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# GLUTENIN SUBUNITS, GLIADIN PATTERNS AND GLUTOPEAK CHARACTERISTICS OF TURKEY'S DOUBLED HAPLOID WHEAT LINES

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**Abstract**

In this study, high molecular weight glutenin subunits (HMW-GS), gliadin patterns, some bread-making quality parameters of doubled haploid wheat lines and their relationships were examined. It appeared from the dendrogram, drawn according to gliadin patterns that there was a variation between genotypes. Additionally, γ-, β- and α-gliadin units were correlated with gluten quality. HMW-GS compositions of doubled haploid lines were very diverse. Subunit 5+10 related high bread-making quality potential was observed in 59 genotypes and the Glu-1 scores changed from 8 to10. This indicates that these genotypes may have a great potential for high bread-making quality. In the study, positive correlations were found among the Glu-1 loci and/or score and the gluten strength characteristics and the SDS sedimentation test. Those doubled haploid population has an important potential in elaboration the contributions made by a number of diverse high molecular weight (HMW) glutenin alleles to the gluten strength characteristics in a set of homogeneous lines of wheat, espicially DNA-based researches for example QTL mapping, marker development. This study and information gained from future researches will increase awareness among wheat quality breeders.

**Key words:** Bread-making quality, Glu-1 score, Gluten strength, Protein content.

## Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most produced and consumed cereal crops in the world and it provides almost half of calories and protein need required for human nutrition (Brush, 1995). For this reason, continuity of production of wheat which has good protein quantity and quality must be ensured.

Protein quality of wheat can be determined by sedimentation (Zeleny, 1947), gluten amount and index value tests (Perten, 1990). Gluten is the most important parameter determining protein quality. The two major protein gluten sub-units are gliadin and glutenin which are essential to bread-making performance of wheat flour.

Gliadin is a significant agent to regulate viscoelastic characteristics of gluten (Xu *et al*., 2007) and divided into four groups which are called α-, β-, γ- and ω-gliadins according to their mobility in gel electrophoresis. In addition, it can be identified by band pattern which is genotype-specific by gliadin electrophoresis and not affected by environmental conditions of cultivars (Bushuk & Zillman, 1978).

Glutenins occur of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (GS) according to their mobility on SDS-PAGE. The HMW-GS are encoded by Glu-1 loci located on the long arm of homoeologous group-1 chromosomes (Payne & Lawrence, 1983); while the LMW-GS are encoded by Glu-3 loci on the short arm of the same chromosomes (Singh & Shepherd, 1988). The role of HMW-GS on dough strength is clear, while LMW glutenin subunits also play a significant role on dough viscosity and formation of large polymers (Cornish *et al*., 2001).

HMW-GS band patterns are valuable distinction tools for specific quality wheat breeding programs (Gianibelli *et al*., 2001). For this reason, it is required that genotypes should be identified with regard to HMW-GS along with more detailed data, and they should be associated with important quality analysis. Wheat breeding programs on bread-making quality is focused on generally Glu-1 allele's scores. These scores are based only on the addition of numerical scores assigned to individual Glu-1 alleles (Payne & Lawrence, 1983). HMW-GS are extensively utilized as markers for their diverse quality scores and for selecting the desired quality lines in wheat breeding programs (Flæte & Uhlen, 2003). In addition, breeding strategies including marker-assisted selection involving doubled haploid technology can be successfully utilized for improvement of bread making quality characteristics. Using HMW-GS as molecular markers for screening of haploids would result in reduction in cost and time in quality- focused breeding programs.

This study carried out to examine HMW-GS and gliadin band variations in doubled haploid bread wheat lines and to determine some quality parameters and the relationships between them. In addition, the study aimed to investigate the effect on quality values of allelic combinations among the materials and to identify desirable quality doubled haploid lines are by assigning Glu-1 quality scores.

## Material and Method

Seventy-four doubled haploid lines developed from $F_2$ plants of the different bread wheat crosses using the anther culture method were used to determine the allelic diversity of HMW-GS, gliadin variations and some quality characteristics. Doubled haploid lines were grown at the Transitional Zone Agricultural Research Institute research areas in 2013. Genotypes were sown into plots which were adjusted in 4 rows, 2 m long, spaced 20 cm apart at the mid-October by hand. The plots were fertilized with 80 kg $P_2O_5$ $ha^{-1}$ at sowing and a total of 120 kg N $ha^{-1}$ at sowing, tillering and pre-anthesis stages. Their pedigrees are listed in Table 1.

