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Genetic Characteristic of High Molecular Weight Glutenin Subunits in Somatic Hybrid Wheat Lines—Potential Application to Wheat Breeding

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Analysis of 17 derivatives from a somatic fusion between common wheat (*Triticum aestivum*) and tall wheat grass (*Thinopyrum ponticum*) showed a diversity of high molecular weight glutenin subunit (HMW-GS) compositions. On the basis of the inheritance of HMW-GS patterns, the derivatives were either (i) bred true over four successive generations, (ii) generated a few novel HMW-GS combinations at each generation, or (iii) showed highly unstable HMW-GS compositions. HMW-GS analysis of F_5 seed and each single seed-generated F_6 progenies further revealed that most of the HMW-GS had genetic stability. The variations of HMW-GS were inferred to occur in early generations and were maintained thereafter. Low molecular weight glutenin subunits (LMW-GS) in hybrid lines with high or low bread-making quality, classified into the first pattern, were compared. The result showed that hybrid lines with the uniform HMW-GS patterns also have identical LMW-GS patterns. The *Glu-1* quality score was inferred to be relatively significant to the sodium dodecyl dulfate sedimentation value, as well as to correlate with the gluten exponent and contents of dry gluten and proteins. Sexual hybridization between high-quality somatic hybrid progeny II-12 and Chinese Spring (CS) showed that these high-quality HMW-GS genes could entail progenies. There was not subunit variation in the progenies of II-12 × CS. Therefore, sexual hybridization between somatic hybrid line and cultivars can transfer novel high-quality HMW-GS of somatic hybrids and benefit wheat breeding.

KEYWORDS: ***Triticum aestivum*; *Thinpyrum ponticum*; somatic hybrid lines; HMW-GS variation and inheritances; wheat quality breeding**

INTRODUCTION

Seed storage proteins largely determine the functional quality of wheat flour. They are classified into aggregating glutenins and monomeric gliadins. The glutenins are further subdivided, on the basis of their subunit molecular weight, into the high molecular weight glutenin subunits (HMW-GSs) and low molecular weight glutenin subunits (LMW-GSs), and these are held together in vivo by intra-/interchain disulfide bonds (*1*, *2*). About 10% of the wheat endosperm storage protein is in the form of HMW-GS, and about 40% is LMW-GS. Together, the glutenins are responsible for the elasticity of the gluten, while the gliadins determine its extensibility (*2*). Apart from the overall content of HMW-GS, LMW-GS, and gliadins (*3*), both the ratio of glutenin to gliadin (*4*) and the number/ratio of HMW-GS and LMW-GS (*5*, *6*) affect the bread-making quality of wheat flour. The association of HMW-GS with bread-making quality enabled Payne and co-workers to assign "quality scores" to specific alleles (*1*, *2*, *7*−*9*). Thus, for example, the subunit pair *1Dx5* + *1Dy10* is superior to *1Dx2* + *1Dy12*, while *1Bx17* + *1By18*, *1Bx13* + *1By16*, *1Bx14* + *1By15*, and *1Bx7* + *1By8* are superior to *1Bx7* + *1By9*, *1Bx6* + *1By8*, or *1Bx7* (*1*, *2*, *10*−*13*). For many years, a simple summing of the individual HMW-GS scores has been used as a selection tool in bread-making quality wheat-breeding programs (*1*, *14*−*16*).

HMW-GS genes are not limited to common wheat, and they can in many cases be introduced into wheat by sexual hybridization. The impact of such exotic alleles has not been greatly explored (*17*−*19*). In China, sexual hybridization between common wheat and tall wheat grass [*Thinpyrum ponticum* (Podp.) Barkworth & Dewey, StStEeEbEx, 2n=70] has given rise to the Xiaoyan series of high-quality wheat cultivars, which contain various HMW-GS combinations different from the parent wheat but similar to other bread wheats, such as Xiaoyan no. 6 (1, 14 + 15, 2 + 12), Xiaoyan no. 503 (1, 7 + 8, 2 + 12), Xiaoyan no. 504 (1, 7 + 8, 2 + 12), Xiaobingmai no. 33 (1, 7 + 8, 5 + 10), and Shanmai no. 150 (null, 14 + 15, 5 + 10) (*20*). Besides, we have also reported some new HMW-GS subunits similar to these in common wheat in the somatic hybrids of *Triticum aestivum*/*T. ponticum* (*21*).

Tall wheat grass has a high seed protein content, apart from its manifold resistances to stress and disease, and represents an important wild relative for wheat improvement (*22*). Asymmetric somatic hybridization between the protoplasts of common wheat and tall wheatgrass has been achieved as described elsewhere

