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Frequency of the High-Molecular-Weight Glutenin Allele in Asian Hexaploid Wheat (*Triticum aestivum* L.) and the Transmission Route through Which the Wheat May Have Reached Japan, the Most Geographically Remote Region of Wheat Production in the World

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The frequency of the *Glu-D1f* allele in Japanese, Chinese, and other Asian hexaploid wheat varieties was analyzed in order to investigate a possible transmission route for hexaploid wheat to the Far East, Japan. The 1380 published data sets were compared to the results for 1107 hexaploid Asian wheat varieties which were determined in this study. The frequency of the *Glu-D1f* allele was clearly different between areas; the allele was present from northern and southern Japan, from Xinjiang, Jiangsu, Zhejiang, and Beijing in China, and from Afghanistan. A high frequency of the high-molecular-weight glutenin *Glu-D1f* allele was found predominantly in southern Japan. This distribution of an adaptively neutral character suggests a specific route of transmission for hexaploid wheat to eastern China and the Far East, Japan. It was introduced from Afghanistan, carried to Xinjiang (in northwest China), Jiangsu, and Zhejiang (in southeast China), and then to southern Japan along the so-called Silk Road. It is believed that cultivated hexaploid wheat originated in the Middle East and the Near East and was carried along the Silk Road through China to the Far East, Japan. Japan is the most geographically remote region of wheat production in the world. During the course of its long journey and its adaptation to diverse local environments, Japanese hexaploid wheat has developed a unique composition of glutenin *Glu-D1* alleles. The frequency of this allele in different wheat varieties allowed us to hypothesize a possible route for the transmission of hexaploid wheat into the Far East, Japan.

KEYWORDS: Hexaploid wheat; glutenin; Asia; transmission route; remote region

INTRODUCTION

The high-molecular-weight (HMW) glutenin subunits are characterized by molecular weights between 80 000 and 145 000 and a complex biochemical structure involving disulfide bonds (*1*). This group of endosperm proteins has been extensively explored in the past 20 years (*2*). Genetic analysis revealed that variation in HMW subunits is controlled by alleles at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci on the long arms of chromosomes 1A, 1B, and 1D, respectively (*3*). It is well known that hexaploid wheat (*Triticum aestivum* L., $2n = 42$, AABBDD), appeared about 7000 years ago in the Middle East and the Near East (*4*, *5*). It was then transmitted from its origin to Europe, Africa, southern Asia, and China. It is known that some wheat varieties were transported along the Silk Road through China to the Far East, Japan. Little is known, however, about the actual route of transmission of hexaploid wheat into Japan. The objective of this study was to analyze the distribution of the *Glu-D1* allele throughout Asia, and thus to determine the transmission route by which hexaploid wheat reached Japan, the most geographically remote region of hexaploid wheat production in the world. I concentrated my studies predominantly on the variation of the HMW glutenin *Glu-D1f* allele and the factors that affected its distribution in different parts of the world. The 1380 published data sets were compared to the results for 1107 hexaploid Asian wheat varieties which were determined in this study. In total, 1380 published cultivars from 21 countries and 1107 Asian varieties were included in these comparisons.

EXPERIMENTAL PROCEDURES

I investigated the allelic composition of the HMW glutenin subunit from 131 Japanese improved cultivars and 174 Japanese, 353 Chinese, 150 Turkish, 3 Syrian, 6 Israeli, 4 Iranian, 1 Iraqi, 23 Indian, 15 Pakistani, 7 Butanese, 66 Nepalese, 1 Myanmar, 1 Filipino, 2 Thailand, 3 Indonesian, 46 Taiwanese, and 21 Afghanistan landraces of hexaploid wheat. These were investigated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), according to the procedure described by Payne et al. (*6*). The 353 Chinese wheat varieties were from Heilongjiang, Jilin, Liaoning, Hebei, Beijing, Shandong, Shanxi, Xian, Hangzhou, Zhejiang, Henan, Jiangsu, Ningxia, Gansu, Xinjiang, Sichuan, Anhui, and Jiangxi, respectively. The Japanese, Chinese, and other Asian hexaploid wheat materials were provided by the National Institute of Agrobiological Resources in Japan. Published data for 1380 cultivars were available concerning the worldwide distribution of the *Glu-1* alleles (*3*, *7*−*16*), and the frequency of the HMW glutenin alleles

