Characterization of HMW-GS and evaluation of their diversity in morphologically elite synthetic hexaploid wheats

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High molecular weight glutenin subunit composition and variation in 95 Elite-1 synthetic hexaploid (SH) wheats (Triticum turgidum/Aegilops tauschii; 2n = 6x = 42; AABBDD) were determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis method (SDS-PAGE). Twenty two different alleles at Glu-I loci in SHs were observed. Forty four different patterns of HMW-GS in synthetics were found. This higher HMW glutenin composition was due to higher proportion of D-genome encoded subunits in these SHs. 8% urea/SDS-PAGE better discriminated subunit 2* than 12% gels. However 12% urea/SDS-PAGE allowed differentiated mobility of Glu-D1 subunits. Genetic variability at Glu-D1 locus was greater than Glu-A1 and Glu-B1 loci. The relative high frequency of superior alleles, Glu-B1b and Glu-D1d indicated the superior bread making quality attributes embedded in these synthetic hexaploid wheats. Of the 95 Elite-1 SHs 27.1% possessed superior alleles at Glu-A1 and 51% had superior alleles at Glu-B1 locus. At Glu-D1 frequency of inferior allele 1Dx2+1Dy12 was very low (5.26%) and nine different rare alleles along with the higher frequency (22.1%) of D-genome encoded subunit, 1Dx5+1Dy10, were observed. These superior alleles shall form the priority selective sieve for their usage in wheat improvement efforts.

Key Words: synthetic hexaploid wheats, bread making quality, HMW-GS, Aegilops tauschii.

Introduction

Development of wheat cultivars with good bread making quality is a challenging objective for many wheat breeding programmes. The major wheat endosperm protein, the gluten, is responsible for bread making quality (Branlard and Dardevet 1985). Gluten is comprised of two prolamine groups, gliadins and glutenin. Glutelins consist of low- and high-molecular-weight (LMW and HMW) complex subunits and constitute about 30–40% of flour protein. It has been reported that HMW glutenin subunits have the largest effect on bread making quality, even though they constitute only 10% of the total storage proteins as compared to LMW which contribute 40% (Payne et al. 1984). The HMW-GSs described as Glu-A1, Glu-B1 and Glu-D1 are encoded by multi-allelic genes located on the long arms of chromosomes 1A, 1B and 1D respectively (Payne et al. 1984). Each of these loci encode one x-type and one y-type subunit gene. These loci are highly polymorphic in nature without environmental influence (Payne et al. 1981a). Hence allelic variation results in different combinations of HMW subunits in different genotypes. Due to the consequences of gene silencing, flour particles have a combination of three to five HMW glutenin subunits (Payne 1987). These subunit combinations are used to predict the bread making quality on the basis of the Glu-I scoring system (Payne et al. 1987) which is being used for many years.

Common wheat (Triticum aestivum L. 2n = 6x = 42; AABBD) originated from a limited number of natural events where Aegilops tauschii (D-genome donor) accessions of restricted geographic origin were involved, thus resulting in a narrow genetic diversity within the D-genome (Lagudah et al. 1987). Ae. tauschii conserves a rich diversity source for enhancing the genetic variability of glutenin subunits in bread wheat that can significantly improve bread making properties (Pfluger et al. 2001). So far, 14 x-type and 10 y-type subunits in Ae. tauschii have been identified which combine into 85 different Glu-D1 alleles. Synthetic hexaploid wheats (T. turgidum x Ae. tauschii; 2n = 6x = 42; AABBD) have been developed by utilizing different
Ae. tauschii accessions from diverse geographic origins with durum wheat genotypes through standard wide cross hybridization procedures with the objectivity to enrich/widen the wheat gene pool by unique genetic resources and addressing all areas of wheat improvement including grain quality (Mujeeb-Kazi et al. 2008). Therefore these synthetic hexaploids wheats (SHs) are a valuable source for improving bread-making quality which harnesses genetic diversity from Ae. tauschii.

Keeping in view the above genetic resource diversity scenario the current study has investigated with the objective to identify the HMW glutenin subunit compositions and extent of variability in synthetic hexaploid wheats that are composed of various durum and Ae. tauschii accessions.

