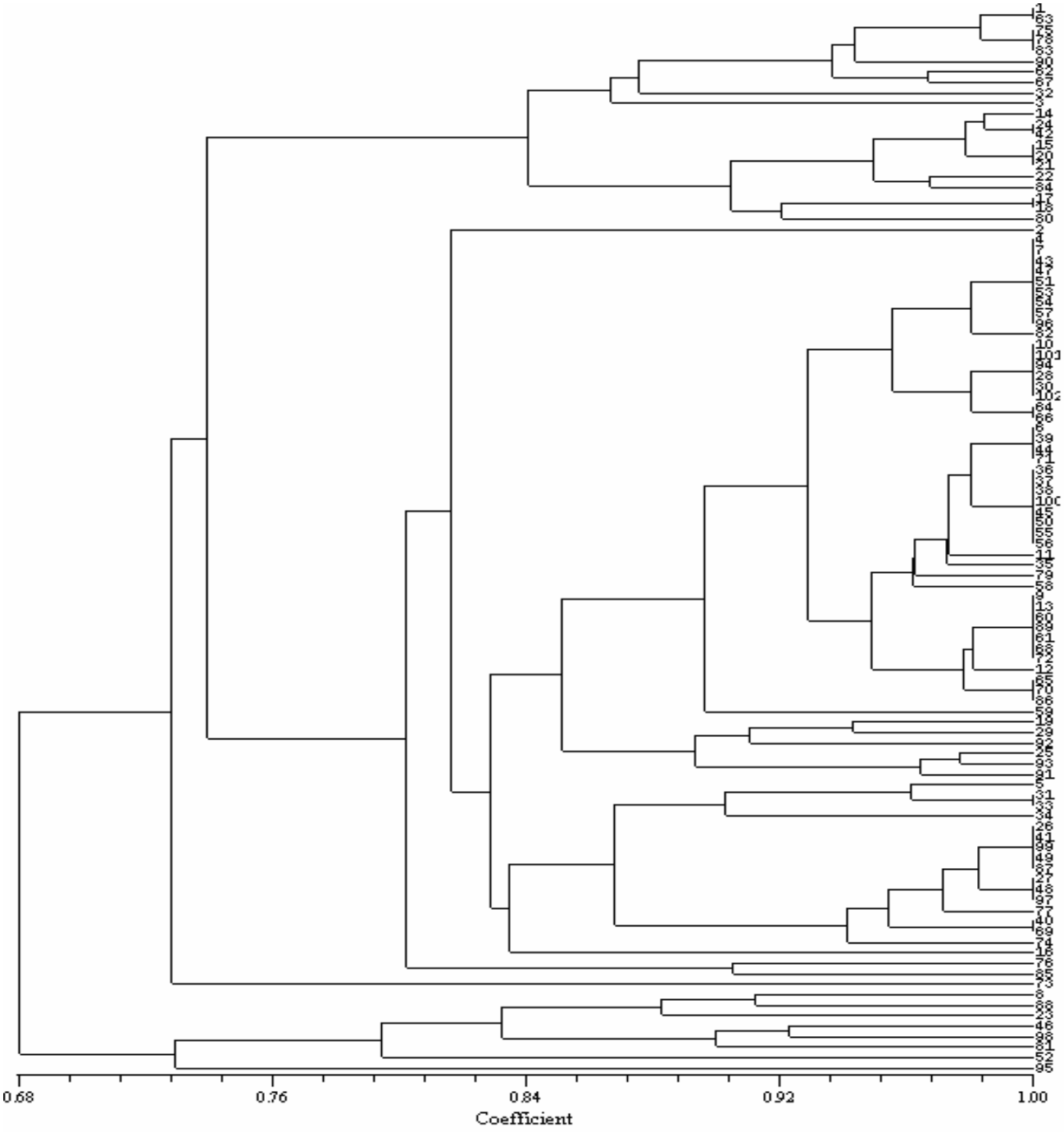

Figure 4. Dendrogram showing the relationship among 102 doubled haploid wheat based on gliadins bands. The scale is based on Nei and Li's similarity coefficient.