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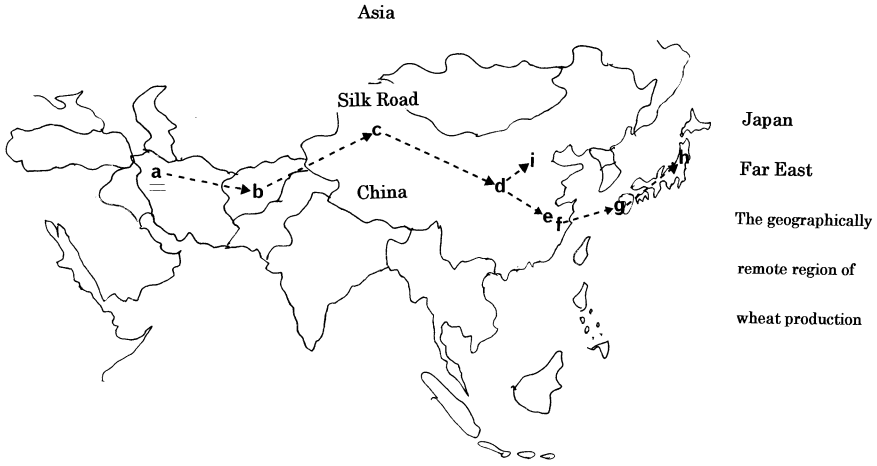

Figure 1. Possible transmission route for hexaploid wheat (*Triticum aestivum* L.) into Japan: a, Middle East and Near East (an origin of hexaploid wheat); b, Afghanistan; c, Xinjiang; d, Xian; e, Jiangsu; f, Zhejiang; g, southern Japan; h, northern Japan; i, Beijing.

was available for Japanese wheat varieties. These data sets were compared to the results for 1107 hexaploid Asian wheat varieties which were determined in this study. In total, 1380 published cultivars from 21 countries and 1107 Asian varieties were included in these comparisons.

Gels were made up to 10% (w/v) acrylamide and 0.2% (w/v) bisacrylamide containing 1.5 M Tris-HCl at pH 8.8 and 0.27% SDS. The stacking gel contained 0.25 M Tris-HCl at pH 6.8. Wheat flour (10 mg) was suspended in 300 μL of 0.25 M Tris-HCl buffer (pH 6.8) containing 2% (w/v) SDS, 10% (v/v) glycerol, and 5% (v/v) mercaptoethanol, and the suspension was shaken for 2 h at room temperature. The suspension was heated at 95 °C for 3 min. The top portion of the supernatant was collected after centrifugation for 3 min at 12 000 rpm, and 30 μL of the extract was loaded into each of the gel slots. The electrode buffer was 0.025 M Tris−glycine at pH 8.3, containing 0.1% (w/v) SDS. Electrophoresis was conducted at 30 mA constant current for 4 h until the bromophenol blue dye ran to the end of the gel. The gels were stained for several hours with Coomassie Blue R in aqueous ethanol and acetic acid. HMW glutenin subunits were identified and numbered according to the system of Shewry and Tatham (*17*). The samples were also analyzed using the milled flour from 30 grains of each variety or landrace. To determine the electorophoretic mobility of each HMW glutenin subunit by SDS−PAGE, standards were included (Bezostaya, Champlein, Chinese Spring, Danchi-komugi, Dunav, Federation, Gabo, Hobbit, Hope, Norin 61, Lancota, Sappo, Serbian)—the masses of these standards reflected the expected masses of the subunits (*18*−*20*). Chi-square values were calculated to test for frequency differences among the *Glu-D1f* alleles. Frequencies of Japanese improved cultivars, Japanese landraces, those from other Asian areas (Turkish, Syrian, Israeli, Iranian, Iraqi, Indian, Pakistani, Butanese, Nepalese, Myanmar, Filipino, Thailand, Indonesian, Taiwanese) and Afghanistan were analyzed relative to the frequencies of Chinese varieties through independent pairwise comparisons. Chi-square values were obtained by considering the frequencies of Chinese varieties as expected values, and the frequencies of the cultivars from those areas were observed in this study.

