Novel High Molecular Weight Glutenin Subunits at the Glu-D1 Locus in Wheat Landraces from the Xinjiang District of China and Relationship with Winter Habit

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Allelic variation of high molecular weight glutenin subunits (HMW-GS) encoded by Glu-A1, Glu-B1 and Glu-D1 in wheat landraces originating from the Xinjiang Uygur Autonomous District (Xinjiang District) of China was investigated by SDS-PAGE and the relationship between the variation and the winter habit was examined. The most frequent allele at the Glu-A1 locus was Glu-A1c (null allele: 75.5%), followed by Glu-A1b encoding subunit 2* (22.3%). These two alleles were distinctive at Glu-A1 in the landraces of both spring and winter cultivars. No difference in allele frequency was found between the spring and the winter wheat cultivars. At the Glu-B1 locus, the most frequent allele was Glu-B1b (90.8%) encoding subunits 7+8, the allele frequency of which was almost the same between the spring and the winter wheat cultivars. Glu-D1a (72.0%) encoding subunits 2+12 was a major allele at the Glu-D1 locus among the landraces. Although no cultivar with Glu-d1f encoding subunits 2.2+12 was found, the novel subunit pair 2.6+12 (24.5%) was observed only in the winter wheat cultivars. Subunit 2.6 was slightly less mobile than subunit 2.2. The major Glu-D1 allele was Glu-D1a (94.5%) among the spring wheat cultivars and this allele characterized the spring wheat cultivars in the Xinjiang District. Both the subunit pair 2.6+12 encoded by the Glu-D1b allele (58.5%) and the subunit pair 2+12 encoded by the Glu-D1a allele (40.7%) predominated among the winter wheat cultivars. The large difference in allelic variation at the Glu-D1 locus suggested that the origin of the spring wheat cultivars was different from that of the winter wheat cultivars. The absence of landrace with Glu-D1f suggested that Xinjiang wheat was not related to Japanese wheat.

Key Words: Triticum aestivum L., allele, gene frequency.

Introduction

Wheat flour mixed with water produces a dough whose viscoelasticity is determined by a protein network called gluten. Gluten consists of glutenin and gliadin. Disulfide bonds of cystein residues between glutenin subunits (GS) form the backbone of the gluten network, which provide the viscoelasticity. GS are composed of high molecular weight (HMW) and low molecular weight GS. The expression of HMW-GS is controlled by alleles at the Glu-1 loci located on the long arm of the group 1 chromosomes (Payne et al. 1987). Various genotypes of GS have been reported (Payne et al. 1983). Differences in GS affect dough strength and bread volume (Payne et al. 1987, Payne et al. 1988, Lukow et al. 1989, Gupta and Sphephard 1990). Among the HMW-GS alleles, Glu-D1d encoding 5+10 subunits is known to improve the bread making quality and has been introduced into bread wheat cultivars in many countries.

Several studies have been carried out on the geographical variations in the composition of HMW-GS. Payne et al. (1983) studied the genetic variations of HMW-GS among 300 wheat cultivars worldwide. They revealed the presence of three alleles at the Glu-A1 locus, 11 alleles at the Glu-B1 locus and six alleles at the Glu-D1 locus. Morgunov et al. (1993) reported the worldwide distribution of HMW-GS in 1380 wheat cultivars from 21 countries. He et al. (1992) identified 35 kinds of HMW-GS among 197 Chinese cultivars. However, their data did not reveal peculiar variations among the landraces in China, since most of their materials consisted of improved cultivars.

Nakamura and Fujimaki (2002) compared the allele frequencies of HMW-GS between 274 Chinese and 131 Japanese cultivars, including both improved and local types. Glu-A1c and Glu-B1b were major alleles in the two countries. As for the Glu-D1 locus, the Glu-D1a allele predominated in China (84.6%) and Japan (55.0%). Allele frequencies of Glu-D1f encoding subunits 2.2+12 differed between China (1.8%) and Japan (35.1%). Five cultivars carrying the Glu-D1f allele were found in Chinese accessions, among

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which were two landraces originated from the Xinjiang District.

Zhang et al. (2002) who studied the genetic variation of HMW-GS using 5129 Chinese cultivars reported that most of the cultivars carried Glu-D1a, while several cultivars from the Xinjiang District carried Glu-D1f.

