Allelic variations in Glu-1 and Glu-3 loci of historical and modern Iranian bread wheat (Triticum aestivum L.) cultivars

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Abstract

Proline and glutamine-rich wheat seed endosperm proteins are collectively referred to as prolamins. They are comprised of HMW-GSs, LMW-GSs and gliadins. HMW-GSs are major determinants of gluten elasticity and LMW-GSs considerably affect dough extensibility and maximum dough resistance. The inheritance of glutenin subunits follows Mendelian genetics with multiple alleles in each locus. Identification of the banding patterns of glutenin subunits could be used as an estimate for screening high quality wheat germplasm. Here, by means of a two-step 1D-SDS-PAGE procedure, we identified the allelic variations in high and low-molecular-weight glutenin subunits in 65 hexaploid wheat (Triticum aestivum L.) cultivars representing a historical trend in the cultivars introduced or released in Iran from the years 1940 to 1990. Distinct alleles 17 and 19 were detected for Glu-1 and Glu-3 loci, respectively. The allelic frequencies at the Glu-1 loci demonstrated unimodal distributions. At Glu-A1, Glu-B1 and Glu-D1, we found that the most frequent alleles were the null, 7 + 8, 2 + 12 alleles, respectively, in Iranian wheat cultivars. In contrast, Glu-3 loci showed bimodal or trimodal distributions. At Glu-A3, the most frequent alleles were c and e. At Glu-B3 the most frequent alleles were a, b and c. At Glu-D3 locus, the alleles b and a, were the most and the second most frequent alleles in Iranian wheat cultivars. This led to a significantly higher Nei coefficient of genetic variations in Glu-3 loci (0.756) as compared to Glu-1 loci (0.547). At Glu-3 loci, we observed relatively high quality alleles in Glu-A3 and Glu-D3 loci and low quality alleles at Glu-B3 locus.

Introduction

Plant storage proteins in general and wheat storage proteins in particular contribute to the major source of the required nutritional protein and carbohydrates to nearly all nations around the globe. Due to their high proline and glutamine contents, the majority of wheat seed endosperm proteins are collectively referred to as prolamins (Payne 1987). Prolamins are comprised of glutenins and gliadins. Glutenins come in two forms or subunits i.e., high molecular weight glutenin subunits (HMW-GSs) and low molecular weight gluten subunits (LMW-GSs). The HMW-GSs, which quantitatively comprise about 20%–30% of the gluten are the major determinants of gluten elasticity (Shan et al. 2003). The LMW-GSs accounts for about 70%–80% of the glutenins and considerably affect dough extensibility (EXT) and maximum dough resistance (Rmax) (Gupta et al. 1994).

The HMW-GSs are encoded by Glu-1 loci on the long arms of homeologous group 1 (Glu-A1, Glu-B1 and Glu-D1) chromosomes and the LMW-GSs are encoded by Glu-3 loci on the short arms of homeologous group 1 (Glu-A3, Glu-B3 and Glu-D3) chromosomes in hexaploid wheat (Triticum aestivum L.). The genetics of glutenin subunits follows the genetic principles of traits with multiple alleles in each locus.
i.e., there are more than two alleles from which one is present for each locus in each homozygous genetic background. Besides the fact that the inheritance in each locus resembles genetic of traits with multiple alleles, the interaction of loci with one another is also important, resulting in epistasis of additive effect on dough resistance between Glu-1 and Glu-3 loci (Gupta et al. 1989; Nieto-Taladriz et al. 1994). To simplify, it means that the weakness of a breeding line in either Glu-1 or Glu-3 can be partially compensated by the other and therefore, the combination of alleles in all loci determines the final phenotype and degree of quality a breeder should be expecting from each breeding line.

