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Characterization of Pakistani Wheat Germplasm for High and Low Molecular Weight Glutenin Subunits Using SDS-PAGE

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High molecular weight (HMW-GS) and low molecular weight (LMW-GS) glutenin subunits play a significant role in bread making quality and extensibility, though they signify merely 10% and 40% of the entire seed storage proteins. For the estimation of bread quality on the basis of allelic difference in HMW-GS and LMW-GS at Glu-1 and 3 loci, wheat germplasm (77 genotypes) was collected from diverse agro-climatic regions of Pakistan and characterized by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Thirty distinct allelic arrangements were identified with a sum of thirteen Glu-1 alleles. Maximum frequency of allele 1 was found in twenty-nine genotypes at Glu-A1 locus while high proportion of subunit pairs 13+16 and 2+12 was detected in 33 and 32 genotypes at Glu-B1 as well as Glu-D1 locus, respectively. Few rare alleles were also separated out. The quality scores ranged from 4–10, however highest quality score of ten was more recurrent (36.36%). A good quality score of 8 and 6 were found in 32.47% as well as 19.48% of genotypes individually. In LMW-GS, seventeen diverse combinations of alleles with aggregate of ten Glu-3 alleles were detected. Glu-A3c and Glu-B3d alleles were observed in 33 (42.85%) genotypes, encoding high sedimentation and protein contents. Hence, this will enable the breeders to utilize both glutenin subunits as biochemical indicator for selecting superior wheat genotypes possessing enhanced bread making quality.

Keywords: wheat, bread making quality, HMW-GS, LMW-GS, SDS-PAGE

Introduction

Wheat is the principal cereal crop globally with respect to utilization as well production. It is the main source of protein, dietary fibre and energy in human food. In Pakistan, it is the basic food which fulfils over sixty per cent of the overall necessities for protein and calories in normal daily life and is consumed in various forms, primarily as bread, chapatti, pizzas, macaroni, doughnuts, spaghetti, noodles and cakes. Mature grains of wheat constitute proteins, i.e. 8–20% (Anjum et al. 2007). Its complex genetic background is responsible for enormous diversity in processing and nutritional qualities among cultivars (Jang et al. 2017).

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Bread making quality of wheat is primarily influenced by its protein content and protein quality. Gluten proteins determine the wheat flour ability to be processed into variety of foods (Zorica Jurkovic et al. 2000). It makes up to 80–85% of total flour protein and impart characteristics of extensibility and elasticity which are indispensable for wheat flours functionality and hence specify bread making quality (Costa et al. 2013). The suitable equilibrium between these two attributes makes wheat gluten acceptable for bread making. Elasticity is defined as stretching capability determination when dough is subjected to a force. On the other hand, extensibility involves stretching in the direction to which force is applied (Jang et al. 2017).

The gliadins and glutenins encompass each about fifty per cent of gluten proteins (Anjum et al. 2007). On the basis of their molecular weight two types of glutenin subunits have been differentiated into high molecular weight glutenin subunit, HMWGSs (97–136 kDa) and low molecular weight subunits, LMWGSs (31–48 kDa) (Zorica Jurkovic 2000). Wheat endosperm comprises of high molecular weight glutenin subunits (HMWGSs), which are primary grain storage protein comprises of only almost 12% of the total protein and has a crucial role in wheat gluten as a skeletal network (Geng et al. 2014). LMW-GS signify almost 40% of wheat gluten and is responsible for defining dough resistance plus extensibility (Park et al. 2011).

Studies have revealed that the HMW-GS have huge impact on the dough's rheological characteristics and bread forming attribute (Peña et al. 2005; Li et al. 2010; Zhang et al. 2011; Hernandez et al. 2012) and LMW-GS from wheat affect bread and noodle processing quality (Lee et al. 2017). Rheological as well as bread manufacturing experiments are arduous and need time, large amount of samples. So, the flour attributes identification through fast and precise methods for all kind of product permits cultivars choice with suitable traits for industrial usage in a smaller amount of time, certifying adequacy and success of fresh cultivar (Jang et al. 2017).

Sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) test is a classical technique employed for protein components separation. It enables the segregation of the gluten proteins subunits by identifying high molecular weight (HMW-GS) and low molecular weight (LMW-GS) glutenin subunits individually. Genes which encode high molecular glutenin subunits are situated at Glu-A1, Glu-B1 and Glu-D1 position on the long arms of A1, B1, and D1 chromosomes, correspondingly (Payne et al. 1984), while those who encode the low molecular glutenin subunits are present at Glu-A3, Glu-B3 and Glu-D3 position on the short arms of A1, B1, and D1 chromosomes, correspondingly. Every locus possesses two strongly associated paralogous genes coding two diverse forms of HMW-GS, viz. x and y type subunits (Jang et al. 2017).

Hence, in current study, aim is to characterize the allelic differences among cultivars through SDS-PAGE and assessment of their association with end-use quality to upgrade the quality of available germplasm which will enable the breeders to develop wheat cultivars using specific HMW-GS, LMW-GS alleles with sufficient gluten stability and resilience for bread production.

Material and Methods

Plant material collection

Wheat germplasm of 77 varieties were gathered from different centres within every single agroclimatic region of Pakistan (Khalid and Hameed 2017), in order to evaluate of should be omitted high molecular weight glutenin subunits. Reference varieties namely, Gabo (2, 2*, 12, 17, 18), Holdfast (1, 5, 7, 8, 11) and Chinese-Spring (2, 7, 8, 12) were employed as references (Payne and Lawrence 1983) while Cheyenne (c, e, f), Chinese Spring (a, a, a), Gabo (b, b, b), Glenlea (g, g, c), Insignia (f, c, c), Marquis (e, b, a), Nanbu-Komugi (d, ab, a), Norin 61 (d, i, c), Orca (d, d, c), Opata (b, ad, a) and Suneca (d, h, e) were used as standard for low molecular weight glutenin subunits (Gupta and Shepherd 1990).

Seed Protein Extraction

For total proteins extraction, five single grains of each genotype were crushed in 50 mM phosphate buffer (pH 7.8) then centrifuged in micro-centrifuge machine (Sigma 1–14) for ten minutes at 14,000 rpm. Dye binding assay was employed to measure amount of protein extracts as defined by (Bradford 1976). Supernatant was combined (4:1) with cracking solution (10 ml having 1 g SDS, 0.01 g bromphenol blue, 2 ml Mercaptoethanol, 1.5 ml 0.5M tris, pH 6.8, 5 g sucrose and 6.5 ml water) and mixed on vortex mixer, heated in a hot water bath (5 min) for proteins denaturation.

Seed Protein Profiling

Sodium dodecyl sulphate-polyacrylamide gels electrophoresis was used to perform samples protein profiling as defined by Laemmli (1970). Same amount of samples were laden into ten per cent gels. Continuous voltage (180 and 160 volts) was applied to conduct electrophoresis. Eventually, gels were fixed in solution comprising 10% acetic acid and 40% ethanol for fifteen minutes and agitated on a shaker persistently. After fixation, gel was washed with distilled water for fifteen minutes with altering water after every five minutes. Gels were then stained with coomassie blue G-250 dye and de stained in water overnight.

Gel Documentation and Analysis

Eventually, gels were photographed via UVIpro platinum gel documentation system (UVItec UK).

Quality Scoring

The HMW-glutenin subunits were recognized through formerly suggested system of numbering (Payne and Lawrence 1983) whereas quality scores were given by scoring system defined by (Payne 1987). Alleles were consigned quality scores of 1 (low quality)

to 4 (high quality) and separate scores for all allele existing in a genotype were combined to render the total quality score for respective genotype.

Statistical Analysis

The produced data matrix was then statistically analyzed using computer software Microsoft Excel along with XLSTAT Version 2012.1.02, Copyright Addinsoft 1995–2012 (http://www.xlstat.com).