Wei et al. (2004) reported 14 kinds of HMW-GS in 169 cultivars from Southern Xinjiang. Glu-A1c at the Glu-A1 locus, Glu-B1b at the Glu-B1 locus and Glu-D1a at the Glu-D1 locus, were the major alleles. However, they studied mostly improved cultivars, and their data did not include the genetic variations of HMW-GS in the Xinjiang landraces.

The Xinjiang District is characterized by diverse geographic and climatic environments. As a result, the spring wheat cultivars are mainly cultivated in Northern Xinjiang, while the winter wheat cultivars predominate in the central Xinjiang and the winter wheat cultivars are cultivated in Southern Xinjiang. As standard cultivars for HMW-GS, we used Chinese cultivars from the Xinjiang District carried Glu-A1b, Glu-B1a, Glu-B1c, Glu-D1a, Glu-D1c, Glu-D1d, Glu-D1f, Glu-A1c, Glu-B1k, and Glu-B1l.

The Xinjiang District was two landraces originated from the Xinjiang District as well as the relationship with the alleles encoding HMW-GS using 5129 Chinese cultivars reported that most of the cultivars carried Glu-D1a, while several cultivars from the Xinjiang District carried Glu-D1f.

In the present report, we studied the genetic variation of the alleles encoding HMW-GS using landraces originating from the Xinjiang District as well as the relationship with the winter habit.

Materials and Methods

Plant materials

A total of 282 accessions of wheat landraces collected in the Xinjiang District and maintained at the Institute of Crop Variety Resources, Xinjiang Academy of Agriculture, were used. They were grouped into spring wheat cultivars (164) and winter wheat cultivars (118). The distinction between the spring and the winter wheat cultivars was made based on the heading performance of the plants sown in the middle of April in the field after low temperature treatment of seeds at 0 to 4°C in a growth cabinet. Compared with standard cultivars, the winter habit was scored as follows: 1 (very weak), 2 (weak), 3 (intermediate), 4 (strong), or 5 (very strong). Score 1 was designated representative of the spring wheat cultivars and scores 2 to 5 were representative of the winter wheat cultivars.

As standard cultivars for HMW-GS, we used Chinese Spring (Glu-A1c: null, Glu-B1b: 7 + 8, Glu-D1a: 2 + 12), Norin 61 (Glu-A1b: 2*, Glu-B1b: 7 + 8, Glu-D1f: 2.2 + 12), Eradu (Glu-A1a: 1, Glu-B1a: 17 + 18, Glu-D1a: 2 + 12), Cadoux (Glu-A1b: 2*, Glu-B1f: 13 + 16, Glu-D1a: 2 + 12), Harunoakebono (Glu-A1b: 2*, Glu-B1c: 7 + 9, Glu-D1d: 5 + 10) and near-isogenic lines of Harunoakebono substituted subunits (Glu-B1d: 6 + 8), (Glu-B1i: 17 + 18), (Glu-B1e: 20), (Glu-D1a: 2 + 12), or (Glu-D1c: 4 + 12) of HMW-GS.

Methods

1) Extraction of glutenin

Ground wheat samples of each cultivar weighing 20mg were put into 1.5 ml tubes. One ml of SA solution (50% propanol and 0.8 M Tris-HCl (pH 8.0)) was added and the samples were incubated at 65°C for 1 hr. The tubes were centrifuged at 2000 rpm for 1 min, and the supernatant was discarded. This manipulation was repeated. Then 200 μl of a SA1 solution (SA solution with 1% Dithiothreitol) was added to the precipitate and mixed with a vortex mixer. After incubation at 65°C for 1 hr, the mixture was centrifuged at 13000 rpm for 10 min, then 100 μl of supernatant was mixed with the same volume of SA2 solution (SA solution with 1.4%-vinylpyridine) in a sample tube and was incubated at 65°C for 30 min. Next, 0.8 ml of aceton was added and centrifuged at 13000 rpm for 10 min. After the supernatant was discarded, the precipitate was dried at 60°C for 5 min, and 100μl of a SC solution (20% glycerol, 6 M urea and 25 mM acetic acid) was added to dissolve the precipitate. Finally, sample buffer (2% SDS, 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 5% 2-mercaptoethanol and BPB) was added, and the mixture was boiled for 3 min and preserved as a sample for SDS-PAGE.

