

## EVALUATION OF PAKISTANI WHEAT GERMPLASM FOR BREAD QUALITY BASED ON ALLELIC VARIATION IN HMW GLUTENIN SUBUNITS

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### Abstract

Seventy six Pakistani wheat genotypes including land races were investigated for Bread quality (BQ) based on allelic variation in HMW glutenin subunits at the Glu-1 loci through SDS- polyacrylamide gel electrophoresis. Twenty five different allelic combinations were detected with a total of 14 Glu-1 loci. Highest polymorphism was revealed by Glu-B locus and some single/ rare sub units were also screened out. The frequencies of dominant subunits were 50% for 2\*, 42.11% for subunit pair 17+18 and 48.68% for 5+10 and 2+12 respectively. The quality scores displayed a range from 4 to 10, however generally good quality score of eight was more frequent (39. 47%). The highest quality scores of 10 and 9 were observed in 22.36% and 19.74% of genotypes respectively. The UPGMA analysis grouped genotypes into three major with two additional sub clusters for each. The cluster "a" "b" and "C" were separated at 73% genetic distance which was further differentiated at a genetic distance of 50% into their sub clusters. Pakistani wheat varieties/land races exhibited large variation in term of HMW-GS. The generated information will lead to the pyrimiding of sub units for high BQ through mission oriented marker assisted breeding programmes for quality improvement of wheat.

### Introduction

Storage proteins account for about 50% of the total protein in mature cereal grains and have a great impact on nutritional quality which is important for humans and livestock. In addition to their nutritional importance, cereal grain proteins also influence the utilization of grain in food processing (Peter *et al.*, 2002). For wheat, processing quality is particularly important, which is largely consumed by humans after processing into bread and other foods. The gluten proteins are the major determinant of end use quality. The isolation of wheat gluten was first described by Beccari, (1745) and by the late nineties it was well established fact that strong i.e., highly visco-elastic doughs contain high proportions of high molecular mass glutenin polymers (Field *et al.*, 1983). However, a breakthrough in understanding came when Payne and co-workers demonstrated that allelic variation in the composition of the HMW prolamins (the HMW subunits of glutenin) was strongly correlated with differences in the bread making quality (Payne, 1987).

The bread making characteristics of wheat flour is closely related to the elasticity and extensibility of the gluten proteins stored in the starchy endosperm, particularly the high molecular weight glutenin subunits (HMW-GS). HMW-GS are of immense importance in breeding and genetical research due to high level of polymorphism. HMW-GS are encoded by two type of genes called (x and y) that are located on three loci (Glu-A1, Glu-B1 & Glu-D1) placed on long arms of the group 1 chromosomes (Lawrence & Shepherd 1981). Research has shown that proportion of inter varietal variation in bread making potential, can be attributed to differences in composition of HMW-GS, fluctuates widely between 15% to 60% (Kolster, 1992). The seed storage proteins are associated with agriculturally significant traits and they are used in a legal protection of cultivars (Knoblochova & Galova 2000). The present investigation was carried out to assess the allelic variation of HMW-GS composition responsible for bread making quality among Pakistani wheat varieties and land races through SDS polyacrylamide gel electrophoresis. The possible outcome of the work may be the utilization of HMW-GS as biochemical marker in breeding for improved bread making quality of wheat by better utilizing resource genotypes.

### Materials and Methods

Experimental material comprised of 76 wheat genotypes including land races collected from different research institutes

of Pakistan. Reference varieties i.e., Chinese-Spring (2, 7, 8, 12), Gabo (2, 2\*, 12, 17, 18) and Holdfast (1, 5, 7, 8, 11) were used as standards (Payne & Lawrence, 1983). Total proteins were extracted from five single grain of each genotype. The grain were grinded into flour and treated with 70% ethanol to remove gliadins. The extraction buffer (0.5M Tris- HCl (pH 6.8) containing 12% (w/v) glycerol, 2% (w/v) sodium dodecyl sulfate (SDS), 0.003% (w/v) bromophenol blue, and 5% 2-mercaptoethanol) was then added to flour and extracted for thirty minutes at room temperature with occasional vortexing. Samples were placed in boiling water for 3 min and centrifuged for 5 min at 14000 rpm to get the supernatant. Proteins to be characterized were separated on 8% denaturing SDS poly acrylamide gels by loading equal volume of samples in the wells following the method of Laemmli (1970). After the completion of electrophoretic run, gels were stained with commasie brilliant blue (G-250).

