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Full Length Research Paper

Genetic variations of m-type LMW-GS genes and their associations with dough quality in *Triticum turgidum* ssp. *turgidum* landraces from China

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Genetic variations of the four subgroups of m-type low-molecular-weight glutenin subunits (LMW-GS) genes and their associations with dough quality parameters were investigated through the group-specific markers in 68 landraces of *Triticum turgidum* ssp. *turgidum* from China. The number of alleles were two, three, three and two respectively by the markers Glu3A.2, Glu3B.1, Glu3B.2 and Glu3A.3, which was one-to-one correspondence to the "MDTSCIP-", "MENSHIP-", "METSHIPS-" and "METSCIP-" subgroups of m-type LMW-GS genes respectively. Results from the sequencing of PCR products further confirmed m-type LMW-GS genes from tetraploid landraces. The widespread variation of dough quality parameters were observed in all accessions and their association with the alleles of m-type LMW-GS genes were also investigated by crosstab analysis. We found that allele variations in "METSHIPS-" subgroup of m-type LMW-GS genes were significantly linked with gluten index at ($K=14.02$, $P<0.05$), time to breakdown at ($K=11.88$, $P<0.05$) and protein content at ($K=24.45$, $P<0.001$) showed that "METSHIPS-" subgroup of m-type LMW-GS genes have profound effects on dough quality. Our results also suggested that group-specific markers for "METSHIPS-" subgroup of LMW-GS genes could be used in wheat quality improvement and marker-assisted breeding programs.

Key words: Genetic diversity, landraces, m-type LMW-GS, quality, *Triticum turgidum* ssp. *turgidum*.

INTRODUCTION

Low-molecular-weight (LMW) glutenins represent about one-third of the total seed storage proteins and 60% of glutenins (Bietz and Wall, 1973). They are classically subdivided into B, C and D groups according to their electrophoretic mobility in SDS-PAGE (Jackson et al., 1983). Coding genes of the LMW-GS are at the Glu-A3, Glu-B3, and Glu-D3 loci, located on the short arms of chromosome 1A, 1B and 1D respectively (Singh and Shepherd, 1988). The general structure of a typical LMW-GS consists of a signal peptide, a short N-terminal domain, a highly variable repetitive domain and a conserved C-terminal domain. Three groups of typical LMW-GS have been found on the basis of the first amino acid residue of N-terminal sequences, namely LMW-m, LMW-s and LMW-i types, which possessed methionine, serine and isoleucine respectively (Lew et al., 1992; Masci et al., 1998; Maruyama-Funatsuki et al., 2004; D'Ovidio and Masci, 2004). Moreover, An et al. (2006) suggested that the divergent time between LMW-i and LMW-m LMW-s was 12.92 MYA while LMW-m and LMW-s were divergent at 11.76 MYA.

The allelic variation of LMW-GS has been widely characterized at protein level in wheat and its relatives

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(Gupta et al., 1989, 1994; Nagamine et al., 2000; Atienza et al., 2002; Masci et al., 2002). Allelic differences in the LMW-GS have also been shown to be significantly related to flour qualities in both durum and bread wheat (Gupta et al., 1989; Pogna et al., 1990; MacRitchie et al., 1990; Figueroa et al., 2011). Incorporation of LMW-GS into wheat dough has shown that they also contribute to dough strength (Lee et al., 1999; Sissons et al., 1998). Further studies by Maruyama-Funatsuki et al. (2004) and Tanaka et al. (2005) confirmed LMW-GS significantly affected dough strength in common wheat. SDS-PAGE (Margiotta et al., 1993) is a routine and conventional method used to determine the allelic composition of LMW-GS. However, LMW-GS are complex polymeric glutenin in nature. Many of the components have similar structure and properties, and the fact that they have similar band pattern in SDS matrix hinders the usage of the technique sometimes (Thomason et al., 1994). Hence, it is necessary to create a functional marker to identify the LMW-GS genes (Anderson and Lubberstedt, 2003)

Recently, analysis of the LMW-GS alleles by PCR (polymerase chain reaction) has become more convenient. Van Campenhout et al. (1995) designed locus-specific markers for LMW-GS genes on each of the group 1 chromosomes of hexaploid wheat. D'Ovidio et al. (1997) and Zhang et al. (2004) developed specific primers for Glu-B3 and Glu-A3 locus. Ikeda et al. (2002) classified LMW-GS genes into 12 groups based on the N- and C-terminal sequences. Long et al. (2005) and Ikeda et al. (2006) also explored nine and ten group-specific markers for LMW-GS genes respectively. Zhao et al. (2006) established the relationships between LMW-GS coding genes and Glu-D3 alleles defined by protein electrophoretic mobility. LMW-m was one of the most commonly encountered polypeptides in wheat (Ferrante et al., 2004; Ikeda et al., 2004). However, more-detailed information is required to understand the relationships between wheat quality and the different alleles of LMW-GS genes, especially, LMW-m type.

