African Journal of Biotechnology Vol. 9 (27), pp. 4286-4289, 5 July, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Analysis of HMWGS of historical set of Pakistani bread wheat varieties using SDS-PAGE

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Accepted 6 November, 2009

In the present study an attempt has been made to characterize thirty bread-wheat varieties of Pakistan for High Molecular Weight Glutenin Subunits (HMW- GS). Glutenin proteins form a continuous proteinaceous matrix in the cells and form a continuous viscoelastic network during the mixing process of dough development. Glutenin consists of High Molecular Weight (HMW) and Low Molecular Weight (LMW) subunits. The HMW Glutenin Subunits (HMW-GS) are chiefly vital for determining dough elasticity. The core objective of our research work was to inspect the glutenin subunits by sodium dodecyl-sulfate polyacrylamide gel-electrophoresis (SDS-PAGE) and compare the banding pattern with Chinese Spring High-Molecular-Weight Glutenin Subunits (HMW-GS). The bands were numbered according to Payne's numbering system and varieties were accordingly assigned theoretical quality scores. All the tested varieties indicated null allele for *gluA1* locus, 17 + 18 for *gluB1* locus and 2 + 12 for *gluD1* locus. This result indicating that all varieties have similar bread making quality alleles at HMWGS loci. The varieties containing 5 + 10 HMWGS allele at *gluD1* locus have better bread making quality. Better bread making wheat varieties may be produced by crossing the local varieties of *gluA1* locus, 17 + 18 for *gluB1* locus and 2 + 12 for *gluD1* locus with 5 + 10 HMWGS allele at *gluD1* locus.

Key words: Wheat varieties, SDS-PAGE, HMWGS, Payne's numbering.

INTRODUCTION

In Pakistan, the wheat (*Triticum aestivum* L.) faces the dual menace of biotic as well as abiotic stresses, which result in lower yields. It has been suggested that information about germplasm diversity and genetic relatedness among elite breeding material is a fundamental element in plant breeding. The value of diverse genetic base for resistance to diseases has recently been advocated in cereals (Garret and Mundt, 1999). Secondly, the future breeding program also depends upon the availability of genetic variability to increase the gain in productivity. Hence to achieve a level of self-sufficiency and sustainability, there is a need to develop cultivars with diverse genetic base.

Most of the newly released wheat cultivars of Pakistan have been developed by crossing exotic parents or the genotypes derived from the exotic material followed by the selection of superior genotypes. The tendency to use of extensive similar parents in breeding programme has led to a concern of lack of genetic diversity (Fouilloux and Bannerot, 1988). It is Glutenins that confer elasticity to dough (Payne et al., 1984). The earlier studies of Bietz and Wall (1972) showed that two types of subunits were present; the low molecular weight (10,000 - 70,000 Da) and the high molecular weight glutenin subunits (80, 000 - 130,000 Da). The high molecular weight (HMW) subunits of wheat glutenin are major determinants of the elastic properties of gluten that allow the use of wheat dough's to make bread, pasta and a range of other foods (Benmoussa et al., 2000). There are both quantitative and qualitative effects of HMW subunits on the quality of the grain. High Molecular Weight Glutenin Subunit (HMW-GS) are encoded at the Glu-1 loci on the long arms of group 1 chromosomes (Glu-A1, Glu-B1 and Glu-D1) (Payne et al., 1980).