**Table 1. Pedigrees of 74 DH lines.**

| Lines | Pedigree | Lines | Pedigree | Lines | Pedigree | Lines | Pedigree |
|---|---|---|---|---|---|---|---|
| **DH-1** | 33IBWSN_S-244/Müfitbey F2.15-1 | **DH-20** | 33IBWSN_S-244/Müfitbey F2.15-19-1 | **DH-39** | Yıldız98/Soyer02 F2.3-4 | **DH-58** | Sönmez/Soyer F2.72-1 |
| **DH-2** | 33IBWSN_S-244/Müfitbey F2.15-2 | **DH-21** | 33IBWSN_S-244/Müfitbey F2.15-19-2 | **DH-40** | Yıldız98/Soyer02 F2.3-5 | **DH-59** | Sönmez/Soyer F2.72-2 |
| **DH-3** | 33IBWSN_S-244/Müfitbey F2.15-3 | **DH-22** | 33IBWSN_S-244/Müfitbey F2.15-20 | **DH-41** | Tosunbey/Müfitbey F2.7-1 | **DH-60** | Sönmez/Soyer F2.99-1 |
| **DH-4** | 33IBWSN_S-244/Müfitbey F2.15-4 | **DH-23** | 33IBWSN_S-244/Müfitbey F2.15-21-1 | **DH-42** | Tosunbey/Müfitbey F2.7-2 | **DH-61** | Sönmez/Soyer F2.99-2 |
| **DH-5** | 33IBWSN_S-244/Müfitbey F2.15-5 | **DH-24** | 33IBWSN_S-244/Müfitbey F2.15-21-2 | **DH-43** | Tosunbey/Müfitbey F2.7-3 | **DH-62** | Soyer /Sönmez F2.99-3 |
| **DH-6** | 33IBWSN_S-244/Müfitbey F2.15-6 | **DH-25** | 33IBWSN_S-44/Müfitbey F2.15-22 | **DH-44** | Tosunbey/Müfitbey F2.7-4 | **DH-63** | Soyer/Sönmez F2.123-2 |
| **DH-7** | 33IBWSN_S-244/Müfitbey F2.15-7 | **DH-26** | 33IBWSN_S-44/Müfitbey F2.15-23 | **DH-45** | Soyer02/Müfitbey F2.164-1 | **DH-64** | Soyer/Sönmez F2.123-3 |
| **DH-8** | 33IBWSN_S-244/Müfitbey F2.15-8 | **DH-27** | 33IBWSN_S-44/Müfitbey F2.15-24 | **DH-46** | Soyer02/Müfitbey F2.164-2 | **DH-65** | Soyer/Sönmez F2.226-1 |
| **DH-9** | 33IBWSN_S-244/Müfitbey F2.15-9 | **DH-28** | 33IBWSN_S-44/Müfitbey F2.15-25 | **DH-47** | WAXY 16 2459-1 | **DH-66** | Sönmez/Soyer F2.226-2 |
| **DH-10** | 33IBWSN_S-244/Müfitbey F2.15-10 | **DH-29** | 33IBWSN_S-44/Müfitbey F2.15-26 | **DH-48** | Harmankaya99/MV8 F2.113 | **DH-67** | Soyer/Sönmez F2.226-3 |
| **DH-11** | 33IBWSN_S-244/Müfitbey F2.15-11 | **DH-30** | Müfitbey/Onearly_S-48 F2.67-1 | **DH-49** | Atilla12/2*Müfitbey F2.117 | **DH-68** | Soyer/Sönmez F2.226-4 |
| **DH-12** | 33IBWSN_S-244/Müfitbey F2.15-12-1 | **DH-31** | Müfitbey/Onearly_S-48 F2.67-2 | **DH-50** | EKG15//Tast/SPRW/3/2*ID800994.W/VEE/4/Sönmez01 F2.64 | **DH-69** | Gerek/Sönmez F2.226-5 |
| **DH-13** | 33IBWSN_S-244/Müfitbey F2.15-12-2 | **DH-32** | Müfitbey/Onearly_S-48 F2.67-3 | **DH-51** | Konya2002/Sönmez01 F2.89 | **DH-70** | Soyer/Sönmez F2.226-6 |
| **DH-14** | 33IBWSN_S-244/Müfitbey F2.15-14-1 | **DH-33** | Müfitbey/Onearly_S-48 F2.67-4 | **DH-52** | Zitnica/GK KALASZ//Sönmez01 F2.103 | **DH-71** | Soyer/Sönmez F2.226-7 |
| **DH-15** | 33IBWSN_S-244/Müfitbey F2.15-14-2 | **DH-34** | Müfitbey/Onearly_S-48 F2.67-6 | **DH-53** | F12.71/COC/KAUZ//ALP01/3/Sönmez01 F2.105 | **DH-72** | Soyer/Sönmez F2.226-8 |
| **DH-16** | 33IBWSN_S-244/Müfitbey F2.15-15 | **DH-35** | Müfitbey/Onearly_S-48 F2.67-7 | **DH-54** | SOM-6//CA8055/GRK/3/Sönmez01 F2.117 | **DH-73** | Soyer/Sönmez F2.226-9 |
| **DH-17** | 33IBWSN_S-244/Müfitbey F2.15-16 | **DH-36** | Müfitbey/Onearly_S-48 F2.67-8 | **DH-55** | Agrı/NAC//MLT/3/SOM-6/4/Sultan95/5/ Sönmez01 F2.122 | **DH-74** | Soyer/Sönmez F2.226-10 |
| **DH-18** | 33IBWSN_S-244/Müfitbey F2.15-17 | **DH-37** | Yıldız98/Soyer02 F2.3-2 | **DH-56** | Sönmez/Soyer F2.5-1 | | |
| **DH-19** | 33IBWSN_S-244/Müfitbey F2.15-18 | **DH-38** | Yıldız98/Soyer02 F2.3-3 | **DH-57** | Sönmez/Soyer F2.5-2 | | |