RESULTS AND DISCUSSIONS

In this study, the 145 kDa HMW glutenin subunit controlled by the *Glu-D1f* allele was frequently found among improved cultivars in Japan, as well as in the landraces (**Table 1**). On the other hand, only a few of the Chinese and Afghani wheat varieties possessed this allele. In this study I compared our results with the results from similar studies of *Glu-1* alleles throughout the world. There were 1380 published data available concerning the worldwide distribution of the *Glu-1* alleles (*3*, *7*−*16*), and the frequency of the HMW glutenin alleles was available for Japanese wheat varieties. These 1380 data sets were compared to the results for 1107 hexaploid Asian wheat varieties which were determined in this study. The published data for 1380 cultivars from 21 hexaploid wheat-producing countries were included in these comparisons, the *Glu-D1f* allele has been reported to be a rare allele in the worldwide distribution of *Glu-1* alleles (*13*). It has also been reported that the product of this allele was more hexaploid in Japanese wheat seed storage proteins than anywhere else in bread-culture zones (*1*). The present study also showed that the *Glu-D1f* allele was more hexaploid in Japan than elsewhere in Asia (**Table 1**). The allelic frequency of this subunit was shown to be in excess of 35% among improved Japanese cultivars and 25.3% among Japanese landraces, while it was found in only 5 Chinese varieties (2 varieties from Xinjiang, **Figure 1c**; 1 variety from Jiangsu, **Figure 1e**; 1 variety from Zhejiang, **Figure 1f**; 1 variety from Beijing, **Figure 1i**) and 2 Afghani varieties (**Figure 1b**) of wheat (see also **Table 1**). This distribution of an adaptively neutral character suggests a specific route of transmission for hexaploid wheat to eastern China and the Far East, Japan. It was introduced from Afghanistan, carried to Xinjiang (in northwest China), Jiangsu, and Zhejiang (in southeast China), and then to southern Japan along the so-called Silk Road. It is believed that cultivated hexaploid wheat originated in the Middle East and the Near East and was carried along the Silk Road through China to the Far East, Japan.

In the present study, the carriers of the *Glu-D1f* allele were found to be distributed across a limited region of Asia, only in southern (Kanto, Tokai, Kinki, Chugoku, Shikoku, and Kyushu

Table 1. Comparison of *Glu-D1f* Allele Frequency for Afghani, Chinese, Japanese, and Other Asian Hexaploid Wheats (*Triticum aestivum* L.)

country	total no. of varieties examined	no. of varieties carrying *Glu-D1f* allele	freq (%)	χ^2 value
other Asian areas[a]	428	0	0.0	1.4[b]
Afghanistan	21	2	9.5	46.86[b]
China[c]	353	5	1.4	
Japanese landrace	174	44	25.3	408.01[b]
Japanese improved variety	131	46	35.1	811.21[b]

[a] Other Asian areas: Turkey, Syria, Israel, Iran, Iraq, India, Pakistan, Bhutan, Nepal, Myanmar, Philippine, Thailand, Indonesia, and Taiwan. [b] Significant at the 0.01 probability level. [c] The *Glu-D1f* allele frequency of Chinese hexaploid wheats: the "expected" class.

areas) and northern (Hokkaido, Tohoku, Hokuriku, and Nagano areas) Japan, in Xinjiang (northwest), Jiangsu, Zhejiang (southeast), and Beijing (northeast) China, and in Afghanistan. However, the allele is rare in wheat varieties from northern Japan, China, and Afghanistan (**Table 1**, **Figure 1**). Results from this study also suggest that there are no other wheat cultivars in any other region in Asia which possess the *Glu-D1f* allele.