Triticum turgidum ssp. *turgidum* (2n = 4x = 28, AABB), a primitive tetraploid wheat species, was widely grown in China before the 1950's. Later, its cultivation area was gradually reduced and now, most of the landraces are only conserved in germplasm banks (Dong and Zheng, 2000). The genetic variation of this primitive wheat widely been studied to evaluate their- gliadin, HMW-glutenin, the crossability with rye, protein content and genomic microsatellite (Li et al., 2006a, b; Liu et al., 1998). The wild and cultivated relatives of bread wheat containing novel alleles of LMW-GS could be utilized as a valuable source with the potential to improve wheat grain properties. Therefore, it is necessary to screen out the additional superior-quality allelic variants in wild and relative species.

The present study was carried out in order to describe the genetic variations of m-type LMW-GS genes in the *T. turgidum* ssp. *turgidum* landraces of China and also their relationships with the dough quality parameters were investigated by using the group-specific markers developed in our previous work (Long et al., 2005).

MATERIALS AND METHODS

Plant materials

A total of 68 accessions of *T. turgidum* ssp. *turgidum* landraces (Table 1), which were conserved in the Germplasm Laboratory of Triticeae Research Institute, Sichuan Agriculture University was collected from 4 separate geographic regions (that is, Sichuan, Shaanxi, Gansu and Henan provinces) in China were used in this study.

DNA isolation, PCR amplification and sequences cloning

Genomic DNA was isolated from 3 to 5-day-old seedlings with CTAB procedure as reported previously (Murray and Thompson, 1980). PCR amplification was carried out by using four m-type LMW-GS group specific primers (Table 2) and conditions in an MJ Research PTC-220 (Programmable Thermal Controller, MJ Research) described by Long et al. (2005). The 25 µl reaction volume consisting of 1.5 U TaqPlus DNA polymerase (TIANGEN), 2.5 µl PCR buffer, 50 ng genomic DNA, 1.5 mM $MgCl_2$ and 100 mM of each dNTP following 5 min of denaturation at 95°C, 35 cycles of 45 s at 95°C, 45 s at 60°C and 45 s at 72°C and a final 10 min extending at 72°C PCR products were separated in 2% agarose gels.

The desired DNA fragments were recovered from the gels (Long et al., 2005) and subjected to ligation with the pMD18-T vector (TaKaRa), and then transformed into *Escherichia coli* DH5α competent cells (Sambrood et al., 1989). Several positive colonies were screened out by restriction digestion and sequenced by a commercial company (TaKaRa). The nucleotide and deduced amino acid sequence analysis were conducted by DNAMAN software DNAMAN 5.2.2 (http://www.lynnon.com) and BLAST (http: //www.ncbi.nlm.nih.gov/ BLAST).

Quality testing

For assuring the dough quality, the protein content, gluten index and farinograph parameters were analysed (Table 3). The protein content of grains at maturity was detected by micro-Kjeldahl method (Allen, 1931) (Büchi, Uzwil, Switzerland). Farinograph, gluten index and dry gluten were detected according to AACC method 38-12 (AACC, 1983). The farinograph (Perten Instruments AB, Sweden) was performed to record water absorption (%), development time (min), stable time (min), mixing tolerance index (BU) and time to breakdown (min).

Statistical analysis

The following parameters were estimated: observed number of alleles per locus, effective number of alleles per locus, observed heterozygosity, average heterozygosity, and Nei's expected heterozygosity (He) (Nei, 1978). These parameters were calculated for all populations using POPGENE 1.32 (Yeh et al., 1997). In order to determine the associations between the dough quality characters and m-type LMW-GS gene alleles, these accessions were divided into different groups based on the value of quality characters, such as two groups (water absorption, development time, stable time), three groups (dry gluten, time to breakdown), four groups (gluten

Table 1. Materials and m-type alleles in this study.