**Analysis of HMW-GS in SDS-PAGE jel:** The compound of the HMW-glutenin fraction was examined at electrophoresis by the method of Payne *et al*. (1980). Firstly, a single grain was grinded in a porcelain mortar and 8 mg of ground samples were transferred into a 1–1.5 ml centrifuge tube with 0.2 ml buffer solution. Then, the samples, were previously extracted with a solution consisted of 10 ml of buffer solution, 4.2 ml of pure water and 0.75 ml of 2-mercaptoetanol, was left for 2 h at room temperature and then boiled along 2 min and centrifuged. Electrophoresis was performed in the SDS-PAGE using 4% stacking gel, 8.7% for separating gel. Four standards (Courtot, Kadet, Chinese Spring, Insignia) containing known HMW-GS were used for comparison and data recording. HMW-glutenins composition was scored according to Payne's catalogue. Payne & Lawrence (1983) named HMW glutenins gene loci as Glu-A1, Glu-1B and Glu-1D and proteins subunits as 0, 1, 2*, 2 + 12, 5 + 10, 6 + 8, 7 + 9 and 17+18 ect. Each of gluten subunits is given of its point determined. The proteins combinations subunit was graded in points between from 3 to 10.

**Analysis of gliadins in A-PAGE jel:** 20 µg flour from single seed was used for extraction. 100 µL of 70% ethanol was added to the flour and incubated at 24°C for 30 minutes with brief vortexing at 10 minutes interval. The tubes were centrifuged at 12.000 rpm during 5 minutes and the supernatant was collected. 26 µL of the dilution buffer was added to 20 µL of the supernatant was obtained. 6µL of the sample was loaded into the well of the gel for A-PAGE separation of gliadins. The electrophorograms of the gliadin proteins are conventionally evaluated according to Bushuk & Zillman (1978) based on relative mobility. The relative mobility (Rm) values are calculated with reference to a specific band, designated 50, of the variety 'Neepawa'. The Rm value of a given band is obtained by dividing the distance the band migrated from the origin by the distance band 50 migrated from the origin and then multiplying this value by 50. The value obtained is the Rm for that band.

**Quality characteristics:** Protein content and PSI thickness value analysis was determined by FOSS NIRS 6500 spectroscopy device according to ICC 105/1 method (Anon., 1980) and Williams *et al*. (1986). SDS (sodium dodecyl sulfate) sedimentation value analyses was performed by Pena *et al*. (1990) and gluten aggregation characteristics were analysed by the method Melnyk *et al*. (2011) using Brabender GlutoPeak device (Brabender GmbH and Co KG, Duisburg, Germany). Torque maximum expressed in Brabender Equivalents ( BEM), maximum torque before breaking down (BM) and peak maximum time (PM) value is pointed out gluten maximum strength, the value of before 15s of maximum strength and the value of after 15s of maximum strength, respectively (Chandi & Seetharaman, 2012). If BEM and BM values are high, gluten strength is also high. The reason of evaluation GlutoPeak characteristics instead of wet gluten rate and index is less than enough material.

**Statistical analysis:** Mean of values, standard error and correlations between quality characteristics and single glutenin allel and gliadin band groups were assessed by Pearson's test using IBM SPSS 20.0 software (SPSS Inc., Chicago, IL). For qualitative separation of gliadin band series was used R 2.13.1 software heatmap procedure (Anon., 2012). HMW-GS was scored using by MASWheat training tools.

## Results and Discussion

Gluto-Peak characteristics and conventional bread-making quality tests such as protein content, PSI, SDS sedimentation value, were examined in 74 genotypes. Results are given in the Table 2. Table 2 showed that there was a wide variation among genotypes in terms of these quality characteristics examined. The highest protein content value was determined in DH-26 genotype with 15.81% while DH-65 genotype gave the lowest protein content value. PSI values of 74 genotypes changed from 58.91 to 93.40 (Table 2). Since the highest SDS sedimentation value was determined in DH-8 with 14.50 ml, it can be accepted to have good bread-making quality. However, DH-50 with the lowest SDS sedimentation value thus had the poor bread-making quality (Table 2). DH-37 and DH-41 genotpes had the greater Gluto-Peak characteristics. As evaluation was based on the quality characteristics, DH-8, DH-26, DH-37 and DH-41 were the best genotypes. These genotypes had high quality scores and desirable HMW-GS patterns as well as (Fig. 1).

