Analysis of the HMW Glutenin Subunits in Immature Seeds for Selection of Bread-making Lines in Wheat (*Triticum aestivum* L.)

Mitsuru Watanabe, Ken Tokuyasu and Akiko Sato

*Tohoku National Agricultural Experiment Station, Morioka, Iwate, 020-01 Japan*

1) Present address: *National Food Research Institute, Kannondai Tsukuba, Ibaraki 305 Japan*

**Summary**

The compositions of the high-molecular-weight (HMW) glutenin subunits affecting bread-making quality were analyzed in immature grains of Recital, Palo Duro, Flamura, Pliska, Monopol, and Nanbukomugi using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). While varietal differences were found in the relationship between the maturity of grains and detection of the HMW glutenin subunits, we could determine all of the subunits in mature grains using immature grains of either of following conditions: (1) on and after 20 days after flowering, and (2) below 55% of water content in whole grains. In this stage, enough plants were obtained from half grains containing embryos. The results suggest that we can efficiently select breeding materials before maturity by the composition of the HMW glutenin subunits. The selection of breeding materials before maturity is useful for breeding bread-making wheat in the Tohoku district that has a short period from harvest to sowing.

**Key Words:** *Triticum aestivum*, immature grains, HMW glutenin subunit, SDS-PAGE, water content in whole grains, the number of days after flowering.

**Introduction**

Payne *et al.* (1987) discussed the relationship between bread-making quality and the compositions of the high-molecular-weight (HMW) glutenin subunits in wheat (*Triticum aestivum* L.). They gave marks on the HMW glutenin subunits based on the contribution to good bread-making. The score of 5 + 10 subunits (4 points) was the highest among all the HMW glutenin subunits. Furthermore, Lookhart *et al.* (1993) reported that 5 + 10 subunits have a strong association with good bread-making quality in the U.S. These results suggest that the presence of 5 + 10 subunits is essential in high quality bread-making wheats. Almost all wheat varieties for bread-making in Canada contain 5 + 10 subunits (Odean *et al.* 1989), whereas Harubikari was the only Japanese registered variety that contained these subunits Yasumuro 1989). Recently, we have analyzed the composition of the HMW glutenin subunits of Harunoakebo-no, which was raised in 1993, and detected 5 + 10 subunits in this variety. At the Tohoku National Agricultural Experiment Station, introduction of the allele Glu-D 1 d encoding 5 + 10 subunits has been tried for breeding new varieties suitable for bread-making (Hoshino *et al.* 1994). In addition, a lot of characteristics, including yielding ability and tolerance to pre-harvest sprouting, have been tested in the period from harvest (July) to sowing (September). If we can select wheat lines containing 5 + 10 subunits before maturity, there is no necessity for examining many characteristics for wheat lines lacking these subunits.

The present work is an attempt to investigate when the HMW glutenin subunits were detectable in the stage of maturity using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for efficient selection of good bread-making wheat lines.

**Materials and Methods**

**Plant materials**

The six wheat varieties, Recital, Palo Duro, Flamura, Pliska, Monopol, and Nanbukomugi were used. Wheat grains of all the varieties were sown in the field of the Tohoku National Agricultural Experiment Station on September 21, 1992. At flowering, spikes were marked and harvested every five days from 10 days to 40 days later. We used five plants in each variety. Five or six grains in the center part of the spike were dried at 60 °C for 24 h and used for analysis of the HMW glutenin subunits. The rest of the grains were used for determining water content in whole grains.

**Water content determination**

Whole grains (2 g) of each variety were taken into an individual aluminum cup, weighed accurately, and dried at 135 °C for 20 h. The cups were left in the desiccator for 30 min and weighed again.

**Identification of the HMW glutenin subunits**

Electrophoresis (SDS-PAGE) was conducted according to Kagawa's system (Kagawa *et al.* 1988) with 7.5% acrylamide gel, since the separation of the HMW glutenin subunits (especially 2* and 5) using this system was better than that using Laemmli’s system (Laemmli 1970). Dried grains were hammer-milled and put through a sieve to remove pericarps. The powdered sample (5 mg) was suspended in 0.2 ml of extraction buffer containing 62.5 mM Tris-HCl, pH 6.8, 5% (v/v)
2-mercaptoethanol, 2 % (w/v) SDS and 10 % (v/v) glycerol. The suspended sample was heated in boiling water for 2 min, and centrifuged at 10,000 x g for 5 min and then the supernatant (0.015 ml) was applied for analysis of the HMW glutenin subunits by SDS-PAGE. After electrophoresis, the gels were stained with Coomassie Brilliant Blue (CBB) R-250. Haruhikari (the composition of the HMW glutenin subunits: chromosome 1 A encoded = null, 1 B = 7+8, 1 D = 5+10) (Yasumuro 1989), Lancer (1 A = 2*, 1 B = 7+8, 1 D = 5+10) (Odean et al. 1989) and Federation (1 B = 20) (Payne et al. 1983) were used as standard reference varieties in order to assign numbers to the HMW glutenin subunits according to the system of Payne et al. (1983).