Electrophoretic studies have indicated appreciable

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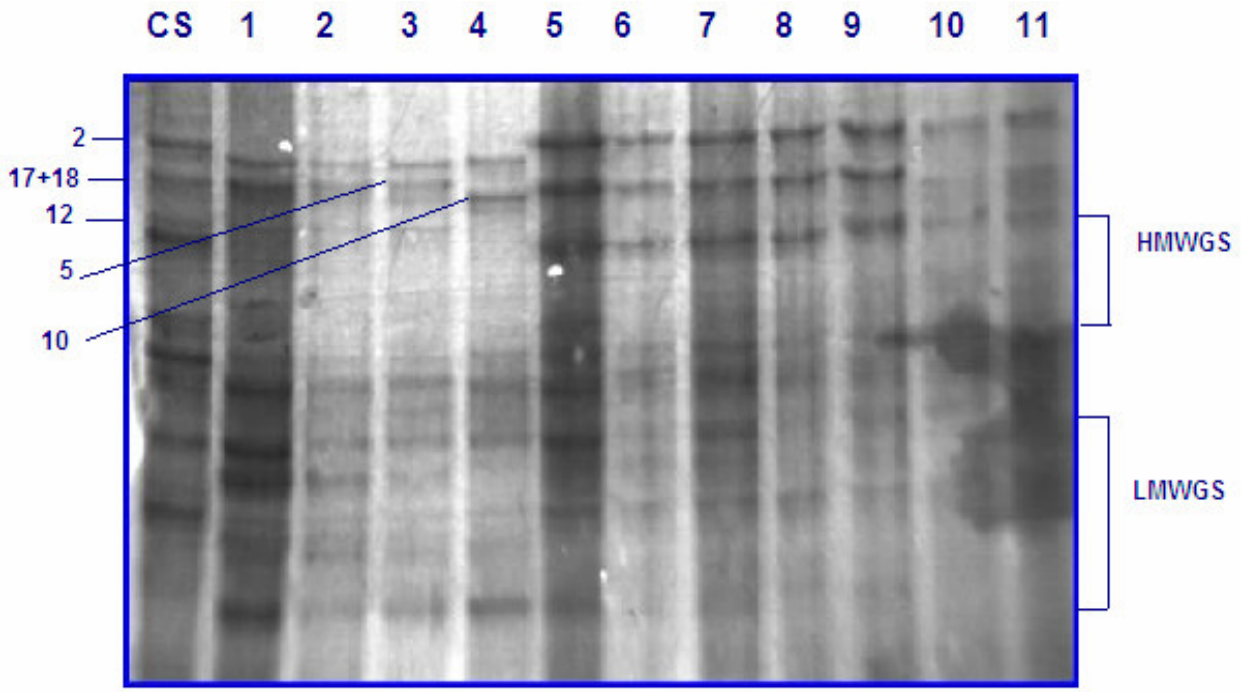

Figure 1. HMWGS pattern of Chinese spring, synthetic hexaploid wheat lines and test entries used during present study. CS = Chinese spring, 1 - 4 = four different synthetic wheat lines used as positive check for HMWGS 5 + 10 (*glud1* locus), 5 = C-518, 6 = C-591, 7 = C-228, 8 = C-271, 9 = C-273, 10 = Mexi-Pak, 11 = Chenab-70. Different alleles of HMWGS are shown left.

polymorphism in the number and mobility of HMWGS in bread wheat (Lawrence and Shepherd, 1980). Bread wheat contain six different HMW-GS but due to the "silencing" of some of the genes, most common wheat cultivars possess three to five HMW-GS. The Low Molecular Weight-Gluten Subunits (LMW-GS) represents about 33 - 34% of the total seed protein and 60% of total gluten (Bietz and Wall, 1972). The LMW-GS are controlled by genes at the Glu-A3, Glu-B3 and Glu-D3 loci on the short arms of chromosome 1AS, 1BS and 1DS, respectively. Due to extensive polymorphism, these proteins have been widely used for cultivar identification in hexaploid and tetraploid wheat (Payne et al., 1984). Allelic variants differ in the number, mobility and intensity of their components and can be characterized through SDS-PAGE.

The core objective of our research work was to inspect the glutenin subunits by sodium dodecyl-sulfate polyacrylamide gel-electrophoresis (SDS-PAGE) and compare the banding pattern with Chinese Spring High-Molecular-Weight Glutenin Subunit (HMW-GS).

MATERIALS AND METHODS

Plant sample

Wheat varieties were collected from National Agricultural Research Center (NARC) Islamabad, Pakistan. The analysis was conducted at the department of biotechnology and genetic engineering (IBGE) NWFP agricultural university Peshawar, Pakistan.

SDS-PAGE analysis

The variation of the gluten was analyzed by using SDS-PAGE (Damania et al., 1983). For extraction of protein, a single seed was ground to fine powder with mortar and pestle. Total 400 ul sample buffer was added to a 0.01 g (10 mg) seed powder and mixed thoroughly by vortex in an Eppendorf tube (1.5 ml) with a small glass rod. The extraction buffer contained the following final concentration: 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol, kept overnight at 40℃ and centrifuged at 13000 rpm for 10 min. Sample proteins 10 µl was loaded in each well To monitor the movement of the protein in the gel, bromophenol blue (BPB) was used as a tracking dye. Seed protein was analyzed through slab-type SDS-PAGE using 10% polyacrylamide gel. SDS-PAGE of total seed protein was carried out in a discontinuous buffer system following the method of Laemmli (1970). The gels were stained with Coomassie brilliant blue (CBB) and destained till the background became transparent.

