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Full Length Research Paper

Genetic diversity of gliadin pattern, morphological traits and baking quality in doubled haploid wheat

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This study aimed at assessing the genetic diversity of 102 lines doubled haploid wheat (sent from CIMMYT) using acid-polyacrylamide gel electrophoresis (A-PAGE) method, morphological traits and baking quality. Cluster analysis according to morphological traits divided all genotypes into four groups, so that genotypes with high yield were placed in one group. However, by cluster analysis according to their qualitative traits, the genotypes were classified into three main groups, while genotypes with higher amount of protein was placed in a separate group. In the studied lines, 48 bands and 47 different patterns were detected and polymorphism was observed in most of the bands. In the ω area, 18 bands and 19 different patterns were observed and the most amount of band was observed in this area. In γ and β areas, 12 and 9 bands, 19 and 12 patterns were observed, respectively. The least pattern variety was seen in α area, presumably because the bands did not separate properly in the one-dimensional electrophoresis in this area. Seven patterns and nine bands were observed in this area. Using Nei formula and according to the patterns, the genetic diversity for all four areas (α, β, γ, ω) was calculated, according to which γ area with H = 0.872 had the most genetic diversity, then came ω and β areas with H = 0.767 and H = 0.714, respectively, and the least genetic diversity was observed in α area with H = 0.646. Cluster analysis according to protein bands has classified genotypes into 9 main groups. Although the lines studied in this research had the same parents, considerable diversity was observed among them. Therefore, the electrophoresis of polyacrylamide gel of gliadins can be used as a strong system for identifying similar varieties. While comparing the observed patterns, one pattern in ω area was proved to be relevant to the trait of the number of grain per spikelet, which can be used as a marker in order to increase yield.

Key words: Genetic diversity, gliadin patterns, morphological traits, backing quality, doubled haploid wheat.

INTRODUCTION

Because of their homozygosis and higher purity, doubled haploid wheat can be used as bred variety and as base in producing hybrid. Therefore, studying their genetic diversity through morphologic traits, baking quality, DNA and isozymes will be important. Gliadins and glutenins constitute around 80% of the total seed proteins in wheat, of which 40% are gliadins. 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-polyacrylamide gel electrophoresis (A-PAGE) are divided into four groups α, β, γ and ω. Many of the gliadin alleles reside at six main loci on the chromosomes of the first (Gli-1) and the sixth (Gli-2) 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 environ-

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mental adaptation (Metakovsky and Branlard, 1998). This study was conducted in order to examine the genetic diversity of 102 double haploids of wheat (sent from CIMMYT) through studying gliadin protein bands and the relation between gliadin patterns with agronomic traits, studying morphological traits and baking quality.

MATERIALS AND METHODS

During the 2006 - 2007 seasons (102 doubled haploid wheat), yield trial was conducted in plots of 2 m long with between-row distance of 20 cm and plant distance of 5 cm. An augmented design was used. The studied characteristics included: yield per plot, number of grains per spikelets, number of fertile tiller, total number of tillers, biomass, yield per plant, harvest index, number of grains per spike, spike density, spike length, plant height, number of internodes, pedankel length, weight of 1000 grains, weight of grains per spike, internode length, seed length, seed width and number of spikelets per spike, also 6 baking quality traits, protein content, gluten index, SDS sedimentation, sedimentation percentage, water absorption and seed hardness were studied.

Separation of gliadin bands with the A-PAGE method were performed on all the 102 lines of wheat, using the Zillman and Bushuk method modified by Poperelya (1989) (Figure 5). Seeds were individually ground and extracted overnight at room temperature with 0.2 ml solution containing 0.9% acetic acid and 18% urea and 0.01% pyronine. The samples were laid on the polyacrylamide gel and the electrophoresis apparatus was set on 450 v and 0.16 amp 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 (Figure 6). 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 determination of next pattern different from the previous one(s) and identification of varieties with similar band pattern by comparing with it. The strategy was followed for all the genotypes and large numbers of different patterns were identified in each group of gliadins (α, β, γ and ω). The exercise was repeated many a times to confirm the pattern of varieties within each group. Since Anza genotype was used as checks in each gel, comparison of band pattern among different varieties was easy. With this procedure, all the band patterns were identified (Tanaka et al., 2003).

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 band patterns in each area (Nei, 1973).

Also, in order to find the relation between agronomical traits and protein patterns, the t-test was run. 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.