The study of LMW-GS has always been problematic because of its banding patterns which tend to overlap with that of gliadins, albumin and certain globulins (Morgunov et al. 1993). Now, 2-dimensional electrophoresis (2DE) SDS-PAGE and a two-step-1-dimensional SDS-PAGE procedure have overcome the latter technical difficulties in separation of banding patterns (glutenins overlapping with gliadins) and facilitated excellent separation of LMW-GS. Nevertheless, these techniques are tedious and time consuming (Gupta and Shepherd 1990). An even more convenient method for studying LMW-GS is the simultaneous use of sequential extraction of gluten proteins and separation of the extracts on gradient 1-D SDS-PAGE gels (Singh et al. 1991; Morel 1994).

Due to the high precision and accuracy associated with molecular markers (Henry 1997), wheat quality assessment by means of molecular markers can accelerate breeding programmes for simultaneous increases of proteins and sustainable productivity. Here, we report allelic variation of HMW and LMW gluten subunits in 65 hexaploid wheat cultivars introduced, bred and/or purified from the local landraces by the University of Tehran, College of Agriculture and by the National Wheat Breeding Programme in Iran through sequential extraction method and separation in gradient 1-D SDS-PAGE.

Materials and methods

Wheat grain materials

For this study, seeds of 65 bread wheat cultivars (T. aestivum L.) were obtained from the GenBank of Seed and Plant Improvement Institute, Iran. We also included Gabo and Chinese spring cultivars as standard cultivars for identification of banding patterns of wheat HMW and LMW glutenin proteins.

Protein extraction and electrophoresis

For the protein extraction, we employed the sequential extraction procedure described by Singh et al. (1991) with some modifications. For the extraction of unreduced prolamin, half the endosperm of a single wheat kernel was crushed into fine powder. Gliadins were extracted in 200 µL of 70% (v/v) aqueous ethanol either 1 h at 60°C or overnight at room temperature followed by centrifugation for 5 min at 10,000 g, and then the supernatant including gliadins were collected. Sample buffer, 100 µL (containing 80 mM Tris-HCl, 40% glycerol (w/v) and 0.02% bromophenol blue) was added onto 100 µL of the supernatant in a new tube, briefly vortexed, and incubated for 3 min at 60°C just before loading on the gels. For extraction of reduced prolamines, the pellets obtained from the previous step were further sequentially extracted by washing the pellets twice in 1.0 mL of 50% (v/v) n-propanol at 60°C by repeated vortexing (at least twice) for 30 min, followed by 2 min centrifugation, discarding the supernatant. We accomplished nearly complete extraction of gliadins by suspending the remaining flour inside the tubes by using a fine spatula because it is essential to remove the entire gliadins at this stage and therefore, to prevent possible contaminations of gliadins in glutenin preparations later on. The pellets were then washed with 0.5 mL of 50% n-propanol centrifuged for 5 min at 10,000 g, and the liquid was discarded. Glutenin was extracted from the pellet by adding 100 µL of 80 mM Tris-HCl (pH 8.0) containing 1% dithiothreitol (DDT) at 50% n-propanol. After brief vortexing, the samples were incubated for 30 min at 60°C. Reduced glutenin (including HMW and LMW subunits linked to each other by SH groups) was then extracted by centrifugation for 2 min. Because alkylation prevents intermolecular disulfide bonds from reforming and therefore improves the resolution of the LMW bands, the samples were alkylated with 100 µL of 80 mM Tris-HCl (pH 8.0) supplemented with 0.14 M 4-vinylpyridine (4-VP) in 50% n-propanol. The samples were then further incubated for 15 min at 60°C and centrifuged for 2 min at 10,000 g. Supernatant, 50 µL, was transferred into a new tube containing 100 µL of sample buffer (containing 80 mM Tris-HCl (pH 8.0), 40% glycerol (w/v), 2% SDS (w/v) and 0.02% bromophenol blue). After brief vortexing, the samples were incubated for 15 min at 60°C to allow the formation of SDS coupled with the reduced and alkylated glutenin polypeptides. The samples were then centrifuged for 2 min at 10,000 g before gel electrophoresis.