The HMW-GS were identified using the previously proposed numbering system (Payne & Lawrence, 1983) while quality scores were assigned using the scoring system described by Payne *et al.*, (1987). Alleles were assigned quality scores of 1 (poor quality) to 4 (high quality) and individual scores for each allele present in a variety were then pooled to provide the overall quality score for that variety.

The generated data matrix was subjected to the statistical analyses using Statistica software (StatSoft Inc., Tusla, OK). Dendrograms showing the genetic relationships of the varieties/ cultivars/ land races was constructed using the unweighted pair group method on arithmetic averages (UPGMA).

### Results

The results displayed high level of polymorphism for HMW-GS (Fig. 1). Among the 76 genotypes studied, 73 were homogenous (96.05%) and 3 were heterogeneous (3.95%) for HMW-GS. Twenty five different allelic combinations were detected (Table 1). A total of fourteen Glu-1 alleles (3 at Glu-A1 locus, 7 at Glu-B1 locus and 4 at Glu-D1 locus) were detected in evaluated material. At Glu-1A locus of A genome three alleles Null, 1 and 2\* were identified (Table 1). The frequencies of occurrence of two active HMW-GS (2\*, 1) and one null type gene encoding no subunit were 50%, 32. 89% and 17.11%, respectively (Fig. 2a). There was more variation for Glu-B1 locus as compared to Glu-A1 and Glu-D1. The alleles

corresponding to 7 different types of subunits, 4 in subunit pairs i.e., 17+18, 7+9, 7+8, 13+16 and 3 single/rare Glu-B subunits like 15, 20 and 21 in lower frequencies were also screened out in all the genotypes Table 1. The frequency recorded for predominate loci 17+18 and 7+9 were 42.11% and 30.26% respectively (Fig. 2b). Glu-D1 locus exhibited alleles encoding the subunits pair 2+12 and 5+10 as a dominant combination having equal frequencies 48.68%. The existence of subunit combination 2+10 (2.63%) and a single subunits i.e., 10 with low frequencies were also observed (Fig. 2c).

The elaboration of results for quantification of the allelic variation by wheat genomes showed maximum contribution of B genome (50%) followed by D genome (23.53%) as shown in Fig. 3.

Individual scores of HMW-GS were then pooled to calculate quality score of genotypes according to Payne *et al.*, (1987) as illustrated in Table 1. The results of quality score based on the HMW- GS compositions among the varieties and land races revealed greater variation with land races generally showing a low score. The score lies in the range of 4 to 10 with an average of 7. Seventeen varieties gained the highest quality score of 10 (22.36%) and 15 had a good quality score of 9 (19.74%). A generally good quality score of 8 was more frequent (39.47%) in this investigation. The medium quality score of 7 was fixed in 6 genotypes only. The other lower quality scores i.e., 6, 5 and 4 appeared in relatively lower proportions with 2.63%, 2.63% and 5.26%, respectively (Fig. 4).

Cluster analysis of the wheat genotypes performed on HMW-Glutelin subunits revealed additional useful information about the observed variation (Fig. 5). The genetic similarity matrix was used to construct dendrogram by UPGMA method. The dendrogram delineated genotypes into three major clusters "a" and "b" and c separated at 73% genetic distance (Fig. 5). At about 50% genetic distance all three clusters could further be divided into two sub clusters each namely "a1", "a2", "b1", "b2", and "c1" and "c2" respectively. Many varieties in each cluster were 100% similar but the highest number of 100% similar varieties (9) was grouped in sub cluster "b2". Clustering also revealed varieties in each cluster that showed at least 10% dissimilarity from other members of the same cluster. These varieties include Margalla-97 from "a1", Kohistan-97 from "b1", Sarsbaz, S24, Mynas and AS-2002 from "b2", C-217 and Bhakker 2002 from subgroup "c1" and "c2" respectively. These varieties are therefore genetically more diverse and good source of variation.