**Table 2. Minimum, maximum and mean values of 74 DH lines and their variation.**

| | Minimum | Maximum | Mean | Variance | CV% |
|---|---|---|---|---|---|
| PC | 12,61 (DH-65) | 15,81 (DH-26) | 14,08 ± 0,17 | 0,77 | 6,22 |
| PSI | 58,91 (DH-26) | 93,40 (DH-37) | 77,12 ± 1,82 | 86,03 | 12,03 |
| BEM | 24 (DH-16) | 36 (DH-37) | 30,81 ± 0,66 | 11,36 | 10,94 |
| BM | 23 (DH-66) | 46 (DH-41) | 32,85 ± 1,05 | 28,45 | 16,24 |
| PM | 22 (DH-11) | 33 (DH-41) | 27,54 ± 0,62 | 9,86 | 11,40 |
| SDS | 6,0 (DH-50) | 14,50 (DH-8) | 10,43 ± 0,42 | 4,67 | 20,72 |

Although bread-making quality is a complex character affected by the environment, the bread-making quality potential of wheat genotypes can be evaluated by utilization HMW-GS in the first generation of breeding programs (Schuster *et al*., 1997). Thus, HMW-GS could be considered as a molecular marker, which make the selection of favourable genotypes, or eliminate the undesirable ones. HMW-GS composition and the Glu-1 score of 74 doubled haploid lines are presented in Figure 1. Five of 74 lines had null, 31 lines had 1 and 38 lines had 2* allele at the Glu-A1 locus. In addition to this, at Glu-B1 locus, 6+8 allele for 3 genotypes and 7 allele for 8 genotypes, 7+8 allele for 22 genotypes, 7+9 allele for 29 genotypes, 17+18 allele for 12 genotypes were observed. At the Glu-D1 locus, 2+12 and 5+10 alleles were identified in 15 and 59 genotypes, respectively (Fig. 1). Similarly, Tabasum *et al*. (2011) and Ali *et al*. (2013) mentioned the higher frequency of 5 + 10.

Payne & Lawrence (1983) have defined a score for each HMW glutenin subunit, in this way, the HMW glutenin subunits could be used a statistical evaluation of the amount of variation for bread-making quality. Generally, the subunits (2+12 and 5+10) coded by genome D are used as molecular markers for the desirable bread-making quality in wheat. If there is the subunit 5 + 10, it is considered that genotypes have high bread-making quality potential, and a maximum score is given them (Payne & Lawrence, 1983). At the same time, it is accepted that the subunit 2 + 12 is related with poor bread-making quality potential (Dessalegn *et al*., 2011). In this research, the majority of genotypes examined (59) presented subunit 5 + 10 and their scores varied from 8 to 10. Furthermore, the scores of subunit 2+12 for 15 genotypes varied from 4 to 8. Since the 5 + 10 subunits are mostly combined with the 7 + 8 and 7 + 9 subunits, it could be said that 44 of the 74 genotypes got high score. Eighteen diverse subunit combinations at the three Glu-1 loci were detected amongst the 74 doubled haploid lines examined (Fig. 1). The patterns of 2*,7+8,5+10 and 1,7+9,5+10 which were the most detected patterns were observed for 14 genotypes and for 13 genotypes, respectively. HMW-GS and patterns with high quality scores were recognized in the plurality of the lines (Fig. 1). Twenty-nine of the genotypes had the maximal likely score of 10; twenty-four of the varieties had a score of 9. This condition indicated that genotypes examined had a major potential in high bread-making quality.

It is difficult to perform conventional bread-making quality tests in doubled haploid plants; because there is a small amount seed and these tests require seeds in large quantities. So, in the early stages of the double haploid plant production or early generations in breeding programs, it is not possible to test the bread-making quality of the lines in this way. But, determining of HMW-GS helps to selection of desirable quality genotypes in this stage. If the correlation between the conventional quality characteristics and HMW-GS was determined, the selection assisted by the HMW-GS would be facilitated during these segregating generations. Knowing the mean value of single allele glutenin may give us an idea about correlation between the conventional quality characteristics and HMW-GS. Mean comparisons (Table 3) pointed out that 7+8 sub-units had the furthest contribution for protein content. As consistent with our findings, Carrillo *et al*. (1990) remarked that in Glu-B1 gene locus, 7+8 sub-units was major contributor in comparison to other sub-units to protein content. Subunit 7+8 had the greatest contribution for SDS sedimentation as the protein content. The most important contribution to the characteristics associated with gluten strength (BEM, BM, PM) was provided by 17+18 band (Table 3).