These calculations were done using Statistical Package for the Social Sciences (SPSS) 12 program. Calculating similarity co-efficients matrix and dendrogram was done by NTSYS 2.01 program.

RESULTS

Correlation between traits was used to determine the linear relationship between morphological traits. There was a significant correlation between number of grains per spikelet (0.74), number of fertile tillers (0.69), total number of tillers (0.64), biomass (0.63), yield per plant (0.608), harvest index (0.591) and number of grains per spike (0.35) (Table 1), but no correlation was observed between the yield and the other traits. Cluster analysis according to the morphological traits using the Ward method classified all the genotypes into four groups. In this classification, genotypes with high yield were placed in one group (Figure 1). In principle component analysis, 7 components could justify 72.64% of the total variance. The correlation was calculated between the first component and yield per plot (r = 0.708**), biomass (r = 0.698**), yield per plant (r = 0.672**), number of fertile tillers (r=o.708**), total number of tillers (r = 0.66**), number of grains per spike (r = 0.559**), spike density (r = 0.504**), spike length (r = 0.418**), number of spikelets per spike (r = 0.727), as well as harvest index (r = 0.588). This component is known as the yield component.

In the second component, the most important traits were weight of grains per spike and weight of 1000 grains. In the third component, plant height and pedankel length were most important; in the fourth component, internode length and seed length; in the fifth component, number of grains per spikelet; in the sixth component, number of internodes and in the seventh component, seed width was the most important.

Cluster analysis according to 6 qualitative traits classified genotypes into three main groups, so that genotypes with high protein were placed in a separate group (Figure 2). Also 6 traits which were indicative of the baking quality were analyzed in the principle components.

Three principle components could justify 86.9% of the total variance. In the first component, protein percentage, sedimentation percentage and the seed hardness were of higher importance. This component alone could justify 55.34% of the total variance. Therefore, these traits were more important in assessing the qualitative traits. The most important traits were the gluten index and SDS sediment percentage in the second component and water absorption in the third component.

Among the studied genotypes, 48 different bands and 47 different patterns were detected in most of which polymorphism were observed. In the ω area, 18 bands and 19 different patterns were observed. Among the observed bands, ω12 and ω15 with 99.1 and 98.03% respectively, had the most frequency of all among the genotypes, while ω18 with a frequency of 0.98% was observed in one genotype only. 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, 12 bands were observed among which band γ4 with 92.15% and band γ7 with 85.29% had the highest frequencies. The least frequency was that of band γ2, which was observed in one genotype only. 19 patterns were observed in this area

Table 1. Correlation between yield with others traits.

Traits	Yield per plot
Harvest index	0.591**
Yield per plan	0.608**
Biomass	0.63**
Total number of tillers	0.642**
Number of fertile tillers	0.699**
Plant heigth	0.05 n.s
Number of internodes	0.128 n.s
Number of spikelets per spike	0.742**
Internode length	-0.008 n.s
Pedankel length	0.125 n.s
Spike length	0.357 **
Spike density	0.441**
Number of grains per spike	0.476**
Weight of grains per spike	0.052 n.s
Number of grains per spikelet	0.031 n.s
Weight of 1000 grains	0.004 n.s
Seed length	0.077 n.s
Seed width	-0.114 n.s

among which patterns γ4 and γ6 with frequencies 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 997.05% had the most frequencies. Here the least frequency was that of β4 and β9. In this area, 12 band pattern were observed and the highest frequency was that of pattern β4 (44.11%) in 45 genotypes and after that β5 in 24 genotypes. The least frequency was that of patterns β8, β16, β11 and β12. Altogether, however, 7 patterns were observed in this area among which the highest frequencies were that of α2, α3 and α1. In this area, 9 bands were observed.

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).

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

DISCUSSION

Between studied 19 morphology traits, number of grains per spikelets, number of fertile tillers, total number of fertile tiller, biomass, weight of spike, harvest index and number of grains per spike had high correlation with yield. According to this, yield is a polygene trait and it is difficult to increase it directly, then the benefit can be acquired with the help of traits which have high correlation with the yield and indirectly increase the yield.