No.	Accession	Origin	MDT-		MEN-			METSHIP-			METSCIP-	
			B[a]	S[b]	B	M[c]	S	B	M	S	B	S
1	AS2281	Sichuan	0[d]	1[e]	1	0	0	1	0	0	1	0
2	AS2235	Anyue, Sichuan	0	1	1	0	0	1	0	1	1	0
3	AS2236	Anyue, Sichuan	0	1	1	0	0	1	0	1	1	0
4	AS2295	Anyue, Sichuan	0	1	1	0	0	1	0	1	1	0
5	AS2297	Anyue, Sichuan	0	1	1	0	0	1	0	1	1	0
6	AS2298	Anyue, Sichuan	0	1	1	0	0	1	0	1	1	0
7	AS2341	Anyue, Sichuan	0	1	1	0	0	1	0	1	1	0
8	AS2317	Batang, Sichuan	0	1	0	0	0	0	0	0	1	0
9	AS2318	Batang, Sichuan	0	1	1	0	0	0	1	0	1	0
10	AS2309	Baichuan, Sichuan	0	1	1	0	0	1	0	1	1	0
11	AS2314	Dajing, Sichuan	0	1	1	0	0	0	1	0	1	0
12	AS2301	Guangyuan, Sichuan	0	1	1	0	0	1	0	1	1	0
13	AS2282	Jianyang, Sichuan	0	1	1	0	0	0	1	0	1	0
14	AS2283	Jianyang, Sichuan	0	1	1	0	0	1	0	1	1	0
15	AS2294	Jianyang, Sichuan	0	1	1	0	0	1	0	1	1	0
16	AS2313	Jinxian, Sichuan	0	1	1	0	0	1	0	1	1	0
17	AS2312	Kaixian, Sichuan	0	1	1	0	0	1	0	1	1	0
18	AS2315	Kangdin, Sichuan	0	1	1	0	0	1	0	1	1	0
19	AS2285	Lezhi, Sichuan	0	1	1	0	0	1	0	1	1	0
20	AS2240	Neijiang, Sichuan	0	1	1	0	0	0	1	0	1	0
21	AS2310	Renshou, Sichuan	0	1	1	0	0	1	0	1	1	0
22	AS2284	Rongxian, Sichuan	0	1	1	0	0	1	0	1	1	0
23	AS2302	Santai, Sichuan	0	1	1	0	0	1	0	1	1	0
24	AS2256	Santai, Sichuan	0	1	0	0	1	0	1	0	0	1
25	AS2303	Santai, Sichuan	0	1	1	0	0	1	0	1	1	0
26	AS2305	Tongnan, Sichuan	0	1	1	0	0	1	0	1	1	0
27	AS2308	Tongnan, Sichuan	0	1	1	0	0	1	0	1	1	0
28	AS2306	Tongnan, Sichuan	0	1	1	0	0	1	0	1	1	0
29	AS2311	Yaan, Sichuan	0	1	1	0	0	1	0	1	1	0
30	AS2299	Yanbian, Sichuan	0	1	1	0	0	1	0	1	1	0
31	AS2300	Yuechi, Sichuan	0	1	1	0	0	1	0	1	1	0
32	AS2307	Zhangming, Sichuan	0	1	1	0	0	1	0	1	1	0
33	AS2304	Zhongjiang, Sichuan	0	1	1	0	0	1	0	1	1	0
34	AS2320	Gansu	0	1	1	0	0	0	1	0	1	0
35	AS2335	Anxi, Gansu	0	0	0	0	0	0	0	0	1	0
36	AS2243	Dinxi, Gansu	0	1	1	0	0	1	0	1	1	0
37	AS2321	Gaolan, Gansu	0	1	1	0	0	0	1	0	1	0
38	AS2368	Gaolan, Gansu	0	1	1	0	0	0	0	1	1	0
39	AS2376	Gaolan, Gansu	0	1	1	0	0	0	1	0	1	0
40	AS2249	Gulang, Gansu	0	1	1	0	0	0	1	0	1	0
41	AS2333	Jinta, Gansu	0	1	1	0	0	1	0	1	1	0
42	AS2328	Linze, Gansu	0	1	1	0	0	1	0	1	1	0
43	AS2373	Linze, Gansu	0	1	1	0	0	0	1	0	1	0
44	AS2370	Minle, Gansu	0	1	1	0	0	1	0	1	1	0
45	AS2336	Weixian, Gansu	0	1	1	0	1	1	0	1	1	0
46	AS2241	Yongdeng, Gansu	1	0	1	0	0	1	0	1	1	0
47	AS2325	Zhangye, Gansu	0	1	1	0	0	0	1	0	1	0
48	AS2374	Zhangye, Gansu	0	1	1	0	0	0	1	0	1	0
49	AS2351	Henan	0	1	1	0	0	1	0	1	1	0
50	AS2258	Enxian, Henan	0	1	1	0	0	0	1	0	1	0