Clustering diagram of gliadin band variations and gliadin band numbers of doubled haploid lines are presented in Figures 2 and 3. In cluster analysis done according to presence or absence of gliadin bands, these 57 bands were divided into three groups; including 30 in the first group, 13 in the second group and 14 in the third group. The genotypes used in clustering analysis based on gliadin band patterns were divided into five groups. The numbers of genotypes in each group were almost equal (Fig. 2). The results showed that the genotypes carried the same gliadin bands were doubled haploid lines derived from same crosses. Nevertheless, there were differences with regard to some bands. It was observed that the genotypes in the same cluster group had similar quality characteristics (Fig. 2).

Gliadin band number varied between 8 (DH-34) to 27 (DH-18). Among 57 different gliadin patterns, there are 35 bands in ω-gliadin region, 9 bands in γ- and β-gliadin region and 4 bands in α- gliadin region (Fig. 3). While ω-gliadins are divided into several subgroups, α and β gliadins are often grouped as one class of α/β gliadins because of analogous protein structures (Wieser, 1991). In this study, the majority of the bands detected were in the ω-gliadin region, similar to the results of Nizar (2002), Branlard *et al*. (2003) and Baytekin *et al*. (2014).

**Table 3. Mean values of quality characteristics according to single glutenin allele.**

| Locus | Subunit | Number | PC | PSI | BEM | BM | PM | SDS |
|---|---|---|---|---|---|---|---|---|
| GLU- A1 | Null | 5 | 13,38 ± 0,43 | 74,77 ± 2,91 | 25,00 ± 1,26 | 25,00 ± 1,42 | 23,00 ± 1,24 | 6,00 ± 0,81 |
| | 1 | 31 | 14,04 ± 0,25 | 78,38 ± 2,54 | 31,14 ± 0,97 | 33,00 ± 1,15 | 27,79 ± 0,83 | 10,30 ± 0,54 |
| | 2* | 38 | 14,18 ± 0,25 | 75,73 ± 2,90 | 30,91 ± 0,84 | 33,36 ± 1,15 | 27,64 ± 0,96 | 11,00 ± 0,60 |
| GLU- B1 | 6+8 | 3 | 13,58 ± 0,13 | 81,74 ± 2,87 | 27,00 ± 0,74 | 30,00 ± 1,25 | 23,00 ± 1,03 | 8,00 ± 0,42 |
| | 7 | 4 | 13,42 ± 0,35 | 78,41 ± 3,64 | 26,50 ± 1,50 | 24,00 ± 1,00 | 24,50 ± 1,50 | 7,25 ± 1,25 |
| | 7+8 | 22 | 14,65 ± 0,48 | 73,25 ± 3,03 | 32,00 ± 1,30 | 33,00 ± 1,30 | 29,20 ± 1,46 | 12,9 ± 0,49 |
| | 7+9 | 29 | 14,07 ± 0,23 | 77,31 ± 2,96 | 29,85 ± 0,74 | 34,08 ± 1,30 | 27,00 ± 0,80 | 9,81 ± 0,50 |
| | 17+18 | 12 | 13,89 ± 0,41 | 79,05 ± 4,79 | 34,60 ± 0,93 | 33,60 ± 3,36 | 29,40 ± 1,12 | 11,35 ± 0,46 |
| GLU-D1 | 5+10 | 59 | 14,05 ± 0,20 | 76,53 ± 2,06 | 30,68 ± 0,79 | 32,36 ± 1,19 | 27,36 ± 0,72 | 10,94 ± 0,39 |
| | 2+12 | 15 | 14,19 ± 0,31 | 80,36 ± 3,39 | 31,50 ± 0,87 | 35,50 ± 1,50 | 28,50 ± 0,50 | 7,63 ± 0,83 |

+PC: protein content, PSI: grain hardness, BEM: maximum gluten strength, BM: the value of before 15s of maximum strength, PM: the value of after 15s of maximum strength, SDS: sedimentation value