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Table 1. HMW-GS Pattern and SDS Sedimentation Value of Somatic Hybrid F_5 Progenies between Wheat and *T. ponticum*

hybrid line no.	SDS sedimentation value	HMW-GS pattern
1	44.0	2*, 13 + 16, 5 + 12
2	45.8	2*, 13 + 16, 5 + 12
3	44.5	2*, 13 + 16, 5 + 12; null, 13 + 16, 2 + 12; null, 7 + 9, 2 + 12
4	49.0	2*, 13 + 16, 5 + 12; null, 13 + 16, 2 + 12; null, 7 + 9, 2 + 12; 2*, 7 + 9, 5 + 12
5	47.5	2*, 13 + 16, 5 + 12; 2*, 7 + 9, 5 + 12; null, 7 + 9, 5 + 12
6	54.8	2*, 13 + 16, 5 + 12
7	45.5	2*, 13 + 16, 5 + 12
8	47.0	2*, 13 + 16, 5 + 12; 2*, 7 + 8, 5 + 12; 1, 13 + 16, 5 + 12; 1, 7 + 8, 5 + 12; null, 7 + 8, 2 + 12; null, 13 + 16, 2 + 12; null, 13 + 16, 5 + 12; null, 7 + 8, 5 + 12
9	43.2	1, 7 + 8, 2 + 12; 1, 7 + 8, 5 + 12; 1, 13 + 16, 5 + 12; 1, 13 + 16, 2 + 12; null, 13 + 16, 5 + 12; null, 13 + 16 + 8, 5 + 12; null, 7 + 8, 5 + 12; 1, 7 + 8, 2 + 5 + 12; 1, 13 + 16 + 8, 2 + 12; 1, 16 + 7+8, 5 + 12
10	48.5	1, 7 + 9, 5 + 12
11	67.0	1, 7 + 9, 5 + 12; 1, 7 + 9, 2 + 12; null, 7 + 9, 2 + 12; null, 7 + 9, 2 + 5 + 12
12	17.0	null, 7 + 9, 2 + 12
13	24.5	null, 7 + 9, 2 + 12
14	18.5	null, 7 + 9, 2 + 12
15	23.0	null, 7 + 9, 2 + 12
16	45.0	1, 7 + 9, 5 + 12
17	49.0	2*, 13 + 16, 5 + 12
Jinan177	32.5	null, 7 + 9, 2 + 12

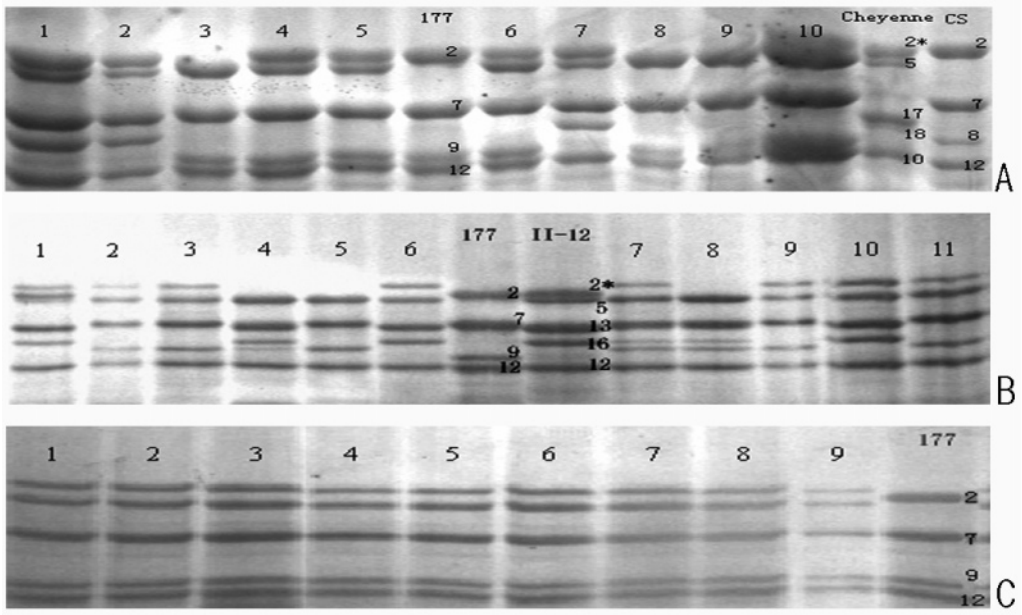

Figure 1. SDS–PAGE analysis of HMW-GS from somatic hybrid F_5 progenies between wheat and *T. ponticum*. (**A**) Sample 5, (**B**) sample 9, and (**C**) sample 10; 1–11, seeds randomly selected from different hybrid lines.

(*22*), and this has resulted in the introduction of many wheatgrass chromosome fragments into the wheat genome (*23*). The hybrid progenies express protein characteristics of both parents but also express some of which differ from both parents but appear similar to species found in other wheat cultivars (*24*). A number of the hybrid derivatives are superior to the parental wheat with respect to functional quality.

In this report, we investigate the inheritance of and variation in HMW-GS and LWM-GS in these hybrid derivatives and explore the relationship between HMW-GS composition and bread-making quality.

MATERIALS AND METHODS

The plant materials derive from a highly asymmetric somatic fusion between wheat cv. Jinan 177 and UV-irradiated tall wheat grass (*22*). The regenerant line II-1 produced five selfed progenies. Over 98% of the F_2 generation was stable in phenotype and propagated to the F_8 generation, which was the somatic hybrid line II-12 (sample 17) in this report. A few F_2 generations (less than 2%) were separated by distinct phenotypes and were maintained thereafter. We bred, respectively, the separate lines and got a series of F_3−F_8 selfed lines (**Table 1**), which were samples 1−16. F_1 crosses were made between line II-12 (reported to be of high quality) (*21*) and cv. Chinese Spring (CS). The parental cv. Jinan 177 (177, with subunits null, 7 + 9, 2 + 12) and other standard cultivars CS (with subunits null, 7 + 8, 2 + 12), Cheyenne (a high-quality breed wheat cultivar with subunits 2*, 17 + 18, 5 + 10), Yanyou 361 (a high-quality common wheat cultivar with subunits 1, 17 + 18, 5 + 10), and 4072 (a high-quality breed wheat cultivar with subunits 1, 13 + 16, 5 + 10) were used to identify individual HMW-GSs. Different plant materials were separately grown in an isolated greenhouse to avoid any cross-pollination from other grasses and wheat cultivars.