Data analysis

Thirty varieties were analyzed for bread making quality, using 10% Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Figures 1, 2 and 3). High Molecular Weight Glutenin Subunits (HMWGS) were studied in detail, because they have major effect on bread making quality (Payne et al., 1981). In all the gels Chinese Spring (CS) variety was used as a standard check to compare HMWGS banding pattern. Banding pattern of Chinese

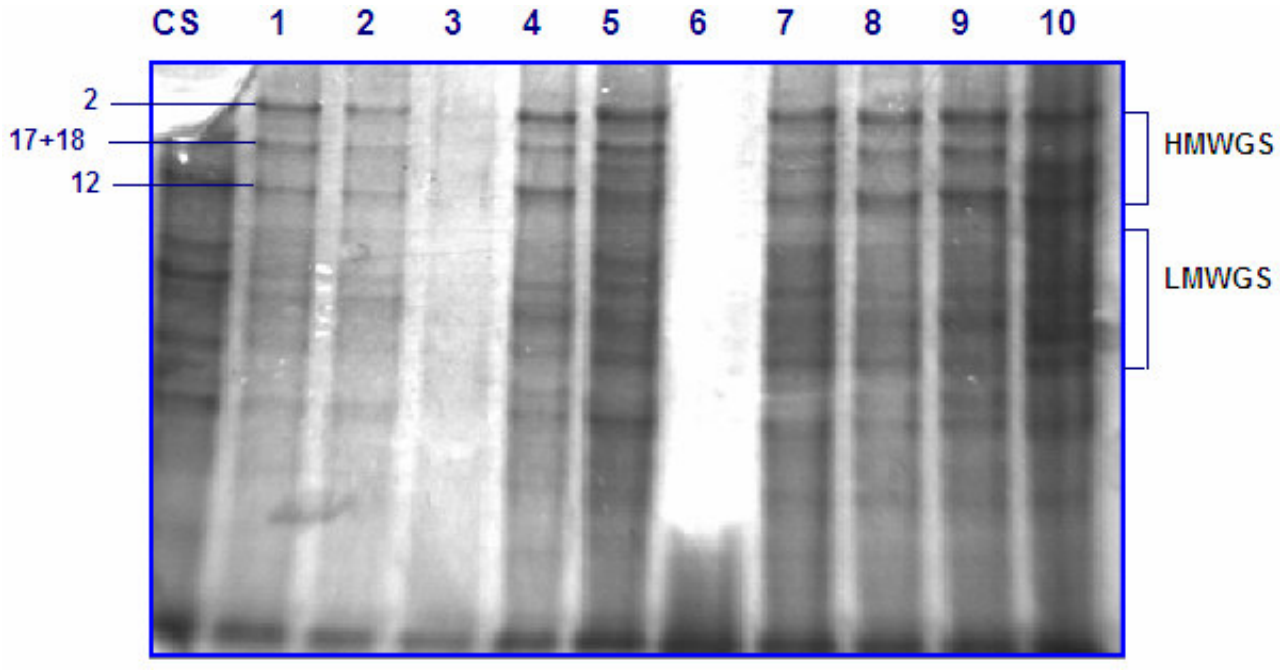

Figure 2. HMWGS pattern Chinese spring and wheat varieties used during present study. CS = Chinese spring, 1 = Barani-70, 2 = Blue silver, 3 = Sonalika, 4 = Yecora, 5 = SA-42, 6 = Pavon, 7 = Sindh-81, 8 = Pak-81, 9 = Punjab-85, 10 = Srsabz. Different alleles of HMWGS are shown left.