Results

The outcomes revealed high polymorphism level regarding high molecular weight–glutenin subunits (Fig. S1). Amongst the 77 tested genotypes, 74 were homogeneous (96.10%) while three were heterogeneous (3.89%) with respect to high molecular weight–glutenin subunits. Thirty distinct combinations of alleles were identified (Table S1*). Overall, thirteen Glu-1 alleles, three at Glu-A1 locus, six at Glu-B1 locus while four at Glu-D1 locus were found in estimated material. Three alleles (1, 2* and Null) were detected at A genome's Glu-1A locus (Table S1). Occurrence rate of two active high molecular weight–glutenin subunits i.e. 1, 2* besides one null form of gene holding no subunit were 37.66%, 35.06% and 26%, respectively (Fig. S2a). Variation was found maximum for Glu-B1 locus in comparison with Glu-A1 besides Glu-D1. Alleles analogous to 6 diverse forms of subunits, 4 in subunit pairs viz., 13 + 16, 17 + 18, 7 + 9, 7 + 8 and 2 single/rare Glu-B1 subunits such as 7 and 20 in lesser proportions were sorted out in entire genotypes correspondingly (Table S1). Rate recorded for prevalent loci 13 + 16 besides 17 + 18 were 42.85% and 28.57% correspondingly (Fig. S2b). Glu-D1 locus displayed alleles responsible for subunits pair 5 + 10 and 2 + 12 as a prevailing combination with occurrences 55.84% in addition to 41.55% respectively. The existence of single subunits viz. 10 with low frequencies and a subunit combination 2 + 10 (3.89%) were also detected (Fig. S2c).

Results elaboration for allelic difference quantification in genomes of wheat exhibited highest input of genome B (46%) pursued by genome (31%) as shown in Fig. S3.

Afterwards, distinct scores of high molecular weight–glutenin subunit were combined to determine genotype's quality score in line with Payne et al. (1987) as shown in Table S1. The outcomes of quality score on the basis of high molecular weight–glutenin subunit composition amongst varieties and advance lines showed larger alteration with advance lines normally displaying a low score. The score ranged from 4–10 having a mean of 7. Twenty-eight genotypes attained the maximum quality score of 10 (32.47%) and 24 had a good quality score of 9 (5.19%). Generally, a good quality score of 8 was much persistent (39.47%) in current analysis. Average quality score of 7 was stable in one genotype merely. Further poor quality scores viz, 6, 5 and 4 seemed in comparatively lower frequencies with 19.48%, 1.30% and 2.60%, respectively (Fig. S4).

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

In LMW-GS, fourteen different allelic combinations were detected (Table S2). A total of 10 Glu-3 alleles (3 at Glu-A3 locus, 4 at Glu-B3 locus and 4 at Glu-D3 locus) were identified in estimated material. At Glu-3A locus, three alleles, i.e. c, d and e were detected (Table S2) among which Glu-A3c was found in 42.85% of the genotypes (Fig. S5a). There was more variation for Glu-B3 locus as compared to Glu-A3 and Glu-D3. Out of four alleles (b, d, h, i), d and h alleles were predominant and revealed a high frequency of 42.85% and 23.37%, respectively (Fig. S5b), while allele b and i in lower frequencies 11.68% and 22% were also found in all the genotypes (Table S2). At Glu-D3 locus, three alleles were detected having alleles a, b and c where allele a holds a dominant frequency of 77.92% and the existence of allele b and c with low frequencies of 9.09% and 12.98% were also observed (Fig. S5c).