Gel preparation, pattern and quality scores nomenclature

We used a gel system consisting of two layers i.e., stacking and gradient acrylamide separating layers. A linear gradient acrylamide gel from 8.1% to 12.5% with 1% crosslinker concentration (bisacrylamide:acrylamide ratio) allowed the visualization of HMW and LMW glutenin subunits. The main separating gel layer consisted of buffer (45.412 g Tris, plus 1g SDS and 460 mL H2O, made up to 500 mL), adjusted with HCl to pH 8.8, acrylamide solution 40%, ammonium persulphate solution 10% and temed as the polymerization agent. The stacking gel consisted of buffer (6.06 g Tris plus 0.4 g SDS and 190 mL H2O, made up to 200 mL), adjusted with HCl to pH 6.8, with 4% acrylamide. Ammonium persulphate solution and TEMED were also added to these solutions for polymerization. The electrode buffer (10×) for both upper (cathodal) and lower (anodal) tanks, contained 30.33 g Tris,
Glutenin alleles in Iranian bread wheat

144.2 g glycine, 10 g SDS, dissolved in 883 mL H₂O made up to 1 L and adjusted to pH 8.3 with glycine. The prepared protein samples (15 µL) as described earlier were loaded at the cathodal part of gel and electrophoresis were run at a constant current of 30 mA/gel for 130 min for gradient gels and 45 mA/gel for 260 min to 10% in gradient gels. After electrophoresis, gels were stained for at least 3 h in a solution containing one part of 1% (w/v) coomassie brilliant blue R mixed with 40 parts of 6% (w/v) trichloroacetic acid in water : methanol : glacial acetic acid (80 : 20 : 7). The gels were then destained by incubating in deionized water for one day with several changes of water. Identification of banding patterns of HMW-GS and LMW-GS and giving quality scores to studied cultivars were conducted, respectively, according to Payne (1987) and Gupta et al. (1990).

Statistical methods

Genetic variations in all loci were calculated with Nei’s formula (Nei 1978) and cluster analyses were also done using Jaccard’s coefficient (Manly 2004) and UPGMA method (Legendre and Legendre 1998) implemented in the freeware statistical package ‘Palaeontological Statistics package (Legendre and Legendre 1998) freely available at http://folk.uio.no/ohammer/past.

Results and discussion

We dissected the allelic variation of HMW and LMW glutenin loci in 65 hexaploid wheat cultivars representing a historical trend in the cultivars introduced, bred, and/or purified from the local landraces by the University of Tehran, College of Agriculture or by the national wheat breeding programme, Iran from the years 1940 to 1990. The certified seeds were obtained from the Cereal GenBank of Seed and Plant Improvement Institute, Iran. We extracted the endosperm proteins of the Iranian cultivars along with the two standard genotypes Gabo and Chinese spring by a sequential procedure which allows for nearly complete separation of glutenins and gliadins. Although a number of LMW glutenin subunits and gliadins bands demonstrate same motilities on SDS-PAGE, sequential extraction procedure, followed by a linear gradient acrylamide gel of 8.1%–12.5% has allowed us to electrophoretically fractionate and differentiate amongst the subunits on the gel (figure 1). When we compared the electrophoretic separation of glutenin subunits in our gradient gels, as opposed to normal 10% gels, we noticed a higher resolution and less time required for separation of the subunits. For identification of allelic variations we followed the nomenclature proposed by Payne (1987) and Gupta et al. (1990). A representative gel for one-step / one-dimensional electrophoretic separation of glutenin subunits is shown in figure 1. Separation of glutenin subunits (figure 1) confirmed that (i) there is no gliadins contamination in the distance between A (HMW-GSs) and B (LMW-GSs); (ii) there is no overlapping between gliadins and the glutenin subunits in the areas between B and C regions.