Table 1. Count'd

51	AS2355	Yuxian, Henan	0	1	1	0	0	0	1	0	1	0
52	AS2291	Ankang, Shannxi	0	1	1	0	0	1	0	1	1	0
53	AS2292	Ankang, Shannxi	0	1	1	0	0	0	1	0	1	0
54	AS2289	Baihe, Shannxi	0	1	1	0	0	1	0	1	1	0
55	AS2253	Langao, Shannxi	0	1	1	0	0	0	0	1	1	0
56	AS2288	Liuba, Shannxi	0	1	1	0	0	0	1	0	1	0
57	AS2378	Lueyang, Shannxi	0	1	1	0	0	0	1	0	1	0
58	AS2379	Mianxian, Shannxi	0	1	1	0	0	1	0	1	1	0
59	AS2380	Nanzheng, Shannxi	0	1	1	0	0	1	0	1	1	0
60	AS2254	Pingli, Shannxi	0	1	1	0	0	1	0	1	1	0
61	AS2356	Qishan, Shannxi	0	1	0	1	0	0	1	0	1	0
62	AS2250	Shangxian, Shannxi	0	1	1	0	0	0	1	0	1	0
63	AS2354	Xian, Shannxi	0	1	1	0	0	0	1	0	1	0
64	AS2257	Xixiang, Shannxi	0	1	1	0	0	1	0	1	1	0
65	AS2251	Xinping, Shannxi	0	1	1	0	0	0	1	0	1	0
66	AS2381	Zexian, Shannxi	0	1	1	0	0	1	0	1	1	0
67	AS2382	Zexian, Shannxi	0	1	1	0	0	0	0	1	1	0
68	AS2287	Zhengan, Shannxi	0	1	1	0	0	0	0	1	1	0

*Note: a-B means big band, b-S means small band, c-M means middle band, d-0 means no band, e-1 means one band.

Table 2. Group-specific primers of m-type LMW-GS used in this study.

Subgroup	Primer	Sequence(5'-3')	Chromosome location	Annealing temperature (℃)	Alleles
MDTSCIP-	Glu3A.2	AGTGCCATTGCGCAGATGAAT AACGGATGGTTGAACAATAGA	1AS	60	2
MENSHIP-	Glu3B.1	GCACAAATGGAGAATAGCCAC AACAAATGGTATTTGTTGTTG	1BS	59	3
METSHIPS-	Glu3B.2	CCTAGCTTGGAGAAACCATT CAAGATAGATGGCTGAATAG	1BS	50	3
METSCIP-	Glu3A.3	ATGGAGACTAGCTGCATCC CTGCAAAAAGGTACCCTTTT	1AS	60	2

Table 3. The variation of protein content, gluten and farinograph parameters in *T. turgidum* ssp. *turgidum* landraces.

Parameter	Mean	Range	Variation index (%)	K value
				METSHIPS
Protein content (%)	14.39±2.34	10.11-22.01	16.24	24.45***
Gluten index	61.06±15.77	23.58-86.38	25.83	14.02*
Dry gluten (g)	13.11±1.44	10.72-15.38	11.00	5.51^{ns}
Water absorption (%)	61.76±0.88	59.50-63.10	1.43	0.25^{ns}
Development time (min)	3.10±1.40	1.20-7.50	44.10	2.75^{ns}
Stable time (min)	4.00±2.20	0.60-11.40	54.10	2.12^{ns}
Mix tolerance index (BU)	77.81±25.20	20.00-128.00	32.38	8.17^{ns}
Time to breakdown (min)	5.08±2.23	1.90-12.80	43.85	11.88*

*, ***, Significant at P=0.05 and P=0.001, respectively, ns = not significant.