2) Electrophoresis

The glutenin fraction was separated by SDS-PAGE using a 10% running gel (10.3% acrylamide, 1.02% bis-acrylamide, 0.375 M Tris-HCl with pH 6.8, 0.2% SDS, 0.033% APS and 0.05% TEMED) and a 5% stacking gel (5.0% acrylamide, 0.5% bis-acrylamide, 0.125 M Tris-HCl with pH 6.8, 0.02% SDS, 0.033% APS and 0.1% TEMED). The poly-acrylamide gel used measured 14 cm × 10 cm × 1 mm. Electrophoresis was run at 20 mA for 6 hrs using an electrophoresis buffer (192 mM glycine, 25 mM Tris-HCl and 0.1% SDS). The gel was stained with a Coomassie brilliant blue G250 solution, and de-stained with ion-exchanged water until the background became clear.

Results

Electrophoretic patterns of the seed storage proteins revealed by SDS-PAGE of the wheat landraces from the Xinjiang District are shown in Fig. 1. Numbers of cultivars and allele frequencies at Glu-1 loci are listed in Table 1. The allele frequency of Glu-A1c encoding a null subunit was the highest (75.5%) at the Glu-A1 locus, followed by Glu-A1b encoding subunit 2* (22.3%). Glu-A1a encoding subunit 1 showed an allele frequency of 2.1% and was seldom observed (Table 1). Based on the winter habit of the materials used, the variation at the Glu-A1 locus was analyzed. Respective allele frequencies of Glu-A1c, Glu-A1b and Glu-A1a among the spring wheat cultivars were 76.6%, 21.3% and 1.8%, while those among the winter wheat cultivars were 73.7%, 23.7% and 2.5%, respectively. Accordingly, no significant differences in the allele frequencies at the Glu-A1 were observed between the spring and the winter wheat cultivars by the χ² test (P = 0.808).

As for the Glu-B1 locus, most of the Xinjiang landraces (90.8%) carried Glu-B1b encoding subunits 7 + 8. The frequencies of occurrence of Glu-B1a (encoding subunit 7), Glu-B1c (7 + 9), Glu-B1i (17 + 18), Glu-B1k (22) and Glu-B1d
A novel HMW-GS in Xinjiang wheat landraces

Table 1. Allelic variation of HMW-GS in wheat landraces from the Xinjiang Uygur Autonomous District of China

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Subunit</th>
<th>Spring wheat</th>
<th>Winter wheat</th>
<th>Total</th>
<th>Chi-square for uniformity</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. landraces (Freq. in %)</td>
<td>No. landraces (Freq. in %)</td>
<td>No. landraces (Freq. in %)</td>
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<td>No. landraces</td>
<td>No. landraces</td>
<td>No. landraces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu-A1</td>
<td>a</td>
<td>1</td>
<td>3 (1.8)</td>
<td>3 (2.5)</td>
<td>6 (2.1)</td>
<td>0.43</td>
<td>0.808</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>2*</td>
<td>35 (21.3)</td>
<td>28 (23.7)</td>
<td>63 (22.3)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>c</td>
<td>null</td>
<td>126 (76.8)</td>
<td>87 (73.7)</td>
<td>213 (75.5)</td>
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<td></td>
</tr>
<tr>
<td>Glu-B1</td>
<td>a</td>
<td>7</td>
<td>10 (6.1)</td>
<td>0 (0.0)</td>
<td>10 (3.5)</td>
<td>17.04</td>
<td>0.004</td>
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<tr>
<td></td>
<td>b</td>
<td>7+8</td>
<td>143 (87.2)</td>
<td>113 (95.8)</td>
<td>256 (90.8)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>7+9</td>
<td>6 (3.7)</td>
<td>1 (0.8)</td>
<td>7 (2.5)</td>
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</tr>
<tr>
<td></td>
<td>d</td>
<td>6+8</td>
<td>1 (0.6)</td>
<td>1 (0.8)</td>
<td>2 (0.7)</td>
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<td></td>
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<tr>
<td></td>
<td>e</td>
<td>20</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<tr>
<td></td>
<td>f</td>
<td>13+16</td>
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<tr>
<td></td>
<td>h</td>
<td>14+15</td>
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<td>0 (0.0)</td>
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<tr>
<td></td>
<td>i</td>
<td>17+18</td>
<td>4 (2.4)</td>
<td>0 (0.0)</td>
<td>4 (1.4)</td>
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<td></td>
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<tr>
<td></td>
<td>k</td>
<td>22</td>
<td>0 (0.0)</td>
<td>3 (2.5)</td>
<td>3 (1.1)</td>
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<td></td>
</tr>
<tr>
<td>Glu-D1</td>
<td>a</td>
<td>2+12</td>
<td>155 (94.5)</td>
<td>48 (40.7)</td>
<td>203 (72.0)</td>
<td>72.4</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>4+12</td>
<td>1 (0.6)</td>
<td>0 (0.0)</td>
<td>1 (0.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>5+10</td>
<td>2 (1.2)</td>
<td>1 (0.8)</td>
<td>3 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>e</td>
<td>2+10</td>
<td>6 (3.7)</td>
<td>0 (0.0)</td>
<td>6 (2.1)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>2.2+12</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>bp(t)</td>
<td>2.6+12</td>
<td>0 (0.0)</td>
<td>69 (58.5)</td>
<td>69 (24.5)</td>
<td></td>
<td></td>
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</tbody>
</table>