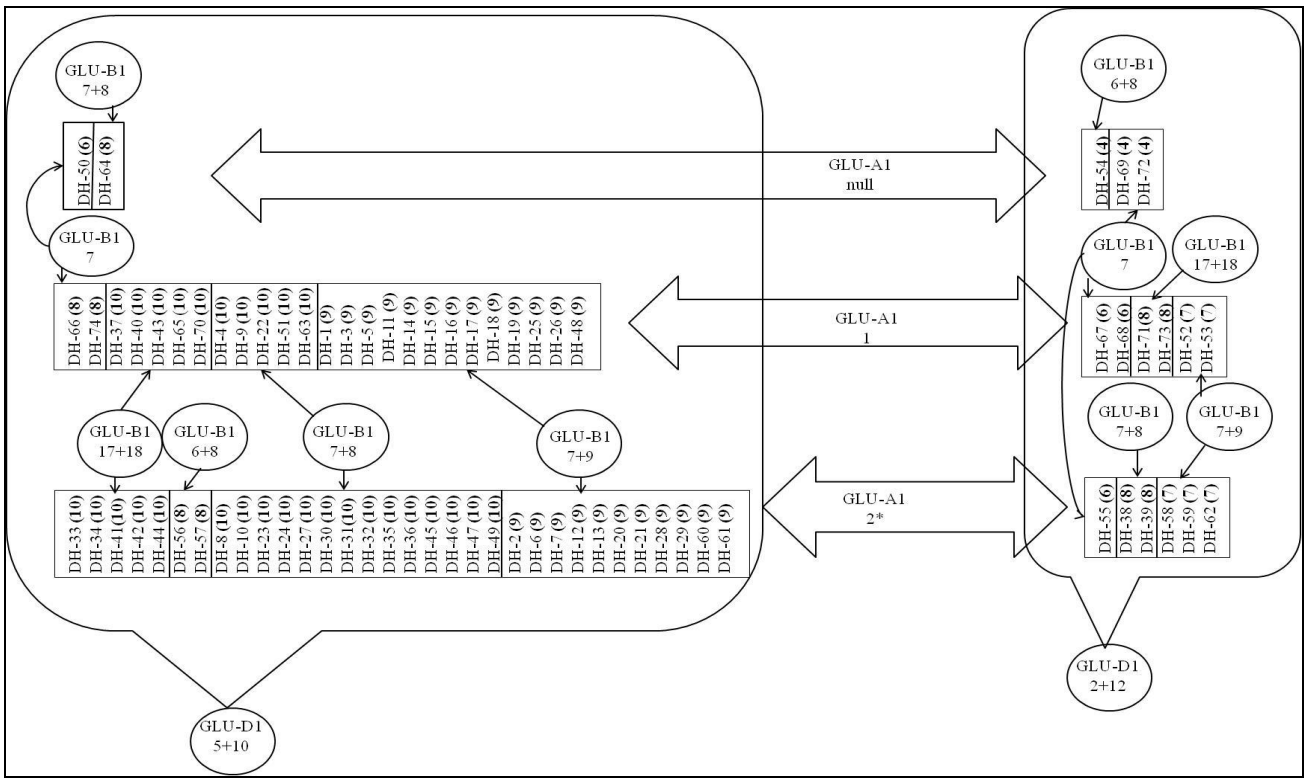


Fig. 1. Variation of doubled haploid lines according to HMW-GS. **(**Glu-1 score was presented in parenthesis)

**Table 4. Correlations between quality characteristics and HMW-GS loci and gliadine band group**

| Quality characteristics | Gliadin Band Group | | | | HMW-GS | | | |
|---|---|---|---|---|---|---|---|---|
| | ω | γ | β | α | Glu-A1 | Glu-B 1 | Glu-D 1 | Scor |
| PC | -0,21 | 0,04 | -0,19 | -0,03 | -0,04 | 0,14 | -0,06 | 0,17 |
| PSI | 0,19 | -0,17 | 0,34 | -0,34 | 0,15 | -0,03 | -0,15 | -0,14 |
| BEM | 0,09 | -0,44* | -0,18 | -0,08 | 0,11 | 0,70** | -0,09 | 0,45* |
| BM | -0,22 | -0,03 | -0,39* | -0,04 | 0,03 | 0,23 | -0,22 | 0,13 |
| PM | -0,02 | -0,37 | -0,20 | -0,18 | 0,09 | 0,50** | -0,13 | 0,33 |
| SDS | -0,09 | -0,25 | 0,02 | 0,47* | -0,07 | 0,59** | 0,57** | 0,92** |

*p≤0.005, **p≤0.01, PC: protein content, PSI: grain hardness, BEM: maximum gluten strength, BM: the value of before 15s of maximum strength, PM: the value of after 15s of maximum strength, SDS: sedimentation value

Nizar (2002) pointed out the high dense band numbers of genotypes which might be due to its high protein content. However, no significant and positive relationship was found between the band density and protein content in this study. This may be due to the fact that the protein content is affected by environmental conditions. The examined correlation between quality characteristics and gliadin band groups, clearly indicated that γ-gliadins and β -gliadins negatively affected to BEM and BM, respectively. Whereas α -gliadins positively affected to SDS value (Table 4). Marchylo *et al*. (2001), Pena *et al*. (2005) noted that dough strength is correlated with the characteristics mentioned. Moreover, Fido et al. (1997) found that α, β and γ gliadins showed the negative effects on dough strength.