In analysis of 6 qualitative traits a bi-plot was drawn according to the first and second components (Figure 3). The bi-plot selected 15 genotypes (B area) which had high amounts of protein content, sediment percentage, seed hardness, gluten index and SDS sedimentation. Among these samples, genotypes 88, 92, 37, 35 and 40 had the most number of the above mentioned traits as well as high yield and therefore, they were introduced as the superior varieties. Genotypes 73, 8, 93, 98 and 50 had high quality proteins, but their yield was medium. Also, genotypes number 2, 4, 5, 11 and 13 had low yield but desirable protein quality; therefore, these were used in breeding programs, especially hybridizing, in order to reach genotypes with high yield and better baking quality.

The assessment of doubled haploid wheat by A-PAGE was shown in ω and γ areas with 19 patterns, β area 12 and α area 7 patterns. Caballero et al. (2004) studied the genetic diversity of Spanish common wheat through pattern variety of gliadin. Among 403 studied genotypes, 61 patterns in ω, 44 patterns in γ, 19 patterns in β and 15 patterns in α was observered. Xu et al. (2008) in their study showed that the greater levels of gliadin variation existed in *Triticum turanicum* and a total of 72 gliadin patterns were found among 87 accessions. In this study, it was observed that ω, γ and β areas had high variety patterns but the least pattern variety was observed in area α. In the present study, α area had the least pattern variety. This may be due to the 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. It could also be due to the least protein variety in the area α of which 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) reported larger variation in γ and ω gliadins than α and β gliadins in Japanese cultivars. Cluster analysis according to gliadin bands classified all the genotypes into 9 main groups (Figure 4). The most similarity index was witnessed 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 witnessed 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