Fig. 1. SDS-PAGE patterns of HMW-GS subunits from Xinjiang wheat landraces and standard cultivar. Lanes 1 to 10: 1, Norin 61 (standard); 2, Shanshanbaidongmai; 3, Liangshandongmai; 4, Datoumai; 5, Nanjiangdongmai; 6, Akesuchunmai; 7, Tutouchunmai; 8, Daomaizi; 9, Jinbaoyin; 10, Chinese Spring (standard)

(6+8) were 3.5%, 2.5%, 1.4%, 1.1% and 0.7%, respectively. These five alleles were seldom observed in the Xinjiang District (Table 1). The frequency of occurrence of the Glu-B1b allele was slightly higher in the winter wheat cultivars (95.8%) than in the spring wheat cultivars (87.2%). Four rare alleles, Glu-B1a (6.1%), Glu-B1c (3.7%), Glu-B1i (2.4%) and Glu-B1d (0.6%) were found within the spring wheat cultivars, while another allele, Glu-B1k (2.5%), was found within the winter wheat cultivars (Table 1). The allele frequencies at Glu-B1 were significantly different between the spring and the winter wheat cultivars (P=0.004).

At the Glu-D1 locus, we identified a novel subunit that was co-expressed with subunit 12. The new subunit was slightly larger than subunit 2.2 (Fig. 2). Then we have tentatively named it subunit 2.6 encoded by a new allele Glu-D1bp(t). At the Glu-D1 locus, the major allele was Glu-D1a encoding subunits 2+12 with a frequency of 72.0%, followed by subunits 2.6+12 (24.5%). Allele frequencies of Glu-D1e (subunits 2+10), Glu-D1d (5+10) and Glu-D1c (4+12) were 2.1%, 1.1% and 0.4%, respectively. The Glu-D1f allele encoding subunits 2.2+12 was not observed in the present study, although its existence was reported by Nakamura and Fujimaki (2002) in wheat cultivars of the Xinjiang District. The differences in the allele frequencies at the Glu-D1 locus between the spring and the winter wheat cultivars were highly significant (P=0.000).

Considering the winter habit, the frequency of Glu-D1a was 94.5% in the spring wheat cultivars and this allele characterized the spring wheat cultivars of Xinjiang. On the other hand, the Glu-D1bp(t) allele encoding subunits 2.6+12...
was the major allele in the winter cultivars (58.5%), followed by the Glu-D1a allele encoding subunits 2 + 12 (40.7%). These two alleles at the Glu-D1 locus were characteristic of the winter wheat cultivars (Table 1).