## Discussion

With the persual of results, it was evident that HMW-GS polymorphism existed among the Pakistani wheat varieties and land races as reported in the genetic stocks of other researchers (Yan *et al.*, 2007; Chaparzadeh *et al.*, 2008). The protein patterns of our genotypes were homogeneous as well as heterogeneous coinciding with the earlier reports (Singh *et al.*, 2007; Kang *et al.*, 2007 and Popa *et al.*, 2003). This heterogeneity could be used in breeding programs for increasing bread-making quality by selection of a glutenin phenotype with a HMW-GS composition associated with good quality. Twenty five different allelic combinations with a total of fourteen Glu-1 alleles depicted not only the presence of allelic variation of HMW-GS responsible for differences in bread-making properties but also its probable utilization in breeding future varieties. Storage protein composition is expected to be a cultivar constant element; being the direct expression of its genotype thus it can provide a useful aid to

cultivar identification. These results are in strong agreements by the previous findings of Popa *et al.*, (2003) Kang *et al.*, (2007) and Tsenov *et al.*, (2009).

In the present study, Glu-1A locus of A genome contributed three alleles Null 1 and 2\* with the dominance of 2\* subunit (50%) and is also comparable to the previous reports of Bahraei *et al.*, (2004); Kang *et al.*, (2007) and Chaparzadeh *et al.*, (2008). The presence of subunit 1 was relatively low as compared to 2\* but higher than the few null alleles. Contrary to present results some researchers have mentioned the lack of subunit 1 in their wheat genotypes (Valizadeh *et al.*, 2001 and Chaparzadeh *et al.*, 2008). These differences may be due to difference in the germplasm used or due to lesser number of varieties included in the previous experiments conducted by Valizadeh *et al.*, (2001); Chaparzadeh *et al.*, (2008). The subunit 1 has good contribution to the quality of wheat; therefore it might be introgressed into to the genetic background of local varieties to improve their quality following continued efforts of breeding efforts of hybridization and selection procedures. Our results are in accordance with an attribute shared by Morgunov *et al.*, (1990) who estimated a few null alleles in Soviet wheat varieties. However, some opposite findings have been reported by Nakamura (2000) and Popa *et al.*, (2003) regarding the frequency of the null allele. The differences may be due to baking requirement of medium elastic dough rather than strong gluten that results in less extensible dough (Barnlard 2003).

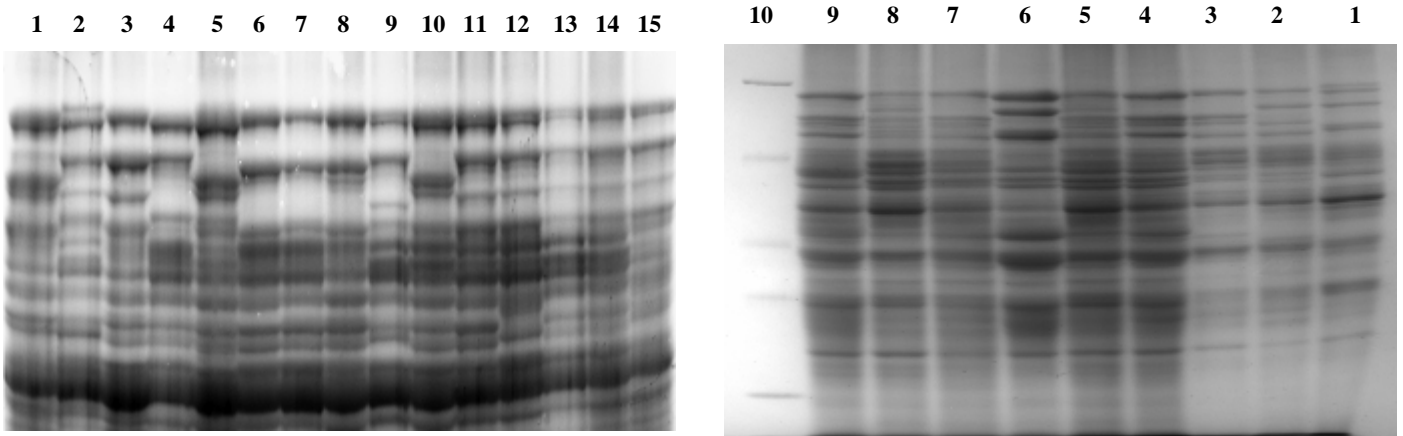
Glu-1B of B genome showed higher level of polymorphism by contributing 7 different types of allelic variants. Previously, maximum glutenin polymorphism at GluB1 locus has also been reported (Pike & MacRitchie 2004; Yan *et al.*, 2007). The most frequent pattern was 17+18 (42.11%) followed by subunits 7+9 (30.26%). The results are in line with the findings of Tahir *et al.*, (1996); Singh *et al.*, (2007) who reported the highest frequency of 17+18 but not in agreement with some other reports (Gregova *et al.*, 1999 & 2006; Popa *et al.*, 2003; Kang *et al.*, 2007) where the highest frequency observed was for subunits 7 +9. The results also revealed the presence of 13+16 subunits with lower frequencies as Mandonlakani *et al.*, (2008) previously reported. A single/rare subunits of 15 and 21 were also detected in some of our genotypes similar to the Urazaliev, (2003); and Dong *et al.*, (2009). Sub unit 20 eluted in five varieties (6.58%) is tenable with the experiments of Yan *et al.*, (2007) who also reported lower frequency for this subunit in their genotypes. However, the present finding does not confirm work of Carrillo, 1993) who opposed our results by observing the allele encoding subunit 20 as more frequent allele of Glu-B1. The scarcity of these subunits is advantageous because they have all been associated with poor bread-making quality (Payne *et al.*, 1987 and Dong *et al.*, 1991).