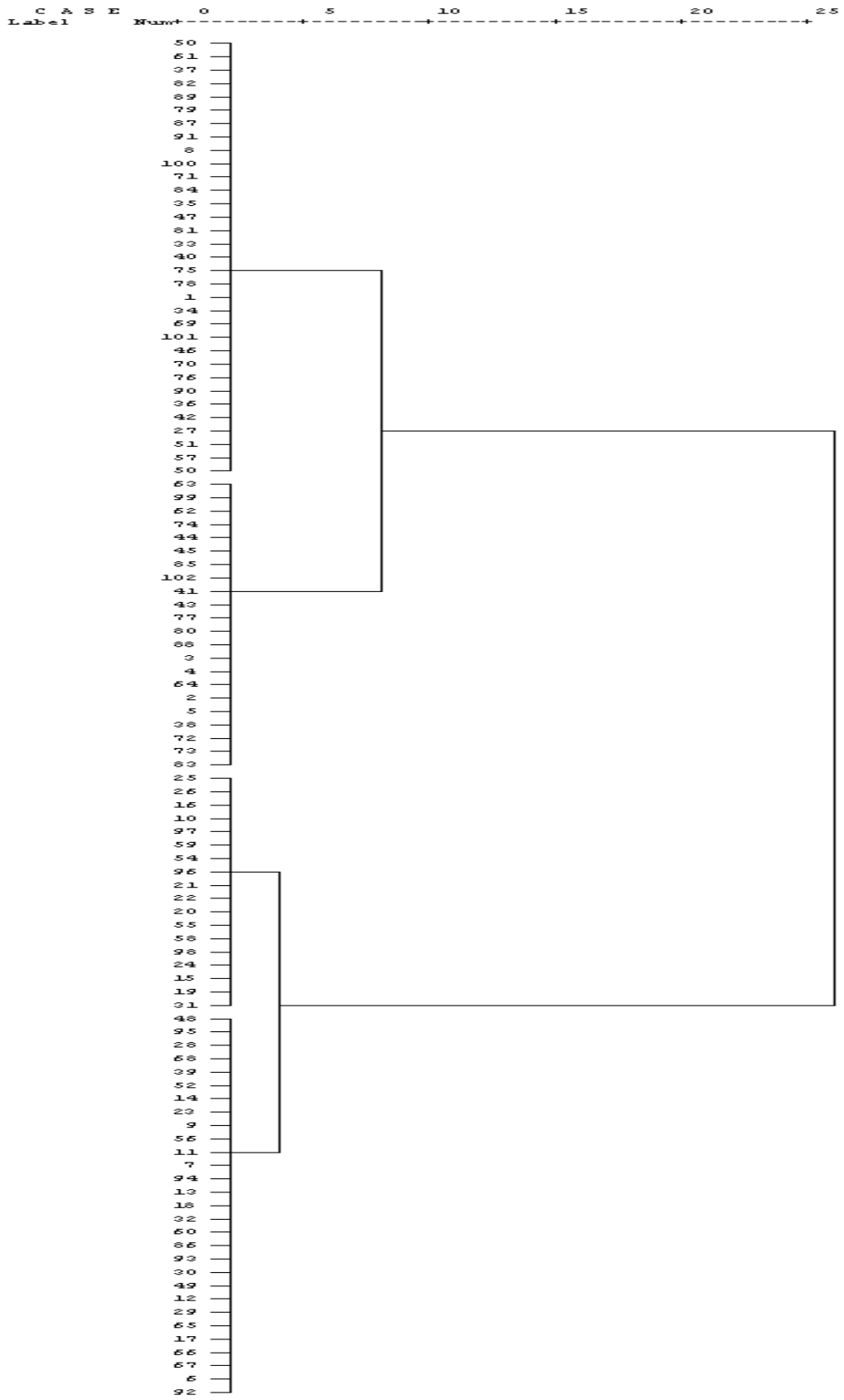

Figure 1. Dendrogram showing the genetic relationships among 102 doubled haploid wheat based on morphological traits.