Allelic composition for each wheat cultivar used in this study is shown in figure 2. This panel includes Glu-1 genotype, quality scores calculated over the subunits of Glu-1, following Payne (1987), Glu-3 genotype, and quality class (i.e. L, low; M, medium; H, high) for Glu-3 after Gupta et al. (1990). The banding patterns observed in Gabo and Chinese spring cultivars facilitated the identification of the banding patterns of the Glu-1 and Glu-A3 glutenin subunits for the cultivars studied. Allelic variations at Glu-B3 and Glu-D3 loci were identified by using the distance location of the sharp bands at zones B and C. We, finally, identified and reported a total of 17 distinct alleles for Glu-1 loci (1 (a), 2* (b), Null (c), and 2** (d) for Glu-A1; 7 (a), 7+8 (b), 7+9 (c), 6+8 (d), 20 (e), 13+16 (f), 13+19 (g), 17+18 (i), and 7+11 (m) for Glu-B1; and 2+12 (a), 3+12 (b), 5+10 (d), 10 (i), and 10* (j) for Glu-D1) and a total of 19 distinct subunits for Glu-3 loci (a, b, c, d, and e for Glu-A3; a, b, c, d, e, f, g, h, and i for Glu-B3; and a, b, c, d, and e for Glu-D3). The occurrence of this wide allelic polymorphism in our panel of cultivars can be considered as an asset in our breeding programmes aimed for wheat quality.

The ‘null’ allele (c) was detected as the consensus allele with the greatest frequency just above 50% for Glu-A1 locus in the Iranian cultivars. At Glu-B1 locus, the allele 7+8 (b) with 64.2% frequency was by far the most frequent allele. Over 70% of the cultivars showed the 2+12 allele (a) at Glu-D1 locus.

High molecular weight glutenin subunits are encoded by three Glu-1 loci on chromosomes 1A, 1B and 1D. Each locus contains two tightly linked genes governing an x-type and a y-type subunit. Therefore, in theory, hexaploid wheat

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Figure 2. Dendrogram of cultivar classification through Jaccard’s similarity criterion of the banding patterns and UPGAMA grouping algorithm is shown. The dendrogram is rooted by Chinese spring as outgroup. Allelic variations in Glu-1 loci, quality scores based on Glu-1 loci, allelic variations in Glu-3 loci and the quality classes based on the Glu-3 loci were also outlined for each cultivar.
Glu alleles in Iranian bread wheat

Figure 3. Allelic frequency distributions for Glu-1 loci are shown. Nei coefficient of genetic variation for each locus is printed below each locus. The frequencies reveal unimodal distributions leading to a moderately lower Nei coefficient of genetic variations.

could contain six different HMW-GSs (Skerritt 1998; Jiang et al. 2009). In common wheat, 1Bx, 1Dx and 1Dy are always expressed, whereas the 1Ax and 1By are not always expressed. 1Ay is interesting because it is always silent in hexaploid wheat while its expression was reported in diploid and tetraploid wheat genotypes (Jiang et al. 2009). Forde et al. (1985) mentioned nucleotide changes in promoter region as the cause for non expression of 1Ay where as transposon-like insertions in the coding regions of the allele could be a possible reason for gene silence Harbred et al. (1987). Our specific observations in this regard include the presence of the allele (7)a without 1By in cultivar ‘Argentine’ and presence of the allele (10) without 1Dx in cultivar ‘Zagros’. We also noticed that the allele we identified (2∗∗) on the locus Glu-A1 for the cultivar ‘Sorkhtokhm’ was previously identified by Lagudah et al. (1987) as the 2/1 variant.

The fact that majority of the wheat cultivars in Iran possess ‘null’ allele in their Glu-A1 locus and consequently relatively lower quality assessment scores (Payne 1987) was found to be one of the key strategies to hold for improved wheat quality in our breeding programmes. Although the allele frequencies at the Glu-1 loci demonstrated unimodal distributions, we observed bimodal or trimodal distributions for the Glu-3 locus. At Glu-3A locus, the most frequent alleles were c (40%) and e (33%). At Glu-B3 the most frequent alleles were a (22%), b (25%), and c (19%). The allele b (36%) and a (31%) were the most and the second most frequent alleles at the Glu-D3 locus in Iranian wheat cultivars. The presence of high quality alleles in Glu-A3 and Glu-D3 loci have led the Iranian wheat cultivars to be classified into medium or high quality classes. However, these germplasms suffer substantially from low quality alleles at Glu-B3 locus.