Glu-D1 locus of D genome also showed considerable amount of polymorphism. The alleles encoding the subunits pair 2+12 and 5+10 were the most frequent patterns. Our findings are in close conformity by some researchers (Schuster *et al.*, 1997; Nakamura 2000; Popa *et al.*, 2003 and Kang *et al.*, 2007) whose investigated genotypes had also dominant proportions of 2+12 and 5 + 10 subunits in several collections. Contrary to these, Barnlard (2003) and Chaparzadeh *et al.*, (2008) mentioned the lower frequency of 5 + 10. Two genotypes in our study had 2+10 sub unit pair that have unknown quality contribution. This is in line with Yan *et al.*, (2007); Solouki & Emamjomeh (2007). These single subunits have no known impact on wheat quality so their lower frequencies are not a matter to worry. However, detailed studies to assess their effects must be conducted in future to solve wheat quality problems.

**Table 1. HMW-GS & quality score for BMQ of wheat varieties bred in Pakistan.**

S. No.	Genotypes	Allelic combination			Quality Score			Total
		Glu-A1	Glu-B1	Glu-D1	Glu-A1	Glu-B1	Glu-D1	
1.	H.D-2009, H.D-2169, H.D-2179, H.D2204 H.D-2236, Shahkar-95, Bays	2*	7+8	2+12	3	3	2	8
2.	H.D-2285, Mexi.Pak-65, Bulbul, Punjab-87, SH-2002,Uqab-2000, Parwaz, Kiran-95, Inqlab 91	2*	17+18	2+12	3	3	2	8
3.	C-217	2*	20/15	2+12	3	1+?	2	6
4.	C-228, C-271	Null	20	2+12	1	1	2	4
5.	WL-711, Abadgar-93, Pavan, Rohtas-90	Null	17+18	5+10	1	3	4	8
6.	Blue silver, H.D2329,MH 97	2*	7+9	2+12	3	2	2	7
7.	S24	2*	17+18	2+12/10	3	3	2+?	8
8.	Pak-81, Bhitai, Mehran-89, Marvi 2000, LU-26, Punjab-76, Punjab-85	1	7+9	5+10	3	2	4	9
9.	Chackwal-86, Chackwal-97	1	7+8	2+12	3	3	2	8
10.	Barani-83, Kohsar-95, Takbeer	1	7+8	5+10	3	3	4	10
11.	Kohistan-97	Null	7+9	5+10	1	2	4	7
12.	Rawal-87, Wafaq, Saleem-2000, Seher, Bakhtwar-92, Kauz, Marwat, Satluj	2*	7+9	5+10	3	2	4	9
13.	Marghalla-97	2*	13+16	5+10	3	3	4	10
14.	Sindh-81, Pasban-90	1	13+16	5+10	3	3	4	10
15.	Fakhr-e-Sarhad,Yacora-70, Sandal , FD-85	1	17+18	5+10	3	3	4	10
16.	Khirman, Soghat-90, Crows, Zardana, Bhakar 2000, Naeem	2*	17+18	5+10	3	3	4	10
17.	C-518, C-591	Null	20	2+12	1	1	2	4
18.	Sarsbaz	2*	17+18	2+10	3	3	2	8
19.	SA-42 ,Chanab-70 Shafaq, Iqbal	1	17+18	2+12	3	3	2	8
20.	Punjab-96	1	17+18	5+10	3	3	4	10
21.	Nishter, Tatara	Null	7+9	2+12	1	2	2	5
22.	Bhakar-2002	Null	7+8	2+10	1	3	2	8
23.	FD-83, Kohinoor	1	7+9	2+12	3	2	2	7
24.	Mynas	2*	17+18,21	2+12	3	3+?	2	8
25.	AS-2002	Null	17+18	2+12	1	3	2	6