Discussion

Wheat glutenin proteins, especially high molecular weight and low molecular weight glutenin subunits accumulated in starchy endosperm have a vital part in defining the bread-making as well as dough characteristics and hence the nutritional quality of flour (Anjum et al. 2007; Peng et al. 2016). According to the research, HMW-glutenin subunits composition variation may be the reason behind rate of inter varietal differences in bread forming capability while LMW-GS have not attained that much attention as HMW-GS because of intricate banding pattern and mobility overlapping with glaidin (Park et al. 2011), so this makes it necessary to evaluate the variation in alleles of high molecular weight as well as low molecular weight glutenin subunits conformation liable for bread manufacturing quality amongst wheat varieties of Pakistan (Tabasum et al. 2011).

In current study, HMW-glutenin subunits polymorphism was observed among Pakistani wheat varieties and has also been stated in the previous studies (Yan et al. 2007; Chaparzadeh et al. 2008). Homogeneous and heterogeneous arrays of protein were detected in our genotypes concurring with the former studies (Popa et al. 2003; Kang et al. 2007; Singh et al. 2007). This heterogeneous state can be utilized in breeding strategies for enhancing bread forming quality through selecting a glutenin physical trait having high molecular weight-glutenin subunit configuration related to good quality. Thirty diverse combination of alleles with aggregate of thirteen Glu-1 alleles illustrated not merely occurrence of allelic difference in HMW-glutenin subunits, accountable for alterations in bread manufacturing attributes nevertheless its possible application for breeding varieties in future.

Storage protein conformation is thought as constant element of cultivar due to direct genome expression therefore; it can help in identification of cultivar. Our results are in accordance with the earlier studies of (Popa et al. 2003; Kang et al. 2007; Tsenov et al. 2009).

In present research, three alleles, i.e. 1, 2* and Null were contributed by Glu-A1 locus of A genome with the prevalence of 1 subunit (37.7%) followed by subunit 2* (35.1) and Null (26%). In contrast to present findings, subunit 2* was found predominant with a percentage of 72.09% and 25% previously (Bahraei et al. 2004; Chaparzadeh et al. 2008)

while (Chaparzadeh et al. 2008) reported lack of subunit one in their genotypes. These variations might be the result of germplasm differences. The subunit 1 has good influence on the wheat quality; thus it may be introgressed into the genetic contextual of native varieties to enhance their quality followed by sustained breeding attempts as well as selection procedures (Tabasum et al. 2011; Costa et al. 2013).

Results obtained in this study coincide with findings of (Morgunov et al. 1990) who assessed a small number of Null alleles in Soviet wheat genotypes. Though, Nakamura (2000) reported high frequency of null allele i.e. 80.4% and 74.1% in Chinese and Japanese wheat varieties while highest frequency of 48.3% was reported by Popa et al. (2003) in Romanian wheat varieties whereas frequency of Null allele was found dominant in French wheat i.e. 69.5% by Branlard et al. (2003).

Glu-B1 of genome B depicted greater polymorphism level by rendering 6 diverse kinds of allelic alternates. Formerly, highest glutenin polymorphism at Glu-B1 locus has also been stated (Yan et al. 2007). The greatest recurrent pattern associated with good bread making quality was of subunits 13 + 16 (42.85%) followed by 17 + 18 (28.57%). Formerly, lower frequencies (3.57%) of 13 + 16 subunits were reported in Iranian wheat cultivars and advanced breeding lines (Mandoulakani et al. 2006) whereas in another study, much lower frequency of 0.7% and 0% in Chinese as well as Japanese wheat varieties (Nakamura 2000) were found. Yet in another study low frequency of about 5% was detected in Pakistani wheat germplasm (Tabasum et al. 2011). However, subunits 17 + 18 were most frequently encountered in hexaploid wheat landraces of Pakistan (Tahir et al. 1996). In another finding, the highest frequencies were observed for subunits 7 + 9 (82.6%) (Popa et al. 2003) still another finding reported the highest frequencies (29%) and (91%) of subunits 7 + 8 in European wheat landraces and obsolete cultivars (Gregová et al. 2006) as well as in Hand-Extended Noodles made from wheat flour (Kang et al. 2007).