Germination test of half grains
Nanbukomugi and Monopol were used for the germination test. Dried 50 half grains, containing embryos, of each variety were treated with 1 % (v/v) H2O2 to break dormancy. They were sown in 30 ml of culture soil in petri dishes (9 cm diameters), and covered with 40 ml of soil. After adding 30 ml of water to each dish, they were stored at 20 °C. Ten days later, the germination rate was tested.

Results and Discussion

Water content in whole grains decreased through maturation, and it differed among varieties on the same day after flowering. The maximum difference was 11.7 % (Pliska: 44.0 %, Monopol: 32.3 %) 40 days after flowering. From this result, we described the number of days after flowering and the water content in whole grains as indexes of the stage of maturation. Therefore, the time of determination of the HMW glutenin subunits was expressed by these two indexes.

The compositions of the HMW glutenin subunits of the six varieties, identified by SDS-PAGE, were listed in Table 1. Except for Nanbukomugi, all of the varieties contained 5 ± 10 subunits. Fig. 1 shows the results of electrophoresis using immature grains of Pliska and Monopol. The HMW glutenin bands, which were controlled by chromosomes 1A, 1B and 1D, appeared at the same stage of maturity.

Fig. 2 shows whether HMW glutenin bands were detectable on the basis of the number of days after flowering and water content in immature grains. In Monopol, distinct HMW glutenin bands appeared 15 days after flowering, and 20 days in other varieties. At the appearance of these bands, the water content in whole grains ranged from 57.3 % (Palo Duro) to 68.7 % (Monopol) on an average. Consequently, we were able to determine all of the HMW glutenin subunits found in mature grains using immature grains of either of the following conditions: (1) on and after 20 days after flowering, and (2) below 55 % of water content in whole grains. Germination rate of half grains in this stage was 78.3 % in Nanbukomugi and 89.3 % in Monopol, so we were able to get about 80 % of plants in the next generation from half grains. For these reasons, we can efficiently select breeding materials for bread-making before maturity by the compositions of the HMW glutenin subunits.

The varietal differences of the time of determination of the HMW glutenin subunits may depend on glutenin content in immature grains. Watanabe et al. (1957, 1958) investigated changes in the content of glutenin and other components at various stages of maturity. According to their results, the content of glutenin in grains increased gradually until 40 days after heading date (heading date is five to seven days before flowering) in semi-hard

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Table 1. The composition of the HMW glutenin subunits of wheat varieties used

<table>
<thead>
<tr>
<th>Variety</th>
<th>Origin</th>
<th>HMW glutenin subunit</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 A</td>
</tr>
<tr>
<td>Recital</td>
<td>France</td>
<td>2 *</td>
</tr>
<tr>
<td>Palo Duro</td>
<td>USA</td>
<td>2 *</td>
</tr>
<tr>
<td>Flamina</td>
<td>Romania</td>
<td>2 *</td>
</tr>
<tr>
<td>Pliska</td>
<td>Bulgaria</td>
<td>null</td>
</tr>
<tr>
<td>Monopol</td>
<td>Canada</td>
<td>1</td>
</tr>
<tr>
<td>Nanbukomugi</td>
<td>Japan</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 1. SDS-PAGE patterns of the HMW glutenin subunits in immature grains of Pliska (A) and Monopol (B) under Kagawa's system (7.5 % acrylamide gel). Numbers above patterns indicate the number of days after flowering.
wheat. Further study is required to analyze whether the accumulation rate of storage protein during maturation differs among varieties.

In the present study, the gels of SDS-PAGE were stained with CBB-R250. Due to the sensitivity of silver stain, it is approximately 100-fold higher than that of CBB stain (Switzer et al. 1979), and a small amount of glutenin may be detected at the early stage of maturity. Therefore, the time of determination of the HMW glutenin subunits with silver stain may be a little earlier than that with CBB-R250 stain.

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Literature Cited