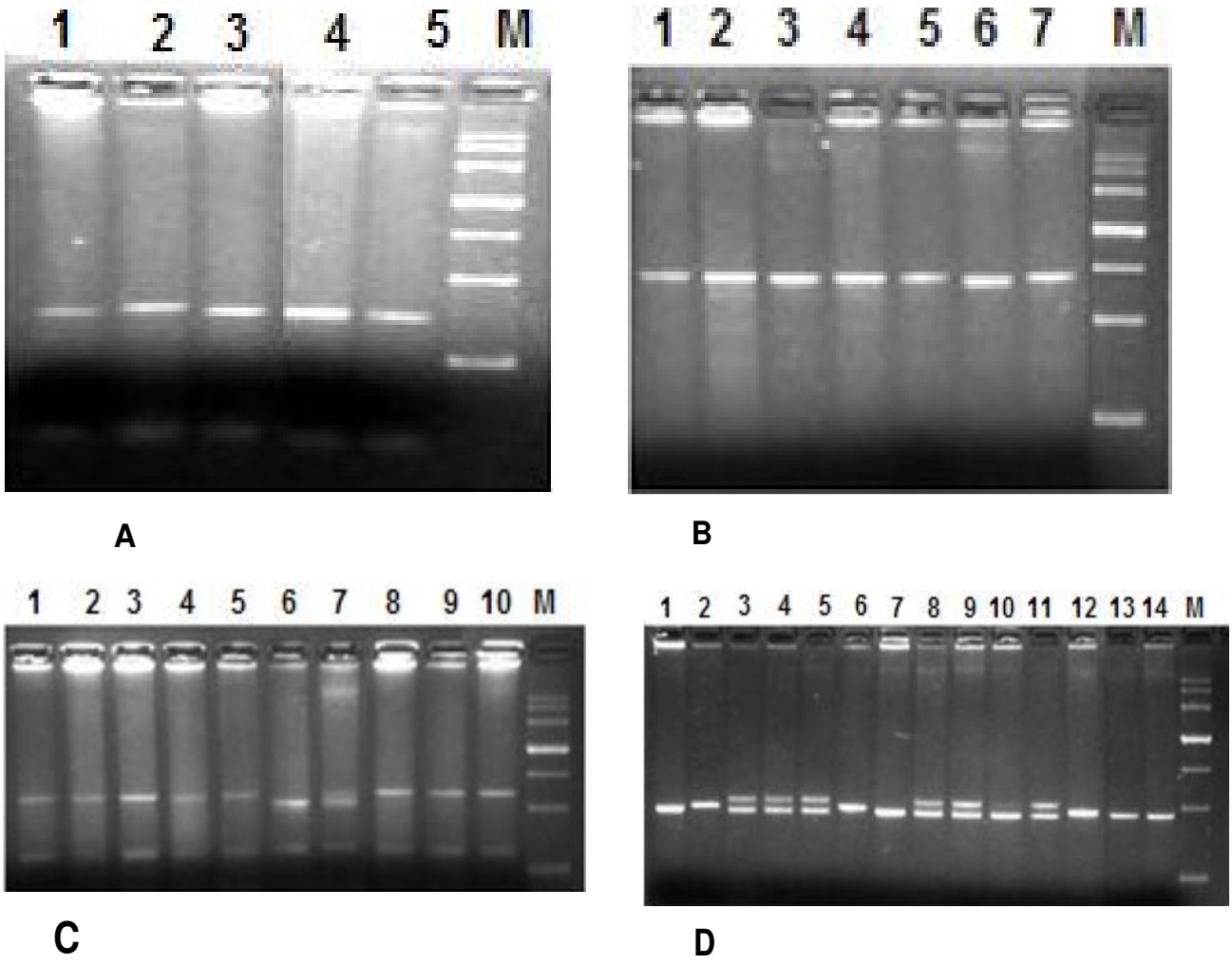

Figure 1. PCR products using different m-type LMW-GS specific primers in *T. turgidum* ssp. *turgidum* landraces (A) using Glu3A.2 primer (about 350 bp): 1. AS2287, 2. AS2241, 3. AS2333, 4. AS2235, 5. AS2236 M. 3000 bp marker; (B) using Glu3A.3 primer (about 700bp): 1. AS2287 2.AS2288 3. AS2293, 4. AS2310, 5.AS2351, 6 .AS2256, 7. AS2257 M. 3000 bp marker; (C) using Glu3B.1 primer (about 530 bp): 1.AS2299 2.AS2312 3. AS2315, 4. AS2333, 5. AS2381, 6. AS2256, 7. AS2336, 8. AS2235 9. AS2236, 10. AS2382 M. 3000 bp marker; (D) using Glu3B.2 primer (about 450 and 500 bp) : 1. AS2253, 2. AS2258, 3. AS2254, 4. AS2235, 5. AS2236, 6. AS2282, 7. AS2287, 8. AS2284, 9. AS2341, 10. AS2382, 11. AS2281, 12. AS2288, 13. AS2343, 14. AS2292, M = 3000 bp marker.

index, protein content, mixing tolerance index). All quality parameter values were transformed to the frequency distribution data. The test for the relationship between quality and alleles of m-type LMW-GS genes was calculated based on the contingency of the table analysis. All analysis was performed using the software DPS (Data Proceeding System) version 6.10 (Tang and Feng, 2001).

RESULTS

Allele variations of m-type LMW-GS

At Glu-A3 locus, two specific primer sets Glu3A.2 and Glu3A.3 were used to detect the allele variations on MDTSCIP- and METSCIP- subgroups of m-type LMW-GS genes (Table 1) respectively. Two alleles including one dominant allele of about 350 bp from 65 accessions and the other allele of over 350 bp from 2 accessions were observed in the MDTSCIP- subgroup of LMW-GS genes (Figure 1A). Similarly, in METSCIP subgroup, two alleles were observed, among them one was dominantly expressed in 67 accessions of size about 700 bp and another poorly expressed allele of less than 700 bp in accession AS2256 (Figure 1B).