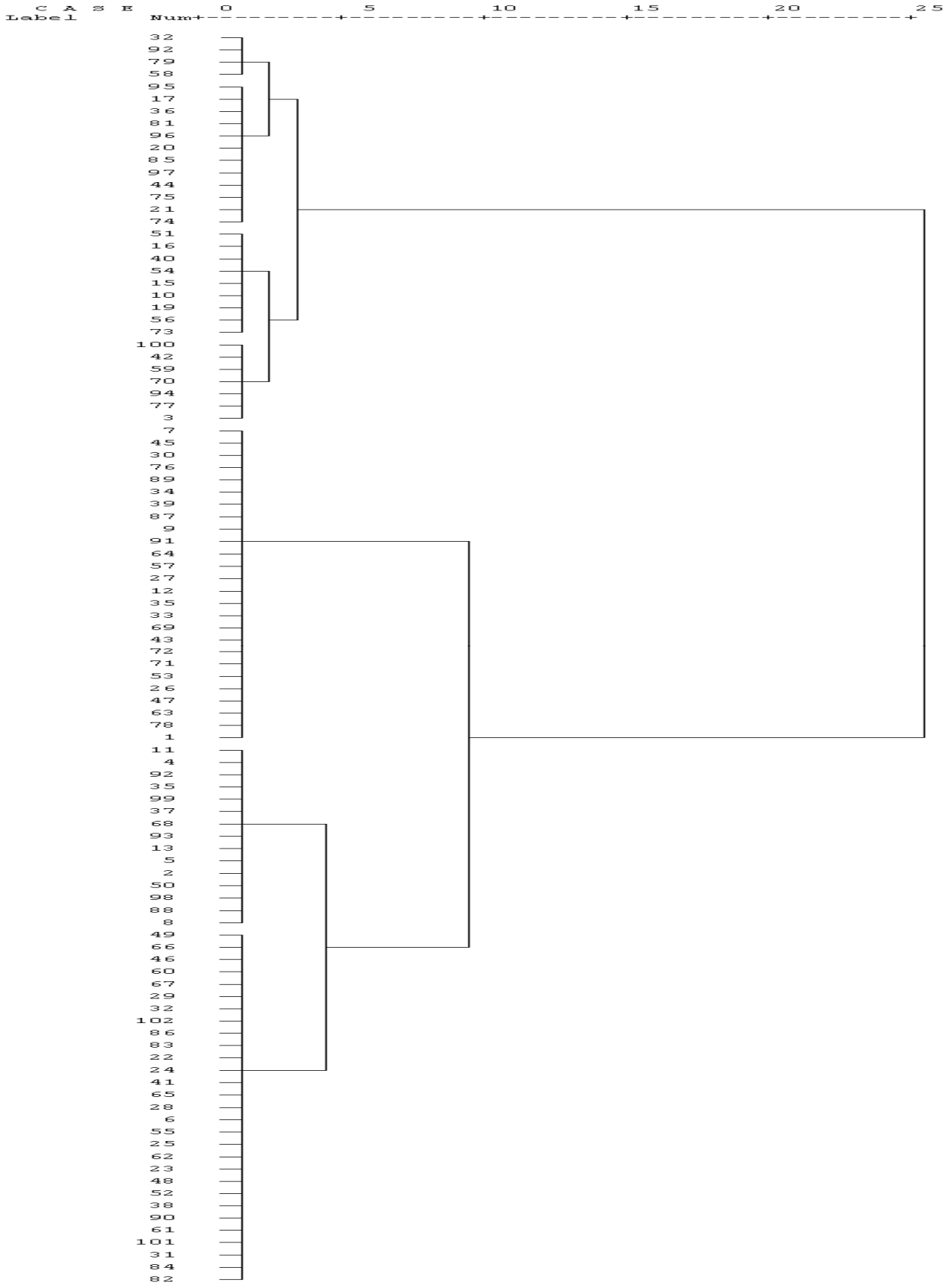

Figure 2. Dendrogram showing the relationship among 102 doubled haploid wheat based on baking quality.

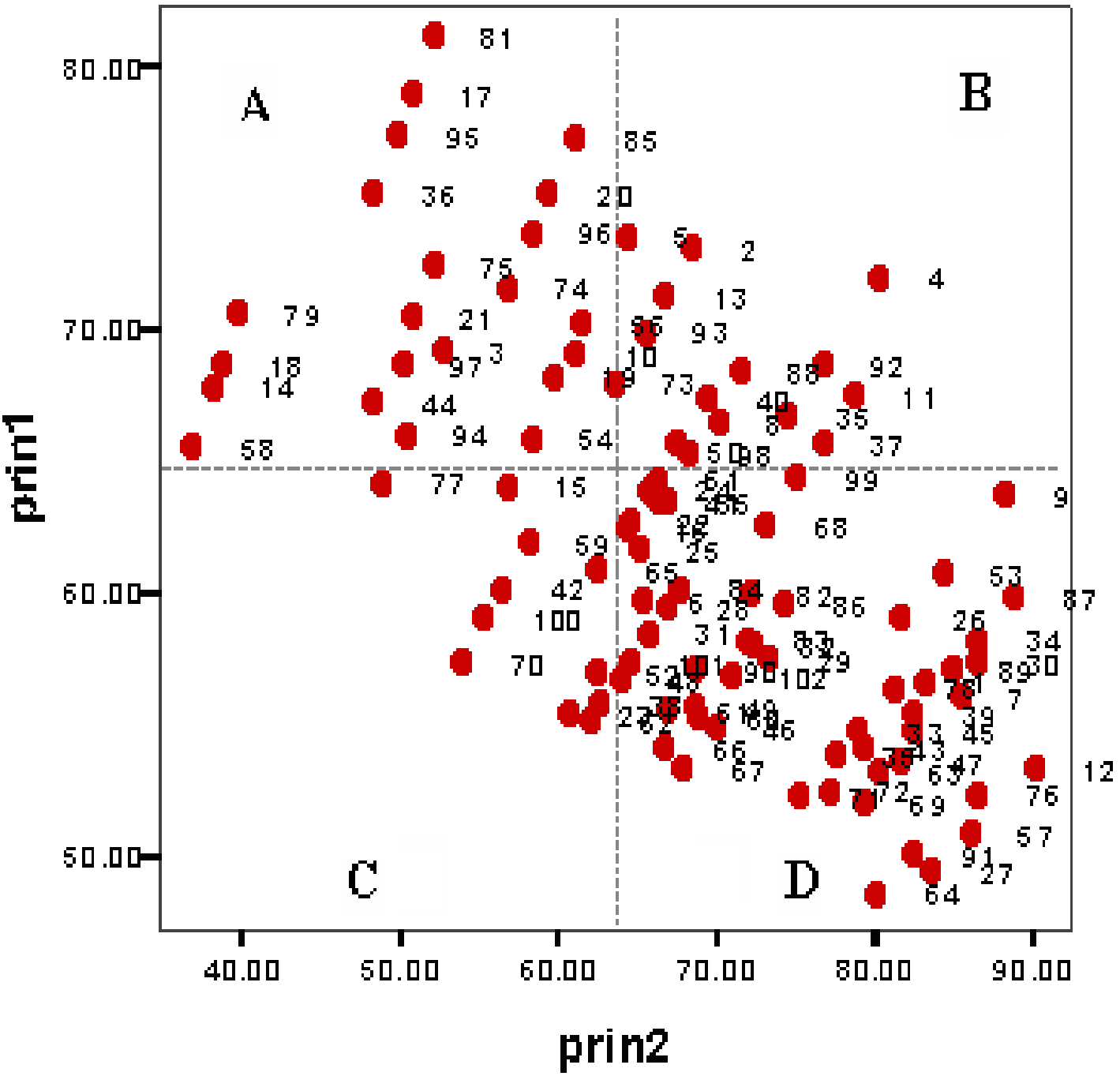

Figure 3. Bi-plot based on baking quality.

parents, gliadin electrophoresis showed considerable diversity among them. However, recently, Dreisigacher 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 analysis of polymorphic markers (Table 2). 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 the wheat germplasm resources.