Materials and Methods

Plant material

The germplasm studied is a subset selected from a wide array of CIMMYT’s synthetic hexaploids produced over the last two decades (Mujeeb-Kazi et al. 2008). This Elite-1 subset is in wide global distribution and utilization. The synthetics in this subset possess an agronomically more desirable grown habit under three Mexican locations; Obregon (27°20′N, 105°55′W, 39 masl), Toluca (19°17′17″N, 99°40′1″W) and El Batan (19°31′N, 98°50′W, 2,249 masl) (Mujeeb-Kazi et al. 2000). Growing the synthetics in these locations enabled selections to be made for this Elite-1 subset. It is comprised of 95 primary synthetic hexaploid wheats derived from the combinations of 33 durum wheats and 74 Ae. tauschii accessions (Table 1). The production and protocol has been reported earlier (Mujeeb-Kazi et al. 1996). Seven wheat genotypes (Chinese Spring, Pavon-76, Pak-17951, SH-231, SH-248, SH-261 and SH-49) and four Ae. tauschii accessions (Claa1, Cla11, Cla25 and PI603250) with known banding patterns were used as standards for the identification and comparison of generated bands. The 34 durum parents within these synthetic hexaploids were also characterized separately for Glu-A1 and Glu-B1 subunits to confirm respective subunits in the synthetic hexaploid wheats.

Protein extraction and SDS-PAGE

A single spike was harvested separately from each of 95 synthetic wheat accessions and their 34 durum parents for SDS-PAGE analysis. A single grain from each spike was crushed, ground to powder and 10 mg of each weighed and taken in a microtube. To extract protein from flour, 1 ml of protein extraction buffer (0.05 M Tris + 0.2% SDS + 5 M Urea, adjusted to pH 8.0 with HCl) was added into the microtube. After few minutes 10 μl mercaptoethanol was added into microtube and mixed well with the help of a Vortex mixer. The HMW glutenin subunits were analyzed through slab type SDS-PAGE using 8% and 12% polyacrylamide gels without urea. Electrophoresis was run at 200 V until the blue line marker passed through the bottom of gel plates. The gels were removed from plates, stained with 0.2% (w/v) Coomassie brilliant blue for 20–30 minutes over a shaker. For de-staining 5% methanol solution was prepared in 7.5% acetic acid.

Nomenclature

The allelic classification at Glu-A1 and Glu-B1 loci and the numbering of HMW glutenin subunits were based on the classification of Payne and Lawrence (1983). The alleles at Glu-D1 locus were identified according to Pena et al. (1995) and William et al. (1993). All the allele names were obtained from MacGenes (McIntosh et al. 2008). The synthetic hexaploid y-type subunit which was initially named T2 was replaced by 12.2 according to Gianibelli et al. (2001). Glu-D1 subunit pair identified as 2.1+12 is not documented in MacGenes therefore its given allelic designation Glu-D1ga which is a combination of individual allelic names of both subunits.

Statistical analysis

The genetic diversity at each locus was calculated using Nei’s index (Nei 1973): \( H = 1 - \sum P_i^2 \), with H and Pi denoting the genetic variation index and the frequency of the number of alleles at the locus, respectively. Allelic frequencies were determined by summing the allelic frequencies in the individual accessions, irrespective of whether the HMW-GS composition was homogeneous or heterogeneous, and then dividing this total by the number of accessions.

Results and Discussions

Allelic variation at Glu-1 loci for HMW glutenin subunits in synthetic hexaploid wheats

The results obtained from this study described as HMW glutenin subunit compositions and allele frequencies in 95 Elite-1 synthetic hexaploid wheats are described in Table 2 and Supplemental Table 1. Unit allelic variability in these synthetic hexaploids at 12% gel is presented in Figs. 1, 2.

Twenty two different Glu-1 alleles were found, three at Glu-A1, six at Glu-B1 and thirteen at Glu-D1 (Table 1). At Glu-A1 locus three x-type subunits 1*, 2* and null encoded by alleles Glu-A1a, Glu-A1b and Glu-A1c, respectively were found. The null allele was found most frequently in 68 (71.6%) genotypes followed by subunit 1 in 15 (15.8%) synthetic wheats while the subunit 2* was observed in 12 (12.6%) genotypes. Previously Pena et al. (1995) reported the presence of null allele in all synthetic hexaploid wheats studied, which may be due to the fact of a small population size. The y-type subunit at Glu-A1 locus always remains absent. However, its activation at this locus can have clear effects on bread-making quality (Ciaffi et al. 1995). Several reports are available on activation of this y-type subunit in A-genome donor species (T. urartu, T. monococcum and T. boeoticum). This allelic richness for y-type subunit has been captured in A-genome amphiploids (Rasheed et al. 2010) which are compatible to be hybridized with common bread wheat and can be transferred through standard
breeding procedures. An et al. (2005) reported the genetic diversity depicted by different co-dominant alleles at Glu-A1 locus to be 0.19 which was very low as compared to our (0.59) finding. This is due to the fact that in these SHs 1 and 2* both covered a major proportion while in the studies reporting less diversity at this locus, major proportion was occupied by the ‘null’ subunit. It has also been determined that quality characteristics of varieties with subunit 1 were better than those of 2* and the null allele (Li et al. 2009).