this study, no correlation was found between agronomical traits, isozyme markers and DNA. Leal et al. (2008) studied *Triticum tauschii* accessions, but found no relation between the matrix obtained from morphological traits and storage proteins. Also in another study, through storage proteins and SSR and RAPD markers, Kumar and Miaja (2007) examined the genetic diversity of 127 wheat genotypes that were collected from various geographical parts of Pakistan. No correlation coefficient was significant in these experiments and no relation was observed between protein markers and DNA markers. At the same time, Magdalena et al. (2007) observed significant correlations between the analysis of gliadins in 59 genotypes of Spanish wheat and spike morphological

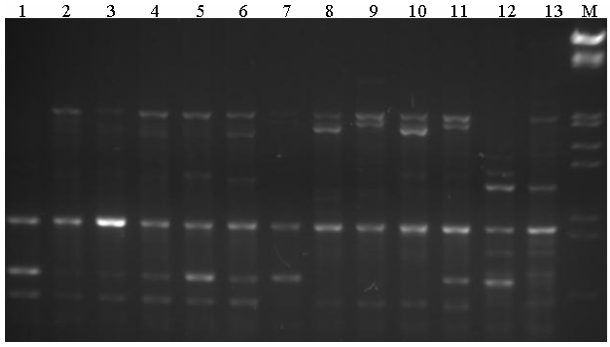

Figure 5. Amplification of DNA for RAPD analysis with primer GLE-12. M- Molecular weight marker (100 bp ladder). Sample numbering as in Table 2.