Branlard *et al*. (2001) and Huang *et al*. (2006) reported that HMW-GS loci and protein content were positively correlated. In this study, there were positive correlation between protein content with Glu-B1 loci and Glu-1 score (Table 4). While the non-significant correlation between PSI, BM value and HMW-GS loci or score, a statistically significant correlation between the BEM, PM value and Glu-B1 loci were observed. These values which are associated with gluten strength supported the some researchers' findings (Flæte & Uhlen, 2003; Oak *et al*., 2004; Nazco *et al*., 2014) about association of gluten and Glu-B1 loci. In addition to this, there were positive and significant correlation between Glu-1 score and BEM value (Table 4). BEM value pointed out maximum gluten strength and determination of bread-making quality is important (Marti *et al*., 2015). Because it is associated with Glu-1 score, this score may be utilized for bread-making quality in the first generation of the breeding programs, when quantities of seeds necessary for the gluten test are not available. Payne *et al*. (1981) demonstrated that the associations of HMW-GS and SDS sedimentation values were as an indicator of bread-making quality. Likewise, Branlard & Dardevet (1985) reported that Zeleny sedimentation value had positive correlation with subunits 7+9 and 5+10, and negative with 2+12 also extensibility had correlation with subunits 2* and 17+18. In the study, a positive correlation was found between Glu-B1, Glu-D1 loci, Glu-1 score and sedimentation value (Table 4).

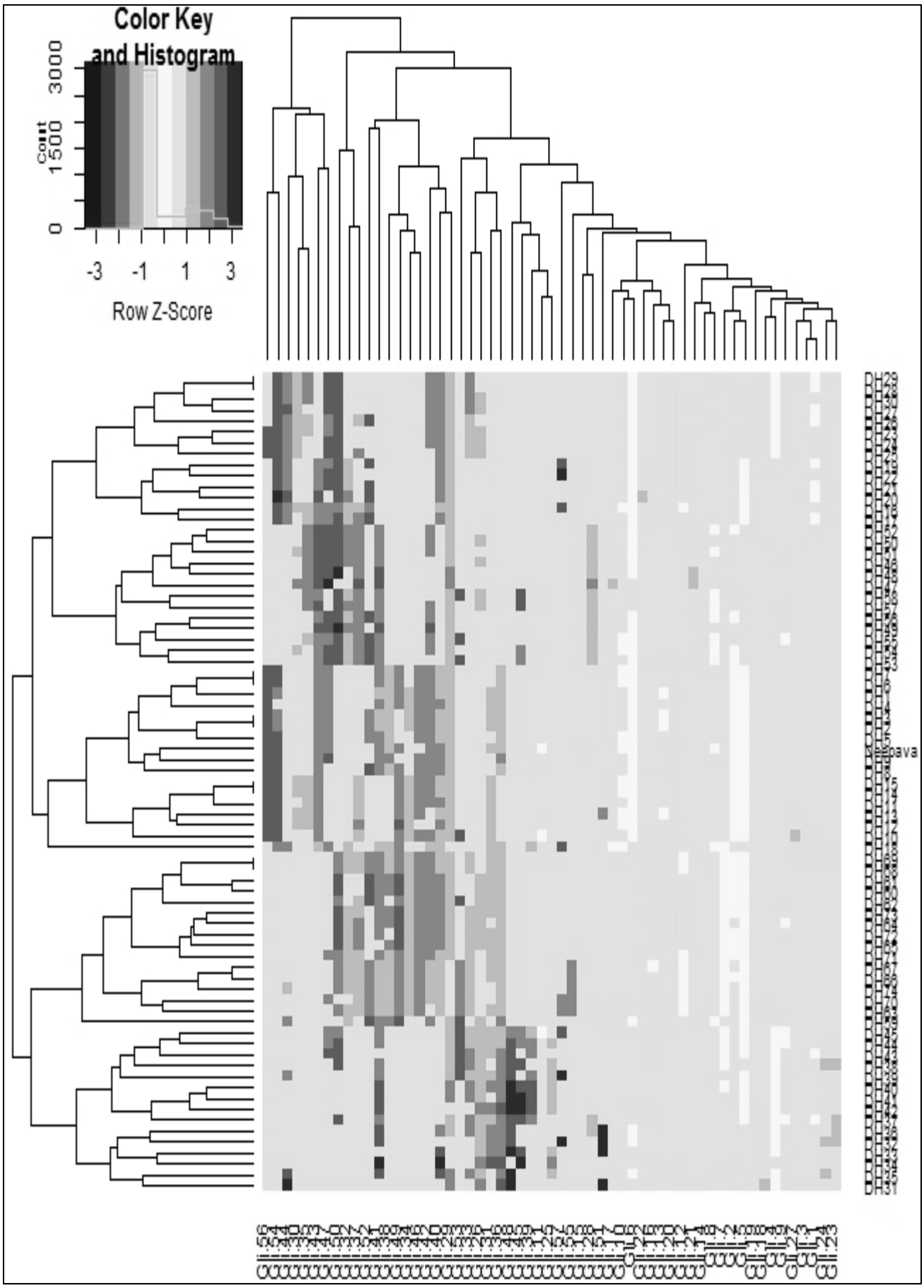


Fig. 2. The two-way cluster diagram (heatmap) based upon their gliadin band diversity for 74 DH lines (dark grey=band exist; light grey=no band).