To identify the allele variation on MENSHIP- and METSHIPS- subgroups of LMW-GS genes at Glu-B3 locus respectively, two specific primer sets, Glu3B.1 and Glu3B.2 were used (Table 1). Three alleles were of little

differ in their sizes were observed in both MENSHIP and METSHIP subgroups. An allele of 530 bp were observed in 62 accessions as dominant and other two alleles near 530 bp were expressed in 3 accessions as rarely in the MENSHIP- subgroup of LMW-GS genes (Figure 1C). As in the case of METSHIP subgroup, among three alleles, two of them were 500 and 450 bp respectively, in 41 accessions and the third allele of about 475 bp from 24 accessions was also detected (Figure 1D). However, no amplified products were found from accessions AS2317 and AS2335.

Genetic diversity of m-type LMW-GS

To further understand the genetic variation of different markers, the genetic diversity of the four subgroups of m-type LMW-GS genes was also estimated by POPGENE 1.32. The effective number of alleles displayed that METSHIPS-type (Glu3B.2, 2.9877) > MENSHIP-type (Glu3B.1, 1.0700) > MDTSCIP-type (Glu3A.2, 1.0303) > METSCIP-type (Glu3A.3, 1.0298). The Nei's expected heterozygosity (He) showed that METSHIPS-type (Glu3B.2, 0.6653) > MENSHIP-type (Glu3B.1, 0.0736) > MDTSCIP-type (Glu3A.2, 0.0294) > METSCIP-type (Glu3A.3, 0.0290). These results indicated that the genetic variations at Glu-B3 locus were higher than that at Glu-A3 locus.

Sequences identified of LMW-GS

To further confirm the type of LMW-GS gene from landraces, we cloned and sequenced the PCR products from accessions AS2241, AS2281 and AS2333 by marker Glu3A.2 and AS2343 by marker Glu3B.1, AS2257 by marker Glu3B.2 (Figure 2) respectively. Five sequences which were termed as EU057592, EU057593, EU057594, EU057598 and EU057597 in GenBank were obtained (Figure 2). Thirteen amino acid residues "MDTSCIPGLERPW" in N-terminal of sequences EU057592, EU057593 and EU057594 indicated that the marker Glu3A.2 can amplify MDT-type LMW-GS genes (Figure 2A). In addition, EU057594 had a deletion of peptide "PFSQQQPILPQGP" in the repeat region when compared to EU057592 and EU057593. Meanwhile, thirteen amino acid residues "MENSHIPGLERPS" in the N-terminal of sequence EU057598 (Figure 2B) and the short peptide "METSCIPGLERPW" in N-terminal of sequence EU057597 (Figure 2C), also suggested that the markers Glu3B.1 and Glu 3A.3 can amplify the MEN-type and MET- type LMW-GS. Therefore, the results indicated that not only were these markers specific and effective but also, there were four m-types of LMW-GS gene in *T. turgidum* ssp. *turgidum* landraces. However, the premature stop codon "TAG" was also found at 104 sites in sequence EU057598 which implied that this m-type gene is a pseudogene.

Association between quality parameters and allele variations

To understand the performance of the quality traits of landraces, protein contents, gluten parameters, and the farinograph parameters were investigated (Table 3). The values of protein content ranged from 10.11% in AS2305 to 22.01% in AS2256, with a mean of 14.39%, while the variation coefficient was 16.24%. The lower variation coefficient was 11.00% and the mean of the dry gluten was 13.11g. The percentage of gluten index was high in AS2379 at 61.06% and when compared with AS2287, it has 23.58%. The variation coefficient of gluten index was 25.83% with a mean of 61.06%. In the water absorption, AS2380 was 59.5%. This was slightly increased to 63.1% in AS2289. The mean and coefficient variation were 61.76 and 1.43% respectively, for water absorption. The lowest variation coefficient observed indicated that water absorption had a stable performance in all *T. turgidum* ssp. *turgidum* landraces of China. The higher variations were also found in the other quality related parameters, which included 44.10% of the development time, 54.10% of stable time, 32.28% of mixing tolerance index and 43.85% of the time to breakdown. In summary, the evaluation of quality traits suggested that there were wide and useful variations in *T. turgidum* ssp. *turgidum* landraces.

Using the test for independence, the associations between the quality parameters and the alleles of m-type LWM-GS genes were estimated. In four subgroups of m-type LMW-GS genes, only the allele variations of METSHIPS- subgroup were significantly associated with 3 quality characters: glutenin index ($P<0.05$), the time to breakdown ($P<0.05$), and protein content ($P<0.001$). The other allele variations of m-type LMW-GS genes were not significantly linked with dough quality characters (Table 3). These results suggested that the allele variations of METSHIPS- subgroup of m-type LMW-GS genes have stronger effects on the dough quality than the other m-type LMW-GS genes.