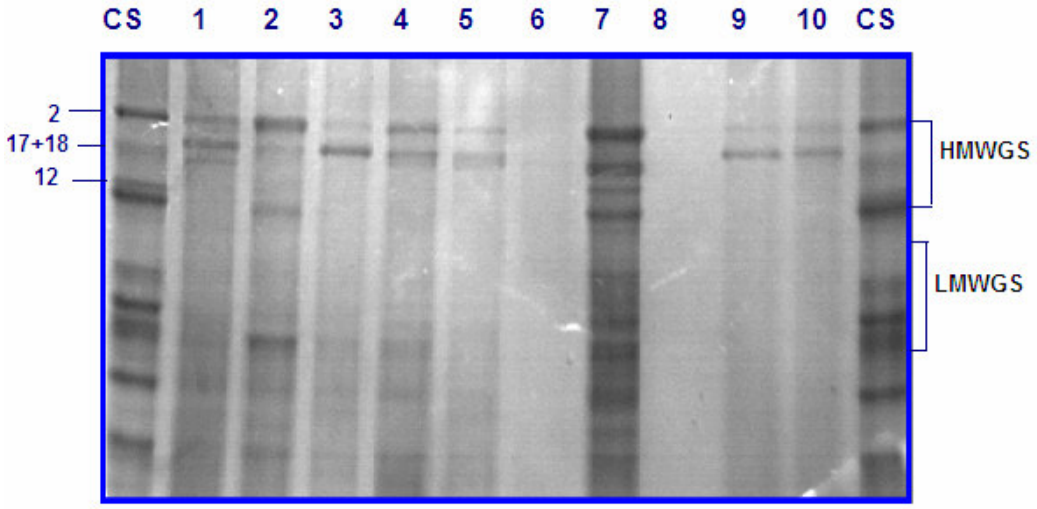

Figure 3. HMWGS pattern Chinese spring and wheat varieties used during present study. CS = Chinese spring, 1 = Shalimar-88, 2 = Pasban-90, 3 = Inqilab-91, 4 = MH-97, 5 = Chakwal 97, 6 = Tatara, 7 = Margalla-99, 8 = Auqab-2000, 9 = G-A 2002, 10 = Momal. Different alleles of HMWGS are shown left.

Spring is '0' for *gluA1* locus, 17 + 18 for *gluB1* locus and 2 + 12 for *gluD1* locus (Dubcovsky et al., 2000).

RESULTS AND

The SDS PAGE analysis of the local (Pakistani) wheat varieties revealed that all the tested varieties have null allele for *gluA1* locus, 17+18 for *gluB1* locus and 2+12 for *gluD1* locus, indicating that all varieties have similar bread making quality alleles at HMWGS loci. The results in the Figures (1, 2 and 3) exhibited that all Pakistani wheat varieties have 2 and 12 band location, which represent their poor breed making capability. The varieties having 5 + 10 HMWGS allele at *gluD1* locus was used as

a standard for HMWGS, which have better bread making quality. So the varieties having 5 and 10 band position possess best bread making quality. In few cases Low molecular weight Glutenin subunit (LMWGS) did not revealed very well but in most of the cases LMWGS pattern was same in the test entries and it was different from LMWGS pattern of Chinese spring. Subunits 17 + 18 and 5 + 10 of the D genome were predominantly found in this set of varieties. The frequency of the appearance of *Glu-I* alleles in the varieties was different from that seen in other countries, especially in terms of the absence of the 'null' form of the A genome and the presence of novel subunits at the and *Glu-DI* loci. Because of the complexity of LMWGS pattern and less effect on bread making quality (Payne et al., 1981) LMWGS pattern were not studied in detail.

DISCUSSION

In the present study an attempt has been made to examine thirty bread wheat varieties for HMW-GS. According to the results the blueprint of HMWGS indicated that all the wheat varieties studied during present study poses 2 + 12 HMGS allele at Glud1 locus. This allele is associated with low bread making quality (Payne et al., 1981) and there is a serious need to incorporate better allele (5 + 10 HMWGS) at this locus. It has also been suggested that the existing wheat varieties should be crossed with wheat lines/varieties having 5 + 10 HMWGS allele at *gluD1* locus, which will add value to the existing wheat varieties. But it should also be considered that *gluA1*, *gluB1* and *gluD1* loci are not solely responsible for bread making quality of wheat flour. Singh et al. (2007) reported that LMWGS and gliadins have also effect on dough quality. However it is necessary that total seed storage proteins (HMWGS, LMWGS and gliadins) should be studied in detail to find out more about bread making quality of Pakistani wheat varieties (Valizadeh et al., 2001).

Conclusion and Recommendations

In the present study, High Molecular Weight Glutenin Subunits (HMWGS) were studied in details. All the varieties showed null allele for *gluA1* locus, 17 + 18 for *gluB1* locus and 2 + 12 for *gluD1* locus indicating that all varieties have similar bread making quality alleles at HMWGS loci. It is recommended that the existing wheat varieties should be crossed with wheat lines/varieties having 5 + 10 HMWGS allele at *gluD1* locus, so that better bread making quality wheat varieties may be produced. A number of exotic wheat varieties are available which have (5 + 10) HMWGS allele at *gluD1* locus and can be used for crossing with local wheat varieties.

ACKNOWLEDGMENTS

We express our genuine thanks to the Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University, Pakistan and Department of Biotechnology, Sarhad University of Science and Information Technology Pakistan for providing research and financial support for this work.

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