Single/rare subunits of 7 and 20 were found similarly in our particular genotypes. Sub unit 20 screened out in nine varieties (11.68%) is in accordance with the results of Yan et al. (2007) who had stated little proportion in their genotypes regarding above mentioned subunit. Contrary to our findings, Carrillo et al. (1995) reported subunit 20 as most recurrent Glu-B1 allele. These subunits scarcity is beneficial because of their poor bread-making quality (Payne 1987; Dong et al. 2009).

Glu-D1 locus of genome D revealed substantial amount of polymorphism as well. The alleles holding the subunits pairs 5 + 10 as well as 2 + 12 were the highest persistent patterns with a frequency of 55.8% and 41.6%. Subunit pairs 5 + 10 impart beneficial effect on bread making quality (Costa et al. 2013). Our results are analogous to the findings of (Nakamura 2000; Popa et al. 2003; Kang et al. 2007) where examined varieties possessed leading frequencies of 5 + 10 and 2 + 12 subunits in numerous collections. In contrast to our results, (Branlard et al. 2003; Chaparzadeh et al. 2008) stated a lower frequency of subunit 5 + 10. One genotype in present study had 2 + 10 subunit whose quality contribution is not known and is in accordance with the findings of Yan et al. (2007).

Various genes in species genome are responsible for seed proteins and their study can offer valuable information regarding evolutionary associations and genome complexity

in species (Schuster et al. 1997; Tabasum et al. 2011). The division of allelic difference into genomic involvement showed that genome B is the basis of genetic diversity though highest contribution towards quality is via genome D.

Bread manufacturing aspect is a composite characteristics swayed through environment. Though, evaluation of bread-making quality potential is possible with the help of HMW-glutenin subunits in wheat genotypes (Schuster et al. 1997). Highest and good quality score of 10, 8 and 6 was found in tested genotypes. On the whole, our germplasm holds a good bread making potential as only one variety and 2 advance lines were detected to enclose a score of 5 or less. Our results varied from Schuster et al. (1997) who proposed mostly poor bread making quality in tested Brazilian wheat genotypes. Genotypes with lower frequency of good quality score specify the varieties development without utilising the comprehensive information of glutenin subunits conformation besides their role in bread making quality.

In present research, seventeen diverse combination of alleles with aggregate of ten Glu-3 alleles illustrated occurrence of allelic difference in LMW-glutenin subunits, accountable for alterations in bread and dough manufacturing attributes. Three alleles i.e. c, d and e were contributed by Glu-A3 locus of A genome. Allele GluA3c was found predominant with a frequency of 42.85%. Contrary to our findings, Park et al. (2011) reported maximum frequency of Glu-A3d in genotypes of Korean wheat while the same frequency was also detected in Japanese and French wheat (Branlard et al. 2001; Tanaka et al. 2005). However, our results are analogous to the previous findings (Branlard et al. 2003; Bellil et al. 2010; Bradová and Štočková 2010; Liu et al. 2010; Ram et al. 2011; Peng et al. 2016) where tested varieties possessed leading frequency of Glu-A3c allele i.e. 49.3%, 47.0%, 44.5%, 72.5%, 40.3%, 64.6% respectively. So, these results showed that the alleles Glu-A3c holds predominance throughout the world with respect to bread wheat. It has found to have high sedimentation volume so it can play vital role in quality improvement (Khan 1984). Formerly, He et al. (2005) reported the Glu-A3d as desirable allele for gluten quality and pan bread quality and it was also found in our evaluated material with a frequency of 35.06%.

Lowest frequency of 22.07% in Glu-A3e was found in present study. This non desirable Glu-A3e allele which is primary cause of reduction in maximum resistance and extensibility of dough (Appelbee 2007) was also found least frequent (3.5%) in Chinese wheat landraces, suggesting the high value of wheat varieties for modern wheat quality improvement.