DISCUSSION

The PCR-based molecular markers have the advantages of being applicable to any developmental stage, giving results that are independent of the environment (Gale, 2005). The coding genes of wheat LMW-GS are not interrupted by introns and are highly conserved at both 5' and 3' terminal domains (Shewry and Halford, 2002; D'Ovidio and Masci, 2004), and these properties made it possible to isolate new LMW-GS genes from wheat and its relatives by PCR amplification thus, developing the PCR-based molecular markers (Masci et al., 1998; Ciaffi et al., 1999; Lee et al., 1999;). At the present, about 30 PCR-based markers for LMW-GS genes have been reported (Long et al., 2005; Ikeda et al., 2002, 2006; Zhang

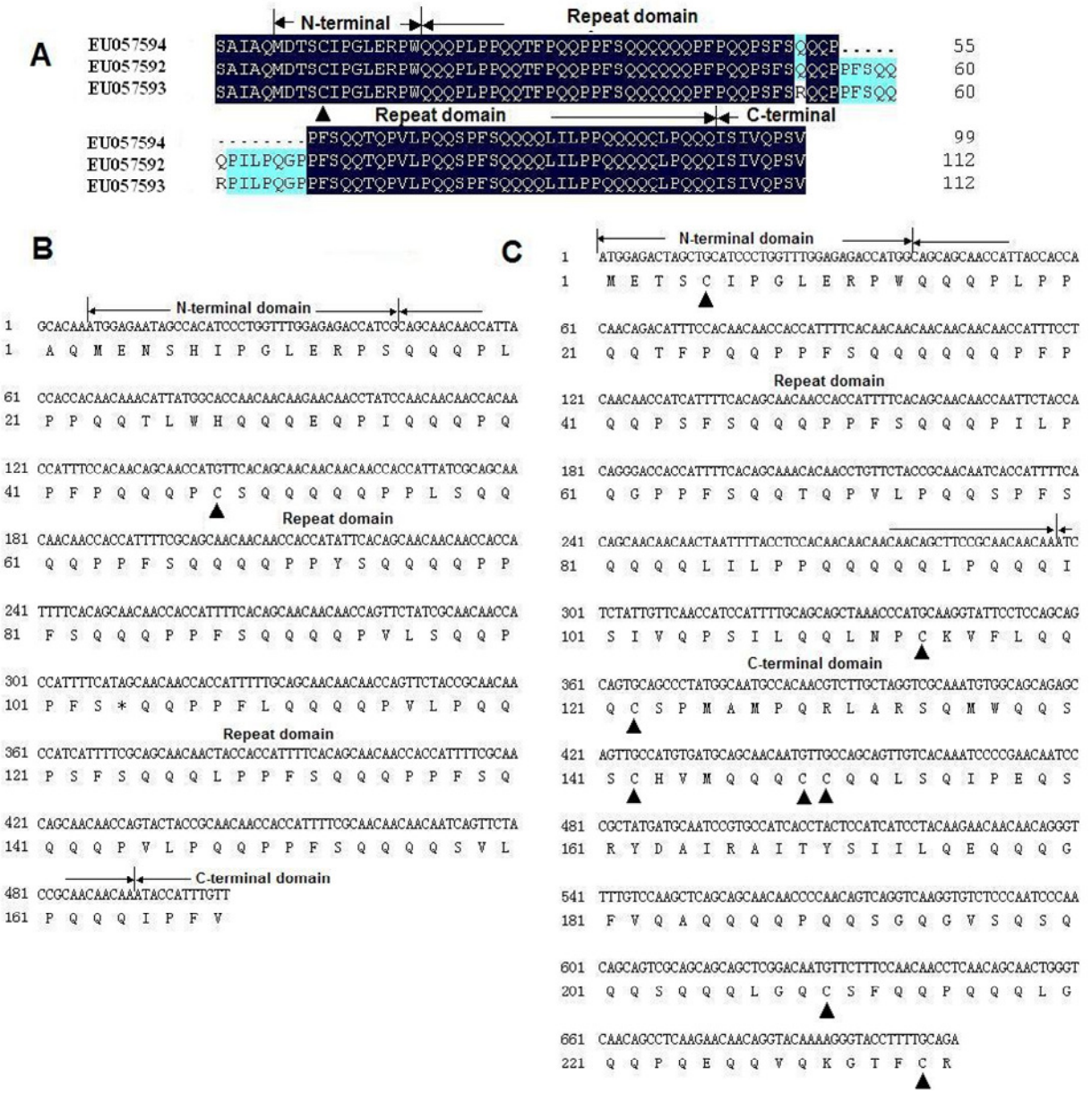

Figure 2. DNA and deduced amino acid sequences; A) The deduced amino acid sequences of EU057594, EU057592, EU057593 by Glu3A.2 marker; B) the sequence EU057598 by Glu3B.1 marker, * represents premature stop codon; C) the sequence of EU057597 by Glu3A.3 marker. Black solid triangle represents cysteine residue.