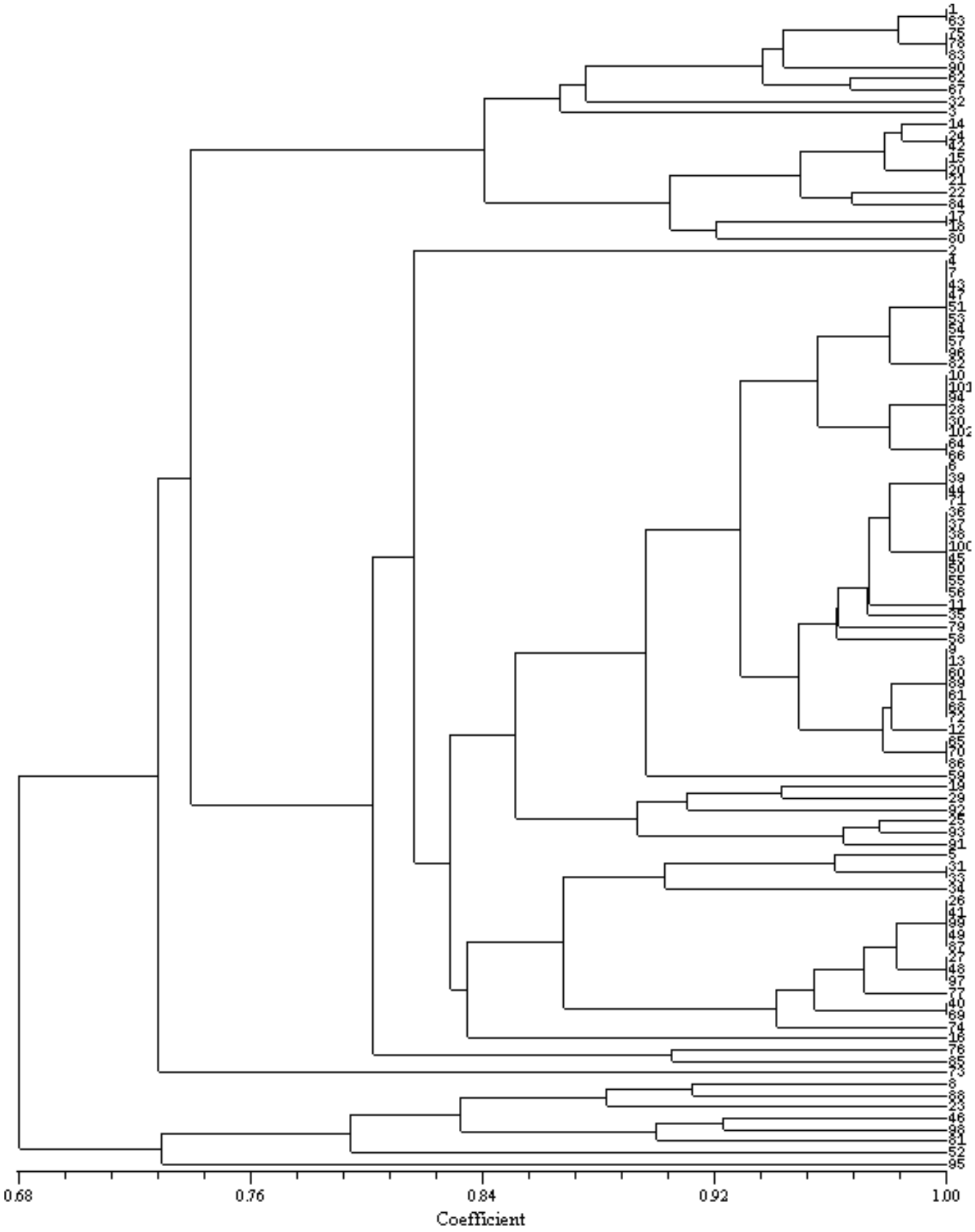

Figure 4. Dendrogram showing the relationship among 102 doubled haploid wheat based on gliadins bands. The scale is based on Nei and Li`s similarity coefficient.