?=Sub units with unknown quality scores



**Note:** lane 1: Gabo, lane 2: Holdfast, lane 3: Chinese spring, lane 4: MH-97, lane 5: WL-711, lane 6: C-271, lane 7: C-228, lane 8: C-217, lane 9: HD-2329, lane 10: HD-2285, lane 11:HD-2236, lane 12: HD-2204, lane 13: HD-2179, lane 14: HD-2169, lane 15: HD- 2009

**Note.** Lane 1- Marquis, Lane 2- Chinese spring, Lane 3- Gabo, Lane 4- Crow’s, Lane 5- Myna’s, Lane 6- Satluj, Lane 7- Parwaz-94, Lane 8- Blue silver, Lane 9- Naeem-82, Lane 10-Marker

**Fig. 1.** SDS-PAGE for HMW-GS in the experimental wheat genotypes.

Seed proteins are product of many genes in genome of species and their analysis can provide usefull information about evolutionary relationships and genome copmplexicity in species. The dissection of allelic variation into genomic contribution revealed that B gnome proved to be a source of genetic variability however, highest quality contribution is through D genome.

Bread-making quality is a complex trait influenced by the environment. However, using HMW glutenin subunits, it is possible to evaluate the bread-making quality potential of wheat genotypes. Highest quality score of 10 or 9 and generally good quality score of 8 was found in tested genotypes. Overall, bread making quality of our collections was better because only some

varieties and mostly land races were found to have a score of 5 or less. The study differed from Schuster *et al.*, (1997) who suggested generally low bread-making quality of the evaluated wheat germplasm. The genotypes having lower proportion of good quality score indicates that varieties have been developed without utilizing the complete knowledge of glutenin subunits composition and their contribution towards bread making quality.

According to cluster analysis, the genotypes were genetically less diverse. The dendrogram elucidated no defined cluster identifying geographical or institutional distribution except for genotypes of indian origin that fall into same cluster and land races forming a close group. However, Nevo *et al.*, (1987) described that composition of subunits in any region

had adaptive value with respect to eco-geographic condition. Some of the genotypes formed clusters with 100% genetic similarity showing tight linkages and thus confirmed the utilization of common exotic breeding lines or sharing of breeding materials at different research stations, conferring loss of genetic diversity. In order to stop genetic erosion, it is necessary to preserve the local wheat germplasm.

The present study determines allelic variants of HMW-GS that correlates strongly with high BMQ. The production of a

new wheat cultivar not only demands a lot of effort but also costly and time-consuming. HMW-GS can be exploited by wheat breeders as biochemical marker for screening wheat germplasm with improved bread-making quality, developing uniformity and improving heterogeneous cultivars by means of selection for the best genotypes. The genotypes with good quality subunits could be combined through different breeding procedures for the production of high quality wheat.

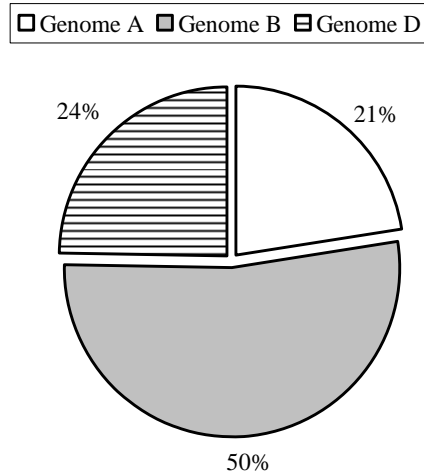


Fig. 3. Percent contribution of wheat genomes towards allelic variation of HMW-GS.