For the extraction of glutenins, a single seed was crushed into a tube, and the flour was suspended in 0.1 M Tris-HCl (pH 6.8), 3.3% (w/v) sodium dodecyl sulfate (SDS), 1% (w/v) dithiothreitol, 17% (v/v) glycerol, and 0.025% (w/v) Pyronin Y. The volume of extraction buffer was 7 μL/mg flour. After incubation for 10 min in a boiling water bath, the sample was centrifuged for 30 min at 12000 rpm. SDS−polyacrylamide gel electrophorersis (PAGE) analysis used a discontinuous buffer system (*25*), with a 4% stacking gel (pH 6.7) and a 7.5−

Table 2. HMW-GS Composition in Generations F_5–F_8 from the Somatic Hybrid between Wheat and *T. ponticum* (Nos. 1–17)

hybrid line no.	hybrid generations	HMW-GS pattern
1, 2, 6, 7, 17	F5–F8	2*, 13 + 16, 5 + 12
10, 16	F5–F8	1, 7 + 9, 5 + 12
12–15	F5–F8	null, 7 + 9, 2 + 12
3	F5	2*, 13 + 16, 5 + 12; null, 13 + 16, 2 + 12; null, 7 + 9, 2 + 12
	F6	2*, 13 + 16, 5 + 12; null, 13 + 16, 2 + 12; null, 7 + 9, 2 + 12
	F7	2*, 13 + 16, 5 + 12; null, 13 + 16, 2 + 12; null, 7 + 9, 2 + 12
	F8	2*, 13 + 16, 5 + 12; null, 13 + 16, 2 + 12; null, 7 + 9, 2 + 12; 2*, 7 + 9, 5 + 12
4	F5	2*, 13 + 16, 5 + 12; 2*, 7 + 9, 5 + 12; null, 13 + 16, 2 + 12; null, 7 + 9, 2 + 12
	F6	2*, 13 + 16, 5 + 12; 2*, 7 + 9, 5 + 12; null, 13 + 16, 2 + 12
	F7	2*, 13 + 16, 5 + 12; 2*, 7 + 9, 5 + 12; null, 7 + 9, 5 + 12
	F8	2*, 13 + 16, 5 + 12; null, 13 + 16, 5 + 12
5	F5	2*, 13 + 16, 5 + 12; 2*, 7 + 9, 5 + 12; null, 7 + 9, 5 + 12
	F6	2*, 13 + 16, 5 + 12; 1, 13 + 16, 5 + 12; 2*, 7 + 9, 5 + 12; 1, 7 + 9, 5 + 12
	F7	2*, 13 + 16, 5 + 12; 1, 13 + 16, 5 + 12; 1, 7 + 9, 5 + 12
	F8	2*, 13 + 16, 5 + 12; 1, 7 + 9, 5 + 12
8	F5	2*, 13 + 16, 5 + 12; 2*, 7 + 8, 5 + 12; 1, 13 + 16, 5 + 12; 1, 7 + 8, 2 + 12; null, 13 + 16, 2 + 12; null, 13 + 16, 5 + 12; null, 7 + 8, 5 + 12; null, 7 + 8, 2 + 12
	F6	2*, 13 + 16, 5 + 12; 1, 13 + 16, 5 + 12; null, 13 + 16, 5 + 12; null, 13 + 16, 2 + 12; null, 7 + 8, 5 + 12; null, 7 + 8, 2 + 12
	F7	2*, 13 + 16, 5 + 12; null, 13 + 16, 5 + 12; null, 13 + 16, 2 + 12
	F8	2*, 13 + 16, 5 + 12; null, 13 + 16, 5 + 12
9	F5	1,7 + 8, 2 + 12; 1,7 + 8, 5 + 12; 1, 13 + 16, 5 + 12; 1, 13 + 16, 2 + 12; null, 13 + 16, 5 + 12; null, 13 + 16 + 8, 2 + 12; null, 7 + 8, 5 + 12; 1, 7 + 8, 2 + 5 + 12; 1, 13 + 16 + 8, 2 + 12; 1, 16 + 7 + 8, 5 + 12
	F6	1, 7 + 8, 2 + 12; 1, 7 + 8, 5 + 12; 1, 13 + 16, 5 + 12; 1, 13 + 16, 2 + 12; null, 7 + 8, 5 + 12; 1, 7 + 8, 2 + 5 + 12
	F7	1, 7 + 8, 2 + 12; 1, 7 + 8, 5 + 12; 1, 13 + 16, 5 + 12; 1, 7 + 8, 2 + 5 + 12
	F8	1, 7 + 8, 2 + 12; 1, 7 + 8, 5 + 12; 1, 13 + 16, 5 + 12; 1, 7 + 8, 2 + 5 + 12
11	F5	1, 7 + 9, 5 + 12; 1, 7 + 9, 2 + 12; null, 7 + 9, 2 + 12; null, 7 + 9, 2 + 5 + 12
	F6	1, 7 + 9, 5 + 12; 1, 7 + 9, 2 + 12; null, 7 + 9, 2 + 12; null, 7 + 9, 2 + 5 + 12
	F7	1, 7 + 9, 2 + 12; 1, 7 + 9, 2 + 5 + 12; null, 7 + 9, 2 + 12
	F8	1, 7 + 9, 2 + 12; 1, 7 + 9, 2 + 5 + 12; null, 7 + 9, 2 + 12; null, 7 + 9, 2 + 5 + 12