It is well known that the frequencies of *Glu-A1*, *Glu-B1*, and *Glu-D1* alleles differ among hexaploid wheat varieties from different countries (*1*, *13*, *20*, *21*). It is believed that cultivated hexaploid wheat originated in the Middle East and the Near East and was carried along the Silk Road through China to the Far East, Japan. Japan is remote from most other wheat-growing areas in Asia. In the course of its long journey and its adaptation to diverse local environments, Japanese hexaploid wheat appears to have depleted its genetic diversity. The frequency of the *Glu-D1f* allele differed between the Japanese and the other Asian hexaploid wheat varieties. Therefore, it is possible that all Japanese wheat varieties show a hexaploid heritage: this would explain the similarities in *Glu-1* patterns for all Japanese wheat. It is said that there were four routes by which people moved across Asia in ancient times. The first of these routes, the so-called Silk Road, ran through Afghanistan, Xinjiang (in northwest China), Gansu, Xian (in northeast China), Jiangsu, and Zhejiang (in southeast China), eventually reaching Japan (**Figure 1**). The second route ran through Pakistan, India, and Myanmar and then to Yunnan in China. The third route ran through Nepal or Pamir, Tibet, and into Sichuan in southwest China or Xian in northeast China. The final route was directly into southern China by boat from India (*4*, *5*, *22*, *23*). With regard to these routes across Asia, the distribution of the *Glu-D1* alleles is very interesting. Of the *Glu-D1* alleles, *Glu-D1a* and *Glu-D1f* alleles were common in Japanese hexaploid wheat; the *Glu-D1a* allele was hexaploid in wheat varieties from all over Japan, whereas the *Glu-D1f* allele was present predominantly in the south (*24*). This finding may also suggest a transmission pattern for hexaploid wheat in Japan. First, hexaploid wheat (characterized by the *Glu-D1a* or the *Glu-D1f* allele) arrived in Japan and became distributed across southern Japan; wheat was then transmitted northward through Japan. As a consequence of this, the northern Japanese hexaploid wheat varieties carried predominantly the *Glu-D1a* allele. This allele is linked to some gene which makes the wheat suitable for cultivation in the colder winters of northern Japan, as compared to the *Glu-D1f* allele, which is not linked to this trait (*24*).

The high frequency of the *Glu-D1f* allele in southern Japan may be due to the selective advantage conferred either by the *Glu-D1f* allele itself or by the action of another linked gene. Whichever gene may be responsible confers a trait which makes the wheat suitable for cultivation in southern Japan, or is responsible for wheat-flour quality (*25*).

The *Glu-D1f* allele has been regarded as a characteristic glutenin allele for Japanese wheat cultivars. In fact, while many hexaploid wheat cultivars in southern Japan possess this *Glu-D1f* allele, most of the northern Japanese cultivars do not. By comparison, consideration of β-amylase isozyme types shows that both types A and J are hexaploid wheat in Japan. Type A was found throughout Japan, whereas type J was present predominantly in southern regions (*23*). This distribution of β-amylase isozyme types is similar to that of *Glu-D1f* alleles in Japanese hexaploid wheat seed storage proteins.

It has previously been reported that *Glu-1* alleles are not associated with ecogeographical parameters in a worldwide context (*13*). However, results from my study suggest that the *Glu-D1f* allele is associated with ecogeographical parameters within Japan, a finding of great interest to Japanese wheat breeders and cereal chemists (*25*). In Afghanistan, Xinjiang in northwest China, Jiangsu Zhejiang in southeast China, and southern Japan, spring and facultative types are sown in the autumn or in the spring, respectively. This style of cultivation is specific to these regions. Genotypes which were suitable for this type of hexaploid wheat cultivation in China may have been selected during the process of transmission to Japan. All of the varieties processing the *Glu-D1f* allele in northern Japan are sown in the autumn, both spring and facultative types (*25*).

The founder principle can explain many instances of rapid speciation and high local frequencies of alleles that appear rarely in other areas (*26*). The hexaploid wheat brought to Japan likely would have included a very limited subset of the wheat found in China; the founder effect described often in evolutionary literature is associated with gene frequencies on islands such as Japan (*24*). It is believed that a cultivated hexaploid wheat that originated in the Middle East and the Near East traveled via the Silk Road through China to the Far East, Japan. Japan is the most remote region in the world for hexaploid wheat production. With this design from China, the hexaploid wheats were exposed to a selective bottleneck induced by the external environment, as well as a founder effect (since all populations went through a bottleneck of small size); consequently, the selective bottleneck was extremely intense and, in fact, most ancestral varieties may be become extinct in Japan (*24*).

In this study, the specific distribution of an adaptively neutral characteristic (the *Glu-D1f* allele) suggested a transmission route for hexaploid wheat into eastern China and Japan. The hexaploid wheat was introduced from Afghanistan, moved through Xinjiang (in northwest China), into Jiangsu and Zhejiang (in southeast China), and then into southern Japan along the so-called Silk Road. The results presented here indicate that *Glu-D1* allele analysis is a powerful tool in the investigation of the real transmission routes of hexaploid wheat across Asia and into the Far East, Japan.

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