Next, we computed the genetic variations in Glu-1 and Glu-3 loci following Nei (1978) method (figures 3 and 4). On an average, the genetic variability in Glu-3 locus (0.756) was significantly greater than that of Glu-1 locus (0.547) at significance level of (P = 0.032). The greater Nei’s coefficient of genetic variation for Glu-3 locus (as compared with that of Glu-1 loci) indicates that the alleles in Glu-3 locus are relatively more distributed amongst the cultivars and representing a bimodal or trimodal distribution whereas, the alleles in Glu-1 locus are relatively less distributed and creating a unimodal distribution. We also generated dummy variables to demonstrate the allelic distribution of Glu-1 and Glu-3 loci and then performed a cluster analysis based on Jaccard’s similarity criterion. Since the cultivar grouping in this dendrogram is based on allelic polymorphism, there are occasions where two cultivars show different allelic pattern.
and yet, equal quality scores. This implies that targeting for promising combinations of allelic variants in Glu-1 and Glu-3 loci would be a strategy in improving quality for this selection of cultivars.

Despite the availability of a huge number of local landraces from a wide range of geographical locations of Iran in the National GenBank, the depth of knowledge in the allelic variation in the Iranian wheat landraces is very limited. The predominant aims in the Iran’s wheat programmes involve improved potential yield, yield stability, and disease resistance. Yield and disease resistance have been the centre of attention over the past three decades because bread constitutes the main energy component of the food basket for the majority of the population. At the beginning of the wheat breeding programmes in Iran, there has also been limited attention given to the quality assessment for Iranian wheat germplasm development because of the fact that, traditionally, flat bread has been almost the only bread style and quality assessment tests were not functionally in place or unconsciously neglected. These statements partially explain why we are behind in exploitation of high score allelic variants in Iranian landraces.

Assessment of bread making quality in our current breeding programme involves measurements of SDS sedimentation volume and gluten strength that are determined in our cereal chemistry laboratories. However, investigation of allelic variations in HMW and LMW gluten proteins provided an indirect assay for quality determination. Najafian et al. (2008) have conducted a classification of Iranian landraces and cultivars based on SDS sedimentation volume where they classified 67 wheat germplasm including hexaploid and tetraploid accessions. In their classification based on SDS sedimentation volume, they grouped the pool of germplasm into two groups of good quality and moderate quality. When we traced the cultivars/landraces we estimated their bread making quality based on Payne et al. (1981) scores of allelic patterns, we detected a reasonable correlation between these two studies. For example, we reported quality score of 10 for HMW allelic patterns of Darab-2 and Sorkhtokhm for which Najafian et al. (2008) reported. We, therefore, think that there is a reasonable correlation between our estimation and the actual SDS sedimentation volumes obtained from these cultivars.

Although the genetic variations observed within the Iranian wheat landraces revealed lower than expected of high score allelic variants and medium-to-low quality scores of genetic combinations amongst different loci, we remain hopeful that there exist suitable germplasm for improved quality traits and high score allelic combinations in hundreds of thousands of Iranian bread wheat landrace accessions.

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References


Table 1. Cross classification of LMW-GSs identified and the quality classes they confer along with their relative frequencies in 65 Iranian wheat cultivars.

<table>
<thead>
<tr>
<th>Glu-A3</th>
<th>Relative frequency</th>
<th>Glu-B3</th>
<th>Relative frequency</th>
<th>Glu-D3</th>
<th>Relative frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>d, b and a</td>
<td>0.268</td>
<td>i and h</td>
<td>0.074</td>
<td>e and b</td>
</tr>
<tr>
<td>Median</td>
<td>c</td>
<td>0.402</td>
<td>b, d, g and e</td>
<td>0.477</td>
<td>c and a</td>
</tr>
<tr>
<td>Low</td>
<td>e</td>
<td>0.328</td>
<td>a, f and c</td>
<td>0.477</td>
<td>d</td>
</tr>
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Glu alleles in Iranian bread wheat


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