10% gradient running gel (pH 8.9). The electrophoresis was conducted at 6 mA constant current at 4 °C for 14−16 h until the Pyronin Y front had reached the bottom of the gel. Staining was achieved by a 20 min immersion in 0.1% (w/v) Coomassie Brilliant Blue R250, 10% (v/v) carbinol, and 50% (v/v) acetic acid and distaining for 60 min in hot water. HMW-GS nomenclature followed that of Payne et al. (*7*−*9*).

For the analysis of bread-making quality, grain samples were milled in a Brabender grinder fitted with a 100 mesh screen. The moisture contents and SDS sedimentation values were tested according to GB 5497-85 and AACC 56-61A, respectively. Wet and dry gluten contents and gluten exponents were determined by a Perten Gluten Index GM 2200 device, and the protein content was determined with a Bûchli AutoKjeldahl Unit K-370. HMW-GSs from somatic hybrids, with electrophoretic mobilities similar to those of common wheat cultivars, were named according to Payne et al. (*7*−*9*).

RESULTS

SDS−PAGE Analysis of HMW-GS and Quality from F_5 Somatic Hybrids. The HMW-GS composition of 40 seeds, randomly selected from each hybrid derivative, was determined (**Table 1** and **Figure 1**). Only lines 12−15 had the same HMW-GS combination as the parent wheat cv. Jinan 177 (null, 7 + 9, 2 + 12), while other hybrid derivatives showed a diversity of HMW-GS types and combinations, including individual subunits not present in either parent but similar to those found in other common wheat cultivars. On the basis of the SDS sedimentation value, we analyzed the relationship between different subunits/combinations and quality. This showed that lines 12−15 with the HMW-GS composition null, 7 + 9, 2 + 12 (**Table 1**) produces a poor-quality flour. Lines 1, 2, 6, 7, and 17 with higher qualities had uniform HMW-GS compositions, 2*, 13 + 16, 5 + 12 (**Table 1**), as described also for the high-quality hybrid line II-12 (*21*). Lines 10 and 16, with the HMW-GS composition 1, 7 + 9, 5 + 12 and higher SDS sedimentation values than Jinan 177 (**Table 1** and **Figure 1**), are thus also potential high-quality lines. The remaining lines were heterogeneous with respect to their HMW-GSs (**Table 1** and **Figure 1**).

Genetic Characteristic of HMW-GS from F_5−F_8 Somatic Hybrid Lines. The HMW-GS composition of 45 seeds from each generation between F_5 and F_8 (**Table 2** and **Figure 2**) revealed that lines 1, 2, 6, 7, and 17 were fixed for 2*, 13 + 16, 5 + 12 in each generation; lines 10 and 16 were similarly fixed for 1, 7 + 9, 5 + 12; and lines 12−15 were uniform for null, 7 + 9, 2 + 12. They are sorted to the first pattern. In contrast, the HMW-GS compositions of lines 3−5 and 11 were variable at F_5 but fixed from F_6 to F_8. The HMW-GS contents of lines 8 and 9 were unstable across the four generations, and some combinations present in F_5 were absent in the later generations (e.g., null, 7 + 8, 2 + 12). In addition, the frequency of some subunits and combinations decreased as the generations advanced.

HMW-GS Inheritance in Somatic Hybrid Lines from a Single Seed. The HMW-GS composition of F_6 progeny from a single F_5 seed from each of lines 3, 12, and 17 was determined, and in every case, all F_6 individuals bred true to their F_5 parental pattern (**Table 5** and **Figure 3**). Thus, inheritance of HMW-GS content is suggested to be stable in lines 1, 2, 6, 7, 10, and 12−17. In the unstable lines 3−5 and 11, segregation was noted in the early generations but fixation was achieved in the later ones.

Genetic Characteristic of LMW-GS from F_5−F_8 Somatic Hybrid Lines. The LMW-GS composition of 45 seeds was obtained for lines 1, 2, 6, 7, 10, and 12−17 over the F_5−F_8 generations line (**Figure 4**). Hybrid lines, including high quality (1, 2, 6, 7, and 17), were compared and contained 2*, 13 + 16, 5 + 12, and 10 and 16 possessed 1, 7 + 9, 5 + 12; low quality (12−15) had *null*, 7 + 9, 2 + 12. These lines had the same LMW-GS structure if their HMW-GS types and combinations were uniform (**Figure 4**).