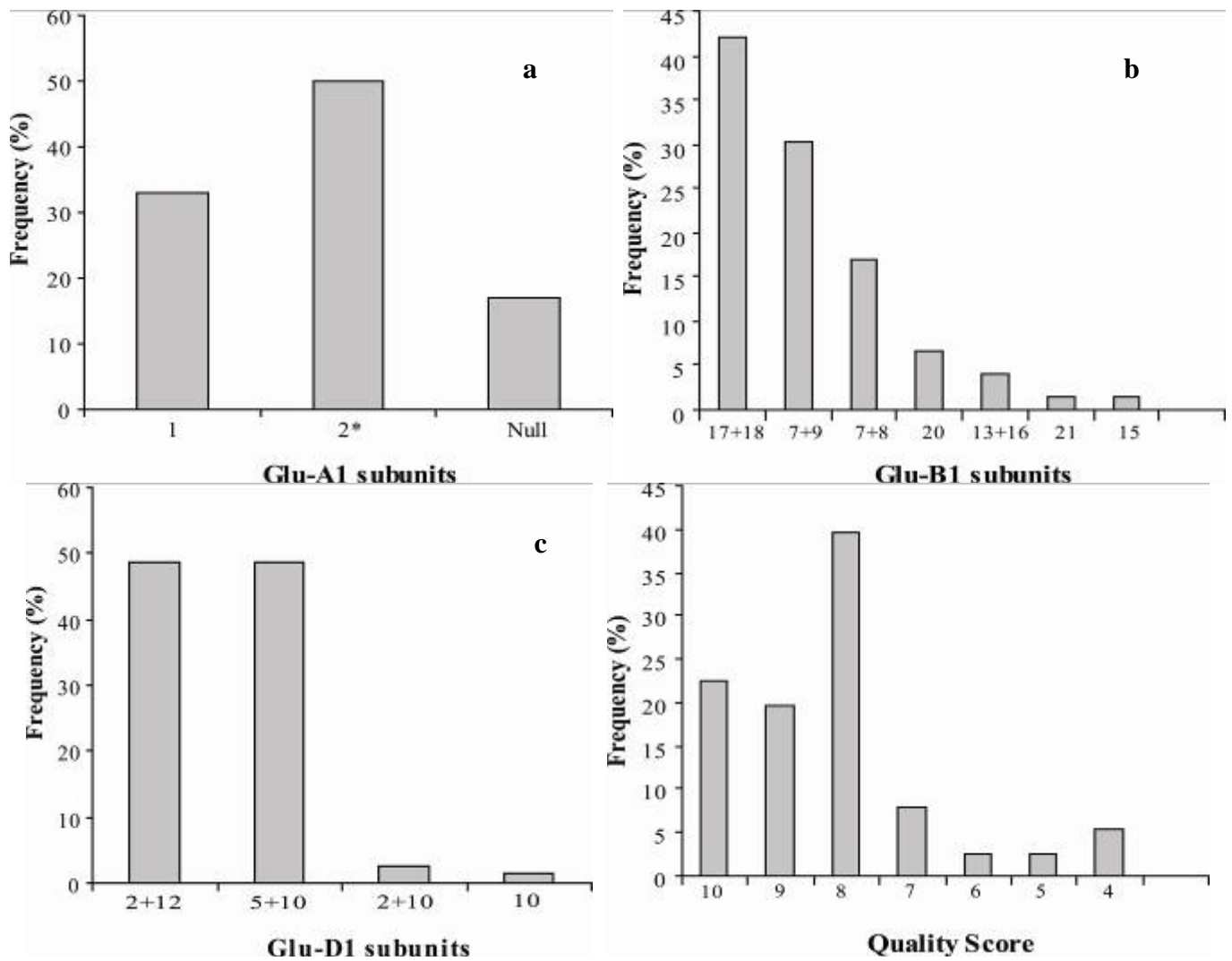


Fig. 2. Frequency of different alleles encoded by Glu-A1 (a), Glu-B1 (b) Glu-D1(c), in wheat genotypes.