At Glu-B1 locus, six different co-dominant alleles were found. The Glu-B1c allele controlling the subunit 7+9 was less frequent (2.1%) among all the subunits at this locus. The most frequent allele was Glu-B1b controlling subunit 7+8 found in 46 (48.4%) genotypes followed by Glu-B1d and Glu-B1e controlling the subunits 6+8 and 20 in 18 (18.9%) and 19 (20%) genotypes respectively. Subunit 6+8 is very common in synthetic hexaploids and durum wheats but its frequency is very low in bread wheat. Tang et al. (2010) analyzed quality effects of 6+8 on 21 quality and noodle test parameters. The overall effect of 6+8 subunit was positive influencing most of the quality parameters particularly if combined with superior subunits from Glu-A1 and Glu-D1. The other HMW-GS alleles found at this locus were Glu-B1f encoding 13+16 subunit and Glu-B1i encoding subunit 17+18 were observed in 7 (7.3%) and 3 (3.2%) accessions respectively. The genetic diversity at this locus depicted by these alleles was 0.70. Earlier, Pena et al. (1995) reported that subunit 7+8 was most frequent in synthetic hexaploids. Similar is the case of Chinese (71.9%) and Japanese varieties (83.2%) in which the subunit 7+8 was most frequent (Nakamura 2000). In this work most of the synthetics (80%) possess the subunits 7+8, 6+8, 17+18, 7+9 or 13+16 which were found to have superior impact on bread making quality. These subunits were considered to have the same quality score at Glu-B1 locus (Gianibelli et al. 2002). The effect of subunit 7 on the quality characteristics was found

| Durum genotypes | *WX accession | *WX accession | *WX accession |
|-----------------|---------------|---------------|---------------|
| ALTAR 84        | WX188         | WX864         | WX174         |
| DECOY1          | WX192(TA1651) | WX878         | WX372(TA2531) |
| CROC 1          | WX193         | WX879(TA2452) | WX409         |
| CPI/GEDIZ/3/GOO/JO69/CRA/4 | WX198       | WX882(TA2455) | WX502         |
| D67.2/P66.270    | WX205         | WX884(TA2457) | WX517         |
| ROK/KML         | WX208         | WX890(TA2463) | WX1024        |
| YARMUK          | WX211         | WX518         | WX1027        |
| DVERD 2         | WX213         | WX249(TA2391) | WX1030        |
| AC089           | WX214         | WX180         |               |
| GARZA/BOY       | WX217(TA2462) | WX237(TA2401) |               |
| 68.111/RGB-U/WARD/3 | WX218   | WX313(TA2460) |               |
| 68.111/RGB-U/WARD/3/FGO/4/RABI/5 | WX219 | WX324(TA2473) |               |
| 68112/WARD      | WX220(TA2470) | WX358(TA2516) |               |
| FGO/USA2111     | WX221(TA2472) | WX408(TA1645) |               |
| YAV3/SCO/JO69/CRA/3/YAV79/4 | WX222(TA1599) | WX518 |               |
| SORA            | WX223         | WX617         |               |
| PBW114          | WX224         | WX620(TA2394) |               |
| CERCETA (CETA)  | WX224         | WX625(TA2456) |               |
| GAN             | WX309(TA2454) | WX629         |               |
| LC59.61         | WX311(TA2456) | WX633         |               |
| STERNA-DW (SRN) | WX316(TA2464) | WX659         |               |
| SCOOP 1         | WX326(TA2475) | WX700         |               |
| SCAUP (SCA)     | WX369         | WX877(TA2450) |               |
| BOTNO           | WX369         | WX897(TA2470) |               |
| SNIPE/YAV79     | WX447         | WX895(TA2468) |               |
| TRINAKRIA (TRN) | WX498         | WX283(TA2427) |               |
| YAV 2/TEZ       | WX511         | WX312(TA2457) |               |
| ARLIN-1         | WX515         | WX314(TA2461) |               |
| FALCIN          | WX629         | WX333(TA2482) |               |
| RASCON          | WX658         | WX428         |               |
| SCOT/MEXI 1     | WX725(TA1618) | WX452         |               |
| GREEN           | WX781(TA1693) | WX454         |               |
| STY-US/CELTA/PAWS/3/SRN | WX783 | WX458 |               |