HMW-GS Types with Bread-Making Quality Parameters. To investigate the relationship of the SDS sedimentation with

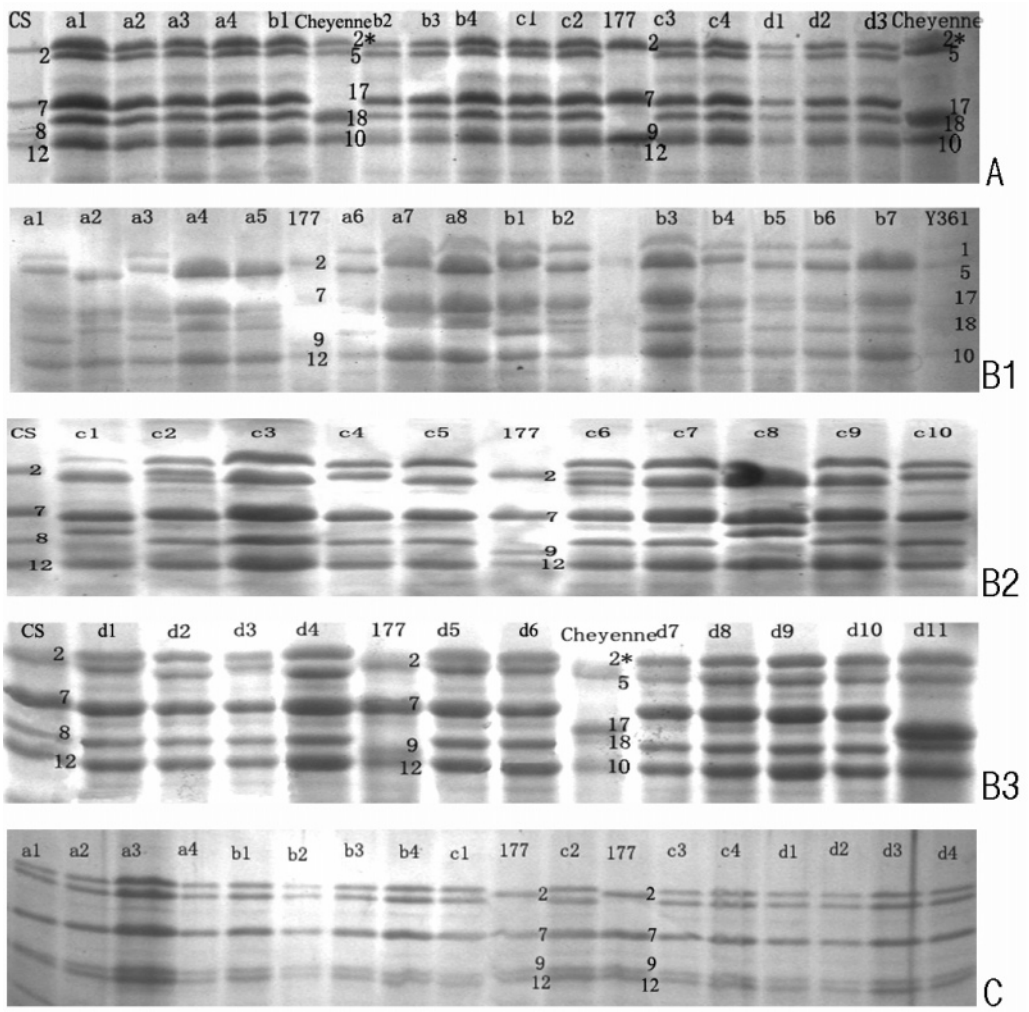

Figure 2. SDS–PAGE analysis of HMW-GS from somatic hybrid lines F_5–F_8. (**A**) Sample 7. (**B1**–**3**) sample 9, and (**C**) sample 10; a1–8, seeds randomly selected from the somatic hybrid F_5 generation; b1–7, seeds randomly selected from the F_6 generation; c1–10, seeds randomly selected from the F_7 generation; and d1–11, seeds randomly selected from the F_8 generation.