Fig. 4. Percentage of quality scores fixed in Pakistani germplasm.

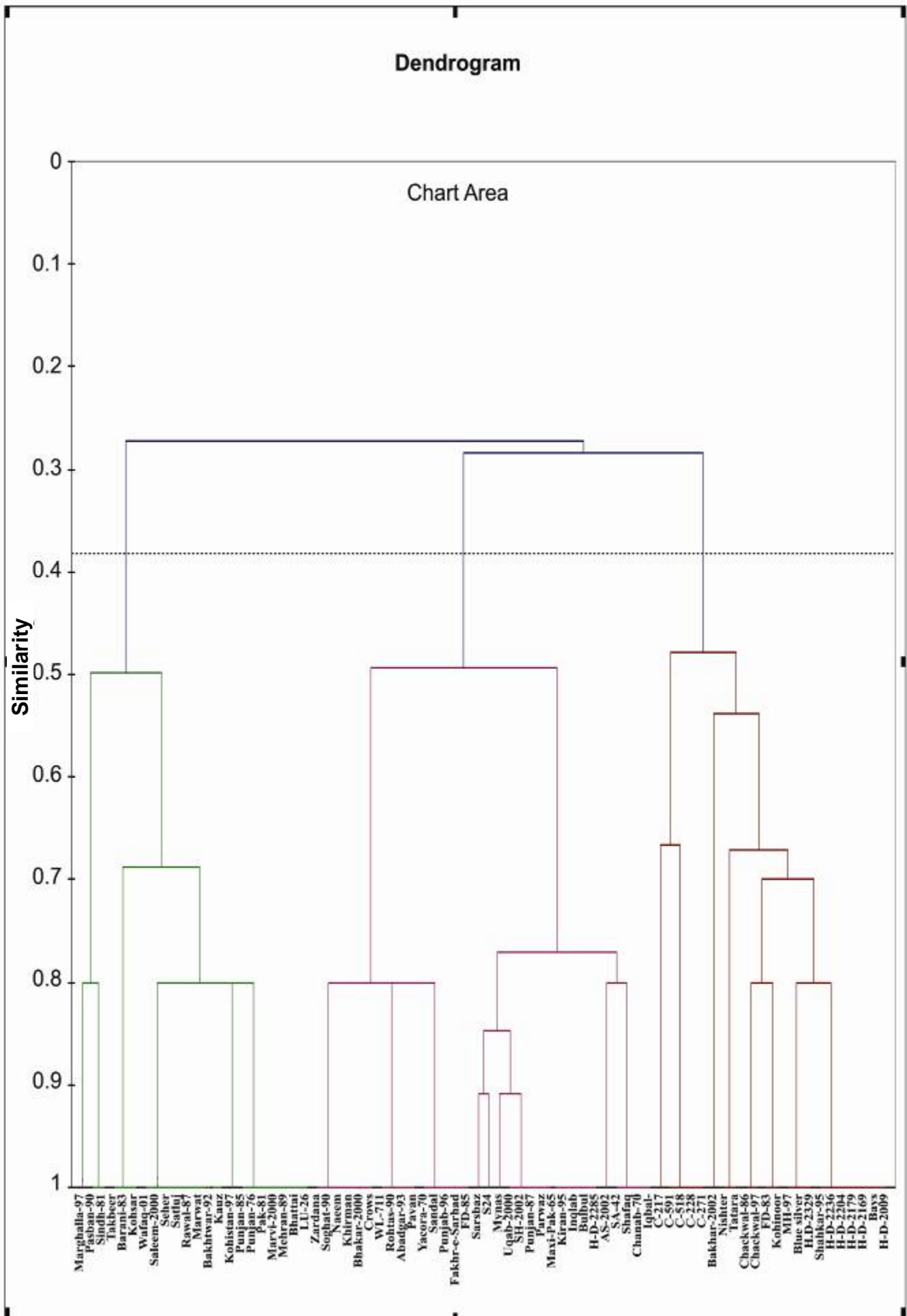


Fig. 5. Dendrogram of 76 wheat varieties/landraces derived from UPGMA cluster analysis based on genetic similarities.

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