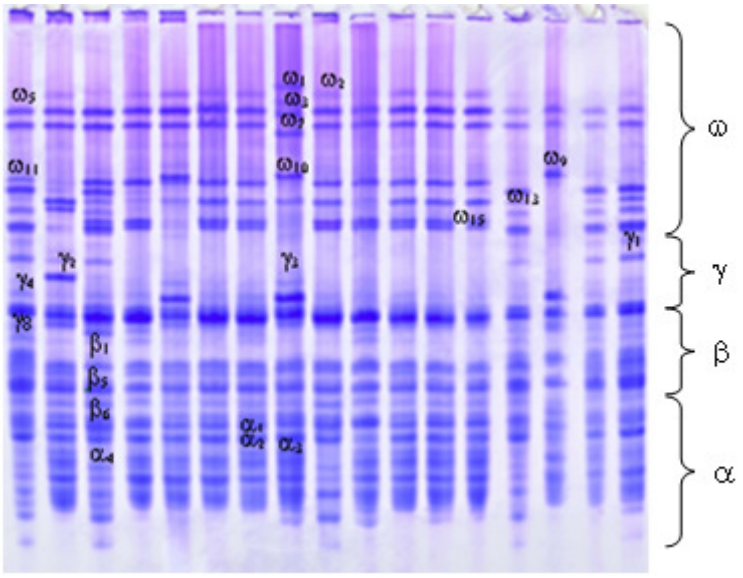

Figure 5. Representative samples for variation detected by A-PAGH separation of gliadins in doubled haploid wheat.

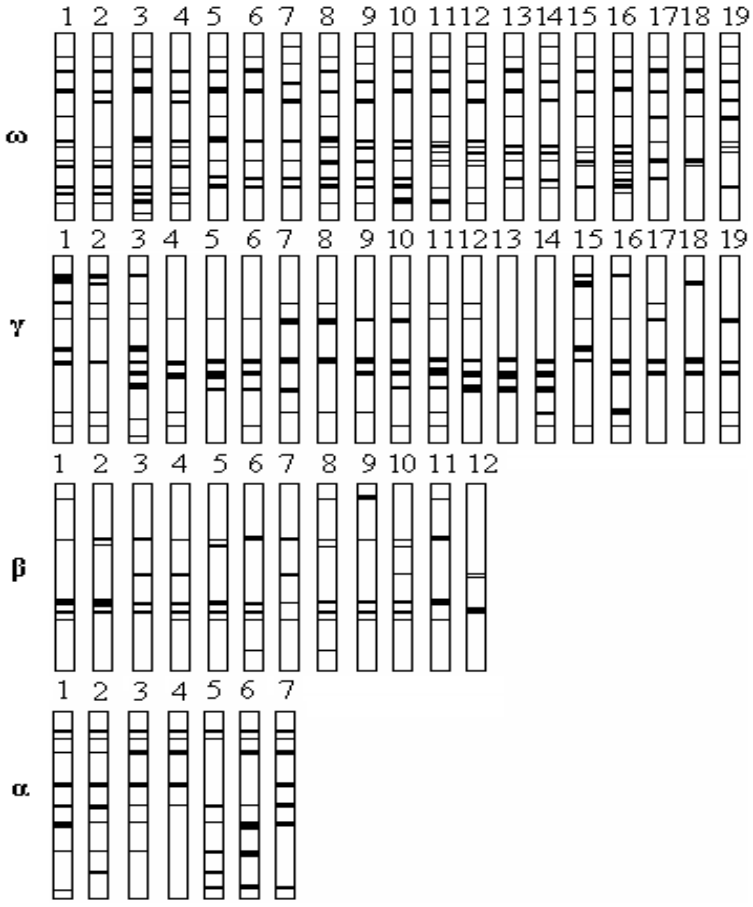

Figure 6. Ideogram of different gliadin pattern in the regions of α, β, γ and ω gliadins observed in the genotypes studied. The numbers shown on the top of ideograms denote the of electrophoretic banding patterns identified among all genotypes.

