Plant Genetic Resources: Characterization and Utilization

cambridge.org/pgr

Characterization of novel low-molecular-weight glutenin subunit genes from the diploid wild wheat relative *Aegilops umbellulata*

Wenyang Wang[1,2,*], Wenjun Ji[2,*], Lihua Feng[3], Shunzong Ning[2], Zhongwei Yuan[2], Ming Hao[2], Lianquan Zhang[1,2], Zehong Yan[2], Bihua Wu[2], Dengcai Liu[1,2] and Lin Huang[2]

[1]State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Sichuan Agricultural University, Wenjiang 611130, China; [2]Triticeae Research Institute, Sichuan Agricultural University, Wenjiang 611130, China and [3]College of Agronomy, Sichuan Agricultural University, Wenjiang 611130, China

Abstract

Low molecular weight glutenin subunits (LWM-GSs) play a crucial role in determining wheat flour processing quality. In this work, 35 novel LMW-GS genes (32 active and three pseudogenes) from three *Aegilops umbellulata* ($2n = 2x = 14$, UU) accessions were amplified by allelic-specific PCR. We found that all LMW-GS genes had the same primary structure shared by other known LMW-GSs. Thirty-two active genes encode 31 typical LMW-m-type subunits. The MZ424050 possessed nine cysteine residues with an extra cysteine residue located in the last amino acid residue of the conserved C-terminal III, which could benefit the formation of larger glutenin polymers, and therefore may have positive effects on dough properties. We have found extensive variations which were mainly resulted from single-nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) among the LMW-GS genes in *Ae. umbellulata*. Our results demonstrated that *Ae. umbellulata* is an important source of LMW-GS variants and the potential value of the novel LMW-GS alleles for wheat quality improvement.

Research Article

*Wenyang Wang and Wenjun Ji have contributed equally to this work.

Cite this article: Wang W *et al*. (2022). Characterization of novel low-molecular-weight glutenin subunit genes from the diploid wild wheat relative *Aegilops umbellulata*. *Plant Genetic Resources: Characterization and Utilization* **20**, 1–6. https://doi.org/10.1017/S1479262122000016

Received: 22 July 2021
Revised: 6 April 2022
Accepted: 7 April 2022
First published online: 12 May 2022

Keywords:
Aegilops umbellulata; LMW-GS; U genome; wheat quality improvement

Author for correspondence:
Lin Huang,
E-mail: lhuang@sicau.edu.cn

Introduction

Low molecular weight glutenin subunits (LWM-GSs) account for approximately 40% of total proteins in wheat grain and primarily determine dough extensibility and strength, thus playing a pivotal role in wheat flour processing quality (Lee *et al*