For LMW-GS, the Glu-3 alleles have been already ranked according to their R_{max} (maximum dough resistance) (Henkrar et al. 2017). In the tested genotypes, Glu-A3c allele represented 42.85%, and as stated by R_{max}, this c allele is associated with least dough resistance and poor quality. Previously, majority of allele c has been reported at Glu-A3 locus in Argentinean (Lerner et al. 2009), Morrocan (Henkrar et al. 2017) and US (Shan et al. 2007) bread wheat cultivars.

The predominant alleles in our study were Glu-B3d with a frequency of 42.85%. Our results coincide with the previous findings (Cornish et al. 1993; Luo et al. 2001; Bellil et al. 2010; Henkrar et al. 2017) who reported maximum frequency of d allele in Saharan

bread wheat cultivars and French cultivars but lowest frequency of Glu-B3b. For the Glu-A3 and Glu-B3, our results differ from Aguiriano et al. (2008) who reported the prevalence of allele a for both locus in Spanish durum landraces. Glu-B3d has been reported to have Superior mixograph characteristics, high sedimentation and strong gluten strength (Branlard et al. 2001; Khan et al. 2016) and hence can be pooled by breeders for quality improvement.

In present research, three alleles i.e. a, b and c were contributed by Glu-D3 locus of D genome with the prevalence of allele a (77.92%) followed by allele c (12.98%) and allele b (9.09%). Our findings regarding Glu-D3 allele are in accordance with frequencies previously found where Liu (2008) reported maximum occurrence of Glu-D3a in 233 Chinese bread wheat while differ from the findings of Shan et al. (2007) and Lerner et al. (2009) with Glu-D3b as a major allele**.** Branlard et al. (2001) reported that allele GluB3a has positive impact on strength of dough. Hence, Glu-A3 and Glu-B3 loci alleles of LMW-GS are noteworthy because of their great influence on wheat processing qualities and in turn gluten elasticity. These alleles are accountable for high protein contents and sedimentation, which defines their important role in wheat quality improvement (Khan et al. 2016).

Wheat germplasm collected from diverse agro-climatic regions of Pakistan was tested in this study. It was major factor responsible for the difference in genetic makeup and for being genetically diverse and is in turn responsible for the variation in distribution of genes/alleles. Difference in allelic variation causes distinct HMW-GS and LMW-GS combinations. So, as the mutual occurrence of diverse alleles from the three loci is significant in terms of scores accumulation as well as quality determination, the assessment of genetic variance concerning loci encoded glutenin proteins, becomes important to characterize existing germplasm for their utilization.

Recent study defined the allelic variants of HMW-glutenin subunits which revealed strong association with high bread making quality as twenty-nine genotypes showed highest frequency of allele 1 at Glu-A1 locus whereas 33 and 32 genotypes revealed high proportion of subunit pairs 13 + 16 and 2 + 12 at Glu-B1 as well as Glu-D1 locus, respectively. Twenty-eight genotypes exhibited maximum quality score of 10 while Glu-A3c and GluB3d alleles of LMW-GS were also found significant with respect to bread processing quality. Based on these novel findings, genotypes having good quality subunits can be pooled by breeders through diverse breeding techniques so as to develop high quality wheat in future.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1*. HMW-GS for bread making quality of wheat varieties in Pakistan

Electronic Supplementary *Table S2*. LMW-GS for bread making quality of wheat varieties in Pakistan

Electronic Supplementary *Figure S1*. Histogram for HMW-GS and LMW-GS in the experimental wheat genotypes

Electronic Supplementary *Figure S2*. Frequency of different alleles encoded by Glu-A1 (a), Glu-B1 (b) and Gu-D1 (c)

Electronic Supplementary *Figure S3*. Percentage of quality scores fixed in Pakistani germplasm

Electronic Supplementary *Figure S4*. Percent contribution of wheat genomes towards allelic variation of HMW-GS

Electronic Supplementary *Figure S5*. Frequency of different alleles encoded by Glu-A3 (a), Glu B3 (b) and Glu-D3 (c)