Table 2. Pedigree of doubled haploid wheat and α, β, γ and ω gliadin patterns identified as per Figure 6.

No.	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

Table 2. Contd.

No.	Pedigree	Gliadin pattern			
		α	β	γ	ω
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
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/SW89-5124*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	16	5

Table 2. Contd

No.	Pedigree	Gliadin pattern			
		α	β	γ	ω
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/STAR/...	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	10	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	17
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

REFERENCES

Branlard G, Dardevet M, Nieto-Taladriz MT, Khelif D (1993). Allelic diversity of omega gliadin as reveald by SDS-PAGE. Their possible implication in quality variation gluten. 93: 234-243.

Caballero L, Martin LM, Alvarez JB (2004). Variation and genetic diversity for gliadins in Spanish spelt wheat accessions. Genet. Resour. Crop Evol. 51: 679-686.

D`Ovidio R, Anzarella OT, Masci S, Lafindra D, Poreceddu E (1992). RFLP and PCR analysis at Gli-2, Glu-1 and Glu-3 loci in cultivated and wild wheats. Hereditas, 116: 79-85.

Dreisigacher S, Zhang P, Warburton ML, Van Ginkel M, Hoisington D, Bohn M, Melchinger AE (2004). SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different megaenvironments. Crop Sci. 44: 381-388.

Hou YC, Zheng YL, Wei YM (2004). Analysis of genetic diversity of hordein in wild relatives of barley from Qing Zong Plateau. Southwest China J. Agric. Sci. 17: 545-551.

Lan X, Wei Y, Wang Z, Zheng Y (1999). Gliadin comparison between Aegilops tanschii cosson from China and middle-East. J. Sichuan. Agric. Univ. 17: 15-20.

Metakovsky EV, Annicchiarico P, Baggini G, Pogana NE (1997). Relationship between gliadin alleles and dough strength in Italian bread wheat cultivars. J. Cereal Sci. 25: 229-236.

Metakovsky EV, Branlard G (1998). Genetc diversity of French common wheat germplasm based on gliadin alleles. Theor. Appl. Genet. 96: 209-218.

Nei M (1973). Analysis of gene diversity in subdivided population. Proc. Nat. Acad. Sci. USA, 70: 3321-3323.

Payne PI (1987). Genetics of wheat storage protein and the effect of allelic variation on bread making quality. Annu. Rev. Plant physiol. 38: 141-153.

Pogna NE, Metakovsky EV, Redaelli R, Raeneri F, Dachkevitch T (1993). Recombination mapping of Gli-5 a new gliadin cooding locus on chromosome 1-A and 1-B in common wheat. Theor. Appl. Genet. 87: 113-121.

Poperelya FA (1989). The analysis of gliadin polymorphism in wheat and their relationship between yield and quality traits. Moskva. Agroprmizdat. pp. 138-149.

Rodriguez QM, Carrillo JM (1996). Linkage map of prolamin loci Gli-D4 and Gli-D5 in hexaploid wheat. Plant Breed. 115: 189-191.

Sozinov AA, Poperelya FA (1980). Genetic classification of prolamines and its use for plant breeding. Ann. Technical Agric. 29: 229-245.

Tanaka H, Tomita M, Tsujimoto H, Yasumuro Y (2003). Limited but specific variation of seed storage proteins in Japanese common wheat (*Triticum aestivum* L.). Euphytica, 132: 167-174.

Wang H, Wei Y, Yan Z, Zheng Y (2006). Genetic variation of gliadin and HMW glutenins subunits in durum wheat. J. Agric. Biotechnol. 14: 721-727.

Weegels PL, Hamer RJ, Schofield JD (1996). Functional properties of wheat glutenins. J. Cereal Sci. 23: 1-17.

Xu LL, Ming Y, Yan ZH, Zheng YL (2008). Genetic variations of gliadin and HMW-glutanin subunits in *Triticum turanicum* Jakubz. Plant Genet. Resour. Newslett. 151: 27-32.

Zhang XY, Yang XM, Dong YS (1995). Genetic analysis of wheat germplasm by acid polyacrylamid gel electrophoresis of gliadins. Sci. Agric. Sin. 28: 25-32.

Zillman RR, Bushuk W (1979). Wheat cultivar identification by gliadin electrophoregrams. II. Effects of environmental and experimental factors on the gliadin electrophoregrams. Can. J. Plant Sci. 59: 281-286.