*WX is *Ae. tauschii* accessions number in the Wheat Wide Crosses working collection at CIMMYT, Mexico and NARC Islamabad, Pakistan; In parentheses accessions numbers are in Wheat Genetic and Genomic resource Center, Manhattan, KS, USA.
Table 2. Allelic frequencies of HMW-GS at Glu-I loci in 95 accessions of the D-genome synthetic hexaploid wheats of the Elite-1 subset

| Locus | Allele | Subunit | Number of Accessions | Frequency (%) | H (Nei’s index) |
|-------|--------|---------|----------------------|---------------|----------------|
| Glu-A1 | a      | 1       | 15                   | 15.8          |                |
|       | b      | 2*      | 12                   | 12.6          |                |
|       | c      | Null    | 68                   | 71.6          | 0.59           |
| Glu-B1 | b      | 7+8     | 41                   | 43.2          |                |
|       | c      | 7+9     | 2                    | 2.1           |                |
|       | f      | 13+16   | 7                    | 7.4           |                |
|       | i      | 17+18   | 3                    | 3.2           |                |
|       | d      | 6+8     | 18                   | 18.9          |                |
|       | e      | 20      | 19                   | 20            | 0.7            |
| Glu-D1 | –      | 1.5+10.5| 1                    | 1.05          |                |
|       | ah     | 1.5+10  | 3                    | 3.15          |                |
|       | aj     | 1.5+12  | 16                   | 16.84         |                |
|       | ag     | 1.5+12.2| 9                    | 9.47          |                |
|       | ai     | 2.1+10.5| 2                    | 2.1           |                |
|       | n      | 2.1+10  | 11                   | 11.57         |                |
|       | ga     | 2.1+12  | 16                   | 15.78         |                |
|       | –      | 2+10.5  | 3                    | 3.15          |                |
|       | a      | 2+12    | 5                    | 5.26          |                |
|       | d      | 5+10    | 20                   | 21.05         |                |
|       | h      | 5+12    | 2                    | 2.1           |                |
|       | x      | 2+12.2  | 3                    | 3.15          |                |
|       | z      | 3+10    | 4                    | 4.21          | 0.86           |

lowest at this locus (Li et al. 2009) and this subunit was not found alone in these accessions. The frequent subunit 7 + 8 was also reported to be associated with extensibility in bread wheat doughs (Pena et al. 1995, Uhlen 1990). So the higher frequency of important alleles Glu-B1b, Glu-B1f and Glu-b1i suggested the inherent potential of these synthetic hexaploids towards bread making quality. The durum parents of these SHs like Croc_1, Dverd2, Ceta, Yar, Gan, Scoop1 and Altar-84 had desirable HMW glutenin composition.

A valuable genetic variability (0.86) was found at Glu-D1 locus for HMW-GS in these synthetics. It also justifies the objectives of development of these synthetics. As the allelic variation of HMW-GS strongly influences the variability in bread making quality and D-genome strongly influences bread making quality (Pfluger et al. 2001, William et al. 1993) therefore a higher level of genetic variability at this locus is a valuable genetic reservoir to improve breadmaking quality. At Glu-D1 locus five x-type subunits viz. 1.5, 2.1, 2, 3 and 5, four y-type subunits viz. 10.5, 10, 12 and 12.2 constitute thirteen different co-dominant allelic combinations. The Glu-D1d allele controlling the subunit 5 + 10, is the most important and superior bread making quality subunit was found most frequent (21.05%) among all the subunits at this locus. Li et al. (2009) reported the superiority of this allele among all the other alleles at Glu-I loci. Luo et al. (2001) reported the association of 5 + 10 subunit with sedimentation volume and longer selshenke time. They also reported that 5 + 10 subunit in a genotype also results in greater wholemeal flour protein. It was also observed that migratory speed of 5 + 10 subunit is slightly slower than 5 + 10. Payne et al. (1981b) established that 5 + 10 subunit has a superior quality affect over 2 + 12 and all other alleles at Glu-D1. The other subunits at this locus were 2 + 12 encoded by allele Glu-D1a found only in 5 (5.26%) genotypes. The other subunits contributed by Ae. tauschii include 2.1 + 12, 2.1 + 10, 1.5 + 12.2 and 1.5 + 12. Subunit designation of 1DyT2 was changed to 12.2 for uniformity in nomenclature and it had higher mobility than 1Dy12. This allele Glu-D1-2l controlling subunit T1 + T2 were first reported by William et al. (1993) in Ae. tauschii and they concluded that T1 and T2 occur together and their presence was designated as T2. The other important subunits at this locus were 1.5 + 12, 2.1 + 12 and 2.1 + 10 found in 16 (16.84%), 16 (16.84%) and 11 (11.57%) accessions, respectively. The subunit pair 3 + 10 was found in six genotypes. This subunit is associated with extensible gluten type and had larger bread loaf volume than 2 + 10 (Pena et al. 1995). The subunit 1.5 + 10 was found in three SHs and this subunit had better overall quality characteristics than genotypes having other subunits. Pena et al. (1995) and Tang et al. (2008) presented evidences that genotypes having the 1.5 + 10 subunit possessed the best bread-making quality. SHs with Ae. tauschii accession numbers 314, 511 and 725 had 1.5 + 10 subunit associated with subunits 7 + 8 and 20 at Glu-B1 locus which indicated the superiority of these SHs apart from the others. The loci in 95 accessions of the D-genome synthetic hexaploid wheats of the Elite-1 subset of the objectives of development of these synthetics. 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This subunit is associated with extensible gluten type and had larger bread loaf volume than 2 + 10 (Pena et al. 1995). The subunit 1.5 + 10 was found in three SHs and this subunit had better overall quality characteristics than genotypes having other subunits. Pena et al. (1995) and Tang et al. (2008) presented evidences that genotypes having the 1.5 + 10 subunit possessed the best bread-making quality. SHs with Ae. tauschii accession numbers 314, 511 and 725 had 1.5 + 10 subunit associated with subunits 7 + 8 and 20 at Glu-B1 locus which indicated the superiority of these SHs apart from the others.
from the SHs with $5 + 10$. Their bread-making quality can be further enhanced by utilizing them with bread wheat genotypes having active 1Ax subunit and selecting the derivatives having 1Dx1.5 + 1Dy10 and 1Ax1/1Ax2* at Glu-D1 and Glu-A1 loci, respectively. The subunit 1.5 + 10.5 and 2 + 10.5 were found only in one and two genotypes, respectively. These do not have any allelic designation in MacGene. The rigorous identification of D-genome encoded subunits requires comparative analysis at different SDS-PAGE concentrations with additional check lines. The present results indicated that 8–12% SDS gels facilitated differentiation migration of these novel subunits.