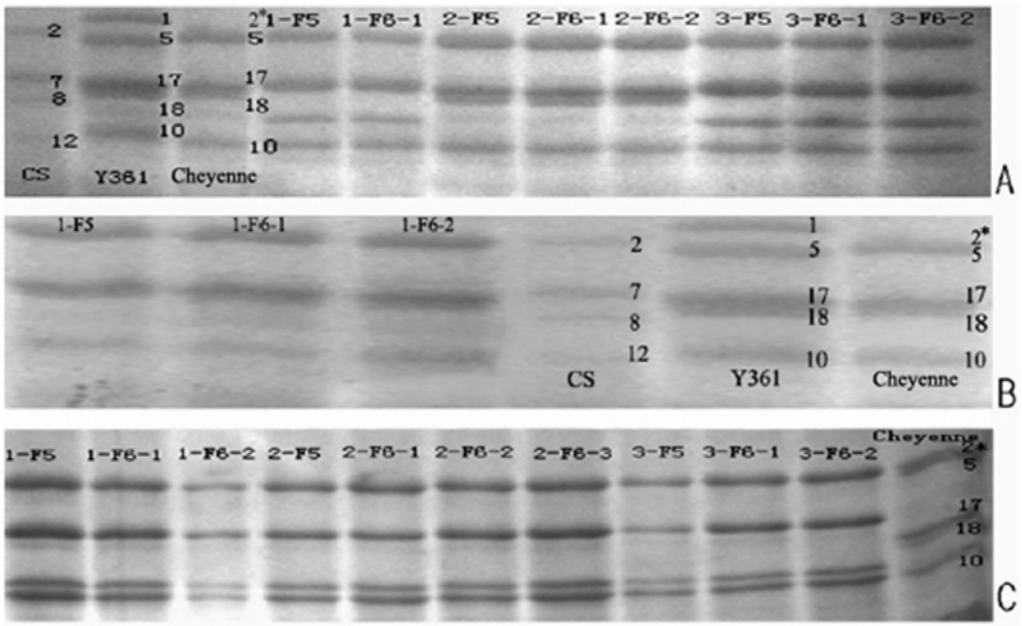

Figure 3. F_5–F_6 HMW-GS analysis of hybrid progenies from single seeds of 3, 12, and 17 lines. (**A**) Sample 3, (**B**) sample 17, and (**C**) sample 12; 1–3-, single F_5 seeds of the somatic hybrid line; 1-F_6-1,2, F_6 progenies from 1-F_5; 2-F_6-1–3, F_6 progenies from 2-F_5; and 3-F_6-1,2, F_6 progenies from 3-F_5.

bread-making quality, some other quality parameters were further detected. The *Glu-1* quality scores from hybrid lines 1, 2, 6, 7, 10, and 12−17 were found relative to some important quality parameters (**Tables 3** and **4**). This indicated that the

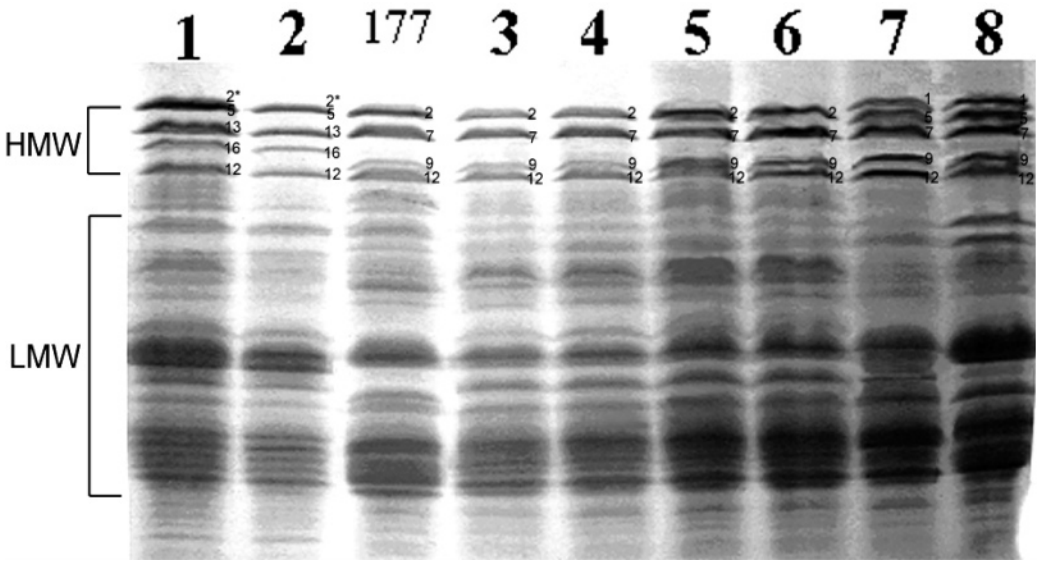

Figure 4. SDS–PAGE analysis of LMW-GS from somatic hybrid lines 1–8. Seeds were randomly selected from somatic hybrid lines 1, 2, 10, and 12–16.

Table 3. Bread-Making Quality Parameters of Some Hybrid Lines

sample	composition of HMW-GS	content (%)				gluten exponent	SDS sedimentation value (mL)
		moisture	proteins	wet gluten	dry gluten		
1	2*, 13 + 16, 5 + 12	13.20	12.814	32.97	10.42	91.05	44
2	2*, 13 + 16, 5 + 12	17.31	12.820	31.81	10.55	90.47	45.8
6	2*, 13 + 16, 5 + 12	14.18	12.765	36.60	12.11	98.90	54.8
7	2*, 13 + 16, 5 + 12	15.86	11.720	32.05	10.19	96.57	45.5
10	1, 7 + 9, 5 + 12	13.96	15.945	42.46	13.96	73.79	48.5
12	null, 7 + 9, 2 + 12	12.92	10.250	9.89	3.23	15.58	17
13	null, 7 + 9, 2 + 12	13.04	11.042	28.00	5.38	29.08	24.5
14	null, 7 + 9, 2 + 12	13.00	11.359	24.04	4.75	31.17	18.5
15	null, 7 + 9, 2 + 12	13.05	10.752	13.34	11.68	22.04	23
16	1, 7 + 9, 5 + 12	12.93	11.646	31.9	12.31	55.60	45