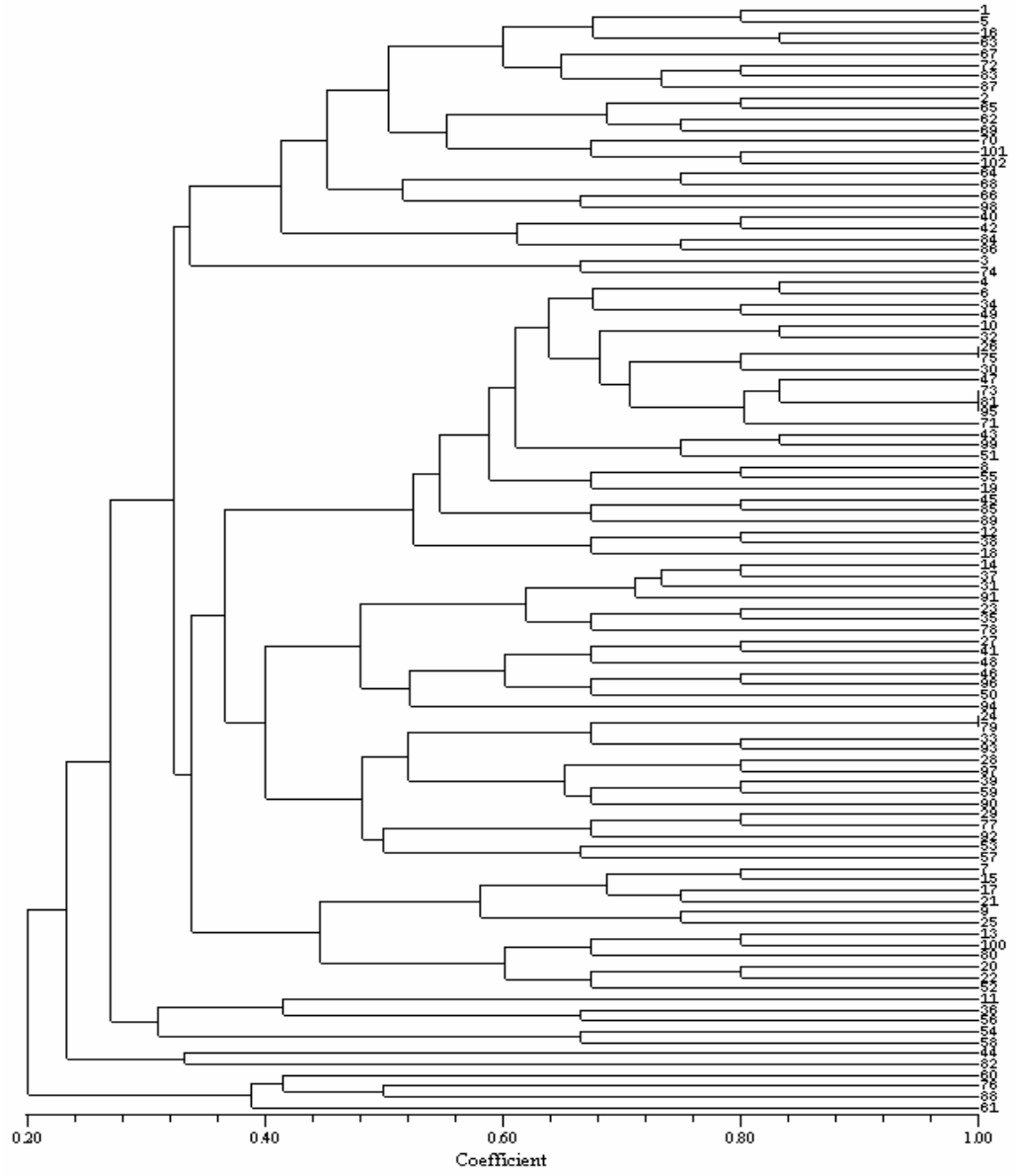

Figure 6. Dendrogram showing the relationship among 102 doubled haploid wheat based on RAPD markers.

traits.

There are various factors that could cause lack of correlation between genetic distances based on morphological traits, storage proteins and RAPD markers. As for morphological traits and storage proteins, since the genes coding these proteins are limited and situated at certain loci on the chromosomes 1 and 6, it is less probable that a great number of genes that code agronomical traits have linkage with the genes that code storage proteins.

As was stated earlier, among 22 agronomical traits, only one trait was found to have relation with protein patterns, and the rest of the genes that code agronomical traits (and are distributed throughout the genome of this plant) had no relation with storage proteins. As for the relation between the storage proteins and RAPD markers, lack of linkage between the RAPD primers used and the genes coding storage proteins could have led to non-relation because primers in RAPD method are random; given a large genome like wheat and using a few primers, it is less probable to observe any correlation between a primer and the gene coding a specified protein. Finally, as for the morphological traits and RAPD markers, different reasons can be presented. For instance, different combinations of the alleles which produce similar phenotypes can lead to morphological similarities, or many of the morphological differences may not essentially be related to the genetic differences of the genotypes.

Another reason may be different ratios of evolutionary variations in morphological traits in comparison with the sequences which are related to RAPD marker's diversity. RAPD diversity is directly dependent on the diversity in the DNA sequence, so that a single nucleotide variation can change the RAPD phenotype, while due to adaptation and in spite of random mutations, morphological traits may be preserved. In addition, RAPD loci may have been situated on non-coding genomic regions (non gene specific).

Since environment has had no effect on them and they show the highest polymorphism, DNA markers seem to be the most appropriate method for studying genetic diversity and identifying genotypes. However, regarding the fact that the results obtained from the analysis of the morphological traits, storage proteins and DNA markers showed that genetic diversity pattern differed according to each one of these methods, and each marker justified similarities and differences among the genotypes under study forming a different point of view. It can be concluded that using only one of these methods to estimate genetic diversity is not sufficient; it is, therefore, suggested that in order to have a more comprehensive picture of genetic diversity in various wheat populations, all the three methods be applied simultaneously and in a complementary way for the maintenance and management of current breeding germplasm as genetic diversity has long been a major role in the pre breeding programme.

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