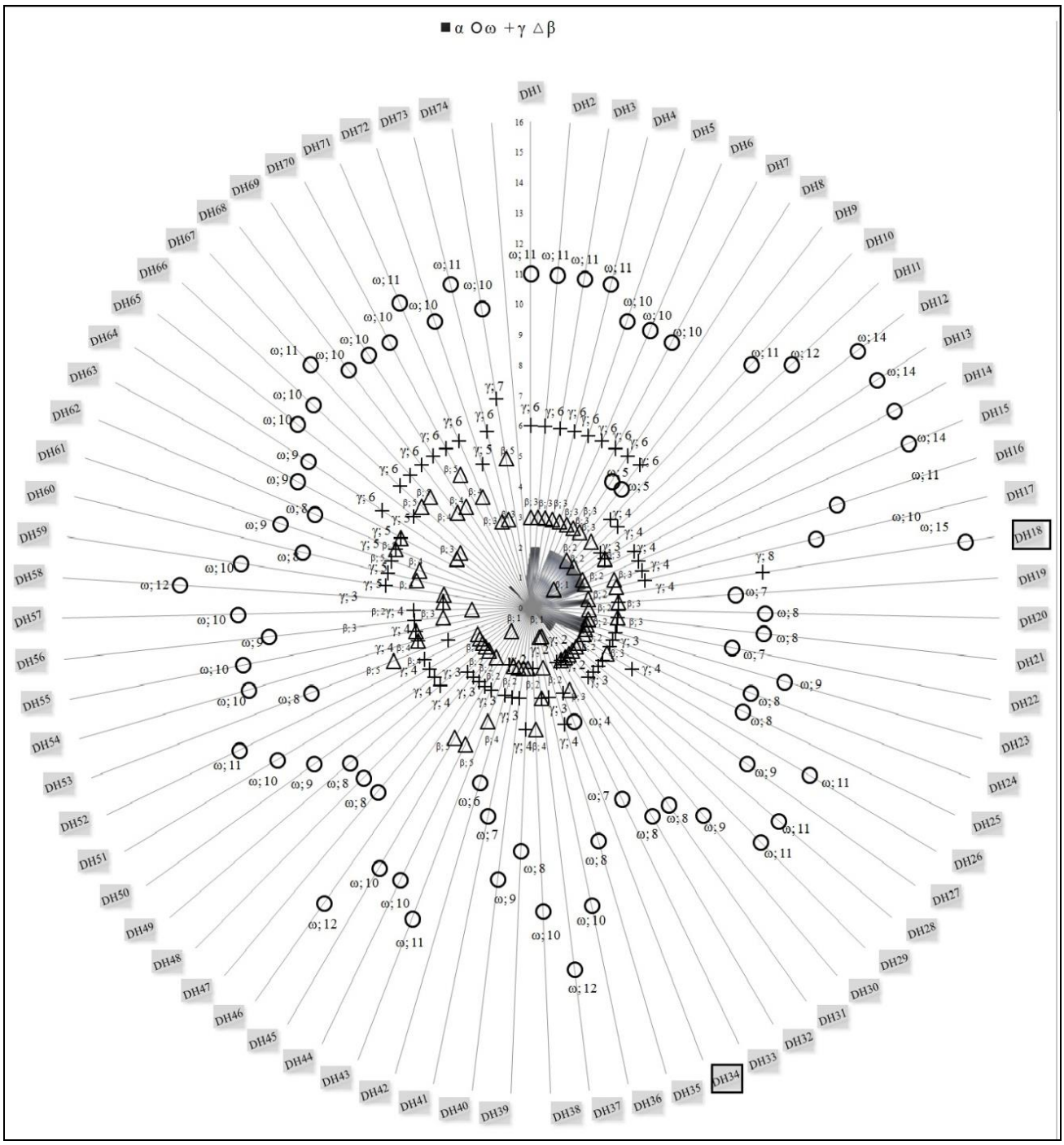


Fig. 3. Gliadin band numbers of 74 DH lines.

## Conclusion

When gliadin band number and dendrogram which was drawn according to gliadin band patterns were examined, it was found that there was a variation between genotypes. Genotypes with same pedigree carried same gliadin bands. These findings were also evident once again that the gliadin bands were the fingerprints of genotypes. Moreover, it was found that γ-, β- and α -gliadins affected to gluten quality.

Since the correlation between the Glu-1 loci and/or score and the gluten strength characteristics and the SDS sedimentation test was positive, the HMW glutenin assisted selection standard can be utilized in those doubled haploid lines for those characteristics. More than half of lines used in this research presented high bread-making quality considering HMW-GS. In conclusion, those doubled haploid lines are an important genetic resource to develop high-quality bread wheat varieties and has a possibility to be selected for good bread making quality. Those doubled haploid lines may be used to investigate further information espicially DNA-based researches (for example QTL mapping, marker development) about the effects of high molecular weight (HMW) glutenin alleles to the gluten strength characteristics. This study and information will be helpful for future researches and also increase awareness of the issue for wheat quality breeders.

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