Table 4. Correlation between Quality Score and Quality Parameters

	Glu-1 quality score	moisture content (%)	proteins content (%)	wet gluten content (%)	dry gluten content (%)	gluten exponent	SDS sedimentation value (mL)
Glu-1 quality score							
moisture content (%)	0.542						
proteins content (%)	0.671[a]	0.303					
wet gluten content (%)	–0.406	–0.240	–0.403				
dry gluten content (%)	0.752[a]	0.298	0.663[a]	–0.597			
gluten exponent	0.921[b]	0.658[a]	0.611	–0.467	0.630		
SDS sedimentation value (mL)	0.966[b]	0.515	0.698[a]	–0.486	0.806[a]	0.922[b]	

[a] Correlation is significant at the 0.05 level. [b] Correlation is significant at the 0.01 level.

quality scoring system could be used efficiently in a breeding program for wheat somatic hybrid lines. It was shown that the *Glu-1* quality score had a significant correlation with the contents of dry gluten and protein, which implied that the system reflected the effect of HMW-GS on bread-making quality properties (**Tables 3** and **4**), in agreement with SDS sedimentation values and gluten compositions.

SDS−PAGE Analysis of HMW-GS from II-12 × CS Hybrid Progenies. The HMW-GS composition of 88 F_1 hybrids from the cross II-12 × CS produced three distinct types: Some progeny were of the II-12 type (2*, 13 + 16, 5 + 12), some were of the CS type (null, 7 + 8, 2 + 12), and some were an additive combination of these (**Figure 5**). The segregation was consistent with a 1:2:1 ratio (II-12:II-12 + CS:CS).

DISCUSSION

Six subunits, assumed to be identical, or at least indistinguishable at the SDS−PAGE level to *Glu1Ax1*, *Glu1Ax2**, *Glu1Bx13*, *Glu1By16*, *Glu1Bx5*, and *Glu1Dy8*, appeared among the derivatives of the fusion, even though they are not present in the parental wheat, with a variation frequency of 34.8%. Of these, only *Glu1By16* product coelectrophoreses with a subunit from the tall wheatgrass parent; the presence of *Glu1By16* is associated with that of *Glu1Bx13*, and this pair is present in

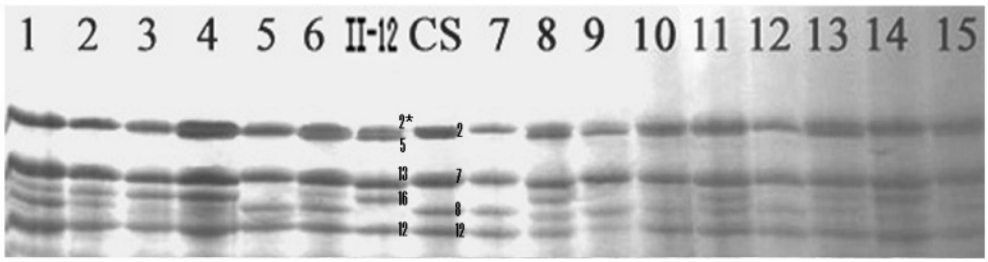

Figure 5. SDS–PAGE analysis of sexual hybrid progenies between II-12 and CS. Lanes 1–15, seeds randomly selected from different II-12 × CS progenies.

lines 1, 2, 6, 7, and 17. Their coding sequences are clustered to *Glu-1Bx7* and *Glu-1By9* in common wheat (*21*). Three discrepant regions in the sequences repeat one time more than *Glu-1Bx7* and *Glu-1By9*, which likely derive from an unequal DNA crossover (*21*). In these superfluous subunits, wheatlike *Glu1Bx13* + *Glu1By16* and *Glu1Dx5* + *Glu1Dy12*, respectively, exist in 10/17 and 13/17 hybrid lines (**Tables 1** and **2** and **Figures 1** and **2**), respectively, but rarely present in Chinese common wheat cultivars. Recent research reveals that 13 + 16 and 5 + 12 subunits play a very important role in the baking quality of Chinese common wheat (*26*, *27*). Quality−correlative parameters indicate that the hybrid lines containing 2*, 13 + 16, 5 + 12 are characteristic of high quality, while the hybrid line and the parent wheat with low-score HMW-GSs null, 7 + 9, 2 + 12 show that of poor quality (*21*).

It has been established that regenerants of parental Jinan 177 from immature embryos passaged through tissue culture showed some variation in HMW-GS composition, as a result of somaclonal variation. In our control experiments, the frequency of this variation was only 10%, and the only change noted was the replacement of *1By9* by *1By8* (*21*). It does not, therefore, seem likely that the extensive variation created in the regenerants from the somatic hybrid process can be caused by the known somaclonal route and that the somatic hybridization itself must induce variation at the DNA level in the HMW-GS. Such factors have been discussed elsewhere (*21*, *23*).

In this experiment, we analyzed HMW-GS of 17 samples from F_5 to F_8. Eleven samples (1, 2, 6, 7, 10, and 12−17, frequency = 64.71%) had identical HMW-GS forms in four successive generations of F_5−F_8; four samples (3−5 and 11, frequency = 23.53%) contained multiform HMW-GS combinations in every generation, with a few changes during four successive generations; two samples (8 and 9, frequency = 11.76%) possessed the most multiform HMW-GS combinations in all samples, appearing as a gradual reduction in the four successive generations.