**HMW-GS composition in synthetic hexaploid wheats**

Forty four different HMW-GS compositions were observed in synthetic wheats (Supplemental Table 1). Pena et al. (1995) reported thirty six different allelic compositions in synthetic hexaploid wheats. Six (6.31%) genotypes possessed the combination of subunits Null, 7 + 8, 2 + 12, Ten (10.57%) genotypes had subunit combination of 7 + 8, 5 + 10; 17 + 18, 5 + 10 or 6 + 8, 5 + 10 indicating superior alleles at both Glu-B1 and Glu-D1 loci. Twelve synthetics showed the presence of a rare allele 1Dy12.2 at Glu-D1 locus with either subunit 1.5 or 2. The quality effects of genotypes with the 1Dy12.2 subunit were not determined because these are rare subunits and their quality effects are yet to be ascertained. The durum cultivars having either of subunit 1 or 2* at Glu-A1 and Glu-B1 encoding subunits 7 + 8, 17 + 18 or 13 + 16 can enhance the bread making quality of these genotypes. Fifteen synthetics have either of 1 or 2* subunit at Glu-A1 locus along with superior (7 + 8, 17 + 18 or 13 + 16) subunits at Glu-B1 locus. Recent findings of Xu et al. (2010) include a wide array of D-genome encoded subunit in synthesized wheat germplasm with same durum background. Our findings presented in this study are important in a way that this set of synthesized genomic germplasms had valuable HMW variability in durum genome of SHs. From these results it is evident that synthetics have a good potential towards bread-making quality and their exploitation in breeding programmes can become a priority choice for the breeder emphasizing on wheat breeding for high grain quality. We propose that such quality descriptors should be the foundation of parental selectivity when a recombination breeding program is initiated.

**Corporating allelic diversity for Glu-1 from these synthetics is preferred due to their promising agronomic features. The improved agronomic features can also reduce the breeder’s efforts of increasing the proportion of domestication gene (Q) by backcrossing with elite conventional bread wheat parents. Conclusively, higher variability at Glu-1 loci is associated with these synthetic hexaploid wheats which could be effectively utilized in a targeted manner in breeding programmes. Moreover the excellent crossability of synthetic wheats with conventional bread wheats facilitates the swift utilization of synthetic hexaploid wheats for introducing new Glu-D1 allelic variations into bread wheat. As SHs have diverse durum in their pedigree, the undesirable qualitative effects associated with Glu-B1 locus prevalent in durum wheat cultivars can be avoided by utilizing satisfactory quality diversity present in the durum cultivars that are within these unique synthetic hexaploid wheat resources.**

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