et al., 2004; Zhao et al., 2006). Long et al. (2005) designed nine LMW-GS group-specific primer sets in order to detect the specific allele variations of LMW-GS genes in the marker-assisted breeding. The validity of the classification was tested in diploid wheat and Aegilops species. In our study, the allele variations of m-type of LMW-GS genes, including MDTSCIP-, MENSHIP-, METSHIPG- and METSCIP- subgroups were detected in *T. turgidum* ssp. *turgidum* landraces of China, and the sequencing and distribution of cysteinne residues further confirmed the different m-type LMW-GS genes according to the previous study of Long et al. (2005). The results also suggested that there were a few allele variations of the 4 subgroup of m-type LMW-GS genes in these landraces of China. The similar results were sustained by the genetic diversity parameters, effective number of alleles and the Nei's expected heterozygosity (He) (Nei et al., 1973, 1978). Moreover, the genetic diversity of Glu-B3 locus was higher than that of Glu-A3 locus in landraces. Most of the dominant alleles of each subgroup of m-type LMW-GS genes displayed the higher frequencies such as, 95.59% in MDTSCIP-type, 98.53% in METSCIP-type and 91.18% in MENSHIP-type. Zhang et al. (2003) observed that the predominant alleles of

HMW-GS were Glu-A1b and Glu-B1-III in *T. turgidum* ssp. *turgidum* landraces of China. The new results and the previous studies both suggested that the landraces of China were a unique tetraploid germplasm.

Due to the complexity of LMW-GS gene family, the contribution of individual LMW-GS to wheat quality is not fully understood. Different allelic forms of LMW-GS seem to play different roles in determining different quality parameters (Luo et al., 2001). Liu et al. (2005) demonstrated that Glu-3 allelic variations were more closely associated with protein quality than with protein quantity. The deduced amino acid sequence of EU057594 has some InDels in the repetitive domain (Figure 2A). Tanaka et al. (2005) inferred that these deletions and substitutions of amino-acid in LMW-GS genes might be correlated with the positive effect on bread-making quality in common wheat. Zhao et al. (2006) also established relationships between LMW-GS coding genes and GluD3 alleles as defined by protein electrophoretic mobility. Ikeda et al. (2006) found that the same gene could encode both the LMW-m and LMW-s but was processed at different sites. These findings further supported the complexity of the relationship between quality and LMW-GS. The sequences of B subunits could be subdivided into LMW-s, LMW-m and LMW-i types (Kasarda et al., 1988; Tao and Kasarda, 1989; Lew et al., 1992; Cloutier et al., 2001; D`Ovidio and Masci, 2004). LMW-m and LMW-s are the most commonly encountered polypeptides (Ferrante et al., 2004; Ikeda et al., 2004). Ferrabte et al. (2006) reported that the incorporation of the LMW-i type into bread wheat dough had minimal effects on dough mixing requirements. Maruyama-Funatsuki et al. (2005) observed five LMW-s glutenin components associated with good bread-making quality. The previous study also proposed that LMW-s glutenin gene encodes LMW glutenin components associated with good bread-making quality (Masci et al., 1998, 2002). However, little information on the association of m-type LMW-GS genes and wheat quality was available. In the present paper, we examined genetic variations of m-type LMW-GS genes in *T. turgidum* ssp. *turgidum* landraces of China and their associations with dough quality parameters. The test for independence between four m-type LMW-GS and dough quality characters indicated that the METSHIPS- subgroup of m-type LMW-GS genes could have more important effects on the quality performance in *T. turgidum* ssp. *turgidum* landraces of China. Lew et al. (1992) proposed that the m-type group was mainly composed of polypeptides having the METSH- or METSC- N-terminal amino-acid sequences, and the former group being both qualitatively and quantitatively better than the latter. Liu et al. (2005) suggested that the quality effects of LMW-GS could be ranked as Glu-B3 > Glu-A3 for SDS sedimentation value, mixing time and mixing tolerance. The similar result was also obtained in our study based on the variation analysis of m-type LMW-GS.

Our study provided a useful attempt in knowing the relationship between the m-type LMW-GS genes and quality characters. These results could also help to improve the marker-assisted breeding of m-type LMW-GS genes in wheat breeding. On the other hand, the *T. turgidum* ssp. *turgidum* landraces which were evaluated by molecular markers could also be used in quality breeding as a special germplasm.

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