The glutenins quality score is significantly correlated with both the SDS sedimentation value and the gluten exponent and is strongly associated with the content of dry gluten and the proteins content (**Tables 3** and **4**). These hybrid lines, consisted of high-score HMW-GSs (e.g., 1, 2, 6, 7, 10, 16, and 17) and have high-quality parameters than other hybrid lines (e.g., 12−15) with low-score HMW-GSs (**Tables 3** and **4**).

LMW-GS in the 10/17 genetic-stable lines was primarily compared. Good accordance between the stable HMW-GS and the LMW-GS has been found in the three kinds of stable hybrid lines among the first pattern (**Figure 4**). There are little LMW-GS variations in different HMW-GS types of hybrid lines. Thus, LMW-GS may be a coimpact on the hybrid quality with the HMW-GS. It is known that there is a very complex interaction of HMW-GS with LMW-GS and gliadin as mentioned above (*1*, *13*, *28*). This is maybe one of the reasons why different quality parameters appeared in the hybrid lines with the same HMW-GS and LMW-GS subunits and combinations, e.g., 1, 2, 6, and 7 (**Tables 3** and **4** and **Figure 4**). The polymorphism of gliadin in these hybrids and the interaction of HMW-GS/LMW-GS/gliadin on the hybrid quality are worthy of further investigation.

Table 5. F_5–F_6 HMW-GS Composition of Single Seeds of Lines 3, 12, and 17

hybrid line no.	F_5 sample	HMW-GS pattern	F_6 sample	HMW-GS pattern
3	1	2*, 13 + 16, 5 + 12	24	2*, 13 + 16, 5 + 12
	1	null, 13 + 16, 2 + 12	28	null, 13 + 16, 2 + 12
	1	null, 7 + 9, 2 + 12	18	null, 7 + 9, 2 + 12
12	1	null, 7 + 9, 2 + 12	17	null, 7 + 9, 2 + 12
	1	null, 7 + 9, 2 + 12	26	null, 7 + 9, 2 + 12
	1	null, 7 + 9, 2 + 12	18	null, 7 + 9, 2 + 12
17	1	2*, 13 + 16, 5 + 12	13	2*, 13 + 16, 5 + 12
	1	2*, 13 + 16, 5 + 12	22	2*, 13 + 16, 5 + 12
	1	2*, 13 + 16, 5 + 12	16	2*, 13 + 16, 5 + 12

As to the single-seed inheritance of three lines (3, 12, and 17) from F_5 to F_6 generations, we discovered that HMW-GS combinations of parent F_5 and filial F_6 generations were completely accordant (**Table 5** and **Figure 3**). This indicates that the HMW-GS in hybrid progenies is genetically stable, which has been supported by the result from SSR markers in these hybrid F_2 and F_6 lines. There are obviously polymorphic SSR loci in different F_2 hybrid lines, but all 62 loci checked showed the same profile in every F_2 and F_6 line (Chen, S. Y. Personal communication). Thus, it appears that segregation in SSR profiles took place in the early generations. Besides, 5−7 *T. Ponticum* chromosome fragments were inserted into different hybrid progeny genomes (*29*), with stable sizes and amounts in the same lines from F_2 to F_6 generations in a GISH experiment (Xiang, F. N. Personal communication). These results accord with the outcome that the extra HMW-GS and LMW-GS produced in the early hybrid generation is able to inherit stably in the succeeding generations in this work.

It was remarkable that multiform subunits and their combinations existing in the hybrid lines (3−5, 8, 9, and 11) were similar to the allele variation in common wheat; the diversification similar to null, 1, 2* occurs in the loci *Glu-1A*; 13 + 16 and 7 + 8 and 7 + 9 in the loci *Glu-1B*; and 2 + 12 and 5 + 12 in the loci *Glu-1D*. So, the variant subunits, i.e., 13 + 16, 7 + 8, and 7 + 9, occurring in a certain locus must have a more similar structure. We have reported that 13 + 16 was homologous to 7 + 9 in the gene sequences (*28*). It was interesting that the samples that express simultaneously six HMW-GSs, i.e., 1, 16 + 7 + 8, 5 + 12 and 1, 7 + 9, 2 + 5 + 12, existed in the hybrid progenies. The line containing 1, 16 + 7 + 8, 5 + 12 gave the highest SDS sedimentation values (**Table 1**), which may favor the new hybrid high-quality trait. It is known that the linkage degree of two genes in loci *Glu-1D* is much higher

than that in *Glu-1A* and *Glu-1B*, so the two genes in loci *Glu-1D* always occur in a pair, and one cannot easily differentiate the unattached effect of two kinds of subunits. In **Tables 1** and **2**, we can find that two kinds of subunit forms (1, 7 + 9, 5 + 12 and 1, 7 + 9, 2 + 12) occur in hybrid progeny line 11; some other subunits can also be distinguished individually (**Tables 1** and **2**). Therefore, it may be possible to detect the contribution of different new subunits to wheat quality.

We have demonstrated that asymmetric somatic hybridization is not only able to effect the introgression of small alien chromosome segments into wheat (*30*) but also appears to induce the expression of additional HMW-GS at a high frequency (*21*). Once induced, these genes appear to be inherited normally through the sexual process. As a result, we suggest that somatic hybridization represents a powerful means of generating de novo genetic variation.

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