Discussion

The present authors studied the genetic diversity among alleles encoding HMW-GS using 282 local wheat landraces originating from the Xinjiang District in China. The most frequent allele at the Glu-A1 locus was Glu-A1c (frequency 75.5%), followed by Glu-A1b (22.3%). These two alleles predominated at this locus. No clear differences in the allele frequencies at the Glu-A1 locus were observed between the spring and the winter wheat cultivars. Zhang et al. (2002) reported that the major allele at Glu-A1 was Glu-A1c (91.2%) among 5129 Chinese cultivars. Nakamura and Fujimaki (2002) also reported that Glu-A1c was the predominant allele in Chinese cultivars. According to a report by Payne et al. (1983), the frequencies of the Glu-A1c, Glu-A1b and Glu-A1a alleles were 44%, 28% and 28%, respectively among 300 cultivars worldwide. Morgunov et al. (1993) reported that the frequencies of the Glu-A1a, Glu-A1b and Glu-A1c alleles in 1,380 cultivars were 33%, 31% and 36%, respectively. Cultivars from China including the Xinjiang District and Japanese cultivars exhibited a distinctive feature, namely, a high frequency of Glu-A1c.

As for the Glu-B1 locus, the major allele was Glu-B1b in the spring wheat cultivars (87.2%) as well as in the winter wheat cultivars (95.8%). Zhang et al. (2002) also reported that the Glu-B1b allele was dominant at the Glu-B1 locus in the Chinese cultivars. Since we identified four other alleles (Glu-B1a, Glu-B1c, Glu-B1i and Glu-B1d) in the spring wheat cultivars, and three other alleles with lower frequencies (Glu-B1k, Glu-B1c and Glu-B1d) in the winter wheat cultivars, the Xinjiang spring cultivars appeared to be more polymorphic than the winter ones in relation to the Glu-B1 locus.

According to the report by Payne et al. (1983), the frequencies of the Glu-B1c, Glu-B1h, Glu-B1b and Glu-B1d alleles in the worldwide wheat collection were 30%, 25%, 19% and 18%, respectively. Morgunov et al. (1993) reported that the frequencies of the Glu-B1c, Glu-B1b, Glu-B1a and Glu-B1d alleles were 31%, 25%, 13% and 10%, respectively. Since the Xinjiang landraces as well as the other Chinese cultivars carried Glu-B1b at a very high frequency, the Chinese cultivars seemed to display a rather restricted allelic variation at the Glu-B1 locus.

As for the Glu-D1 locus, more than 90% of the spring wheat cultivars had Glu-D1a, while around 60% of landraces of the winter wheat cultivars showed a new subunit pair, 2.6 + 12. Since the molecular weight was approximately 160Kd, a larger value than that of subunit 2.2 (Fig. 2), this subunit appeared to be the largest among the known HMW-GS. Further analyses should be carried out to clarify the effect of this subunit on the properties of gluten.

According to Nakamura and Fujimaki (2002), Glu-D1a predominated at the Glu-D1 locus in China (84.6%) and Japan (55.0%), which was supported by the findings of Zhang et al. (2002). However, the relationship between allelic variation and winter habit had not been reported.

In the present investigation, the presence of a large difference in allele frequencies at the Glu-D1 locus between the spring and the winter wheat cultivars of the Xinjiang landraces was disclosed, which suggests that the origin of the winter wheat cultivars is different from that of the spring ones in the Xinjiang District.

Nakamura and Fujimaki (2002) reported the presence of the Glu-D1d allele encoding subunits 5 + 10, which improve the bread-making quality, in two Japanese and 29 Chinese cultivars. In the present study, we detected this allele in only three landraces and concluded that this allele was rare in Xinjiang. They also reported that the allele frequency of Glu-D1d was only 1.8% (5 cultivars) in the Chinese wheat cultivars while 35.1% in the Japanese ones. Since two of the five Chinese cultivars carrying Glu-D1d were Xinjiang landraces, Nakamura (2004) suggested the existence of some relationships between Japanese and Xinjiang wheat in the origin of this allele. Zhang et al. (2002) also reported that some cultivars carried Glu-D1f in the Xinjiang District. However, in the present study in which many Xinjiang landraces were examined, no cultivar carried Glu-D1f, while 69 landraces showed a new subunit pair, 2.6 + 12.

We concluded that there was no relationship between the Xinjiang landraces and Japanese cultivars. Therefore, the origin of the Glu-D1d allele in the Japanese cultivars remains to be elucidated. Further studies on the genetic diversity of HMW-GS in landraces from other regions of China and the Korean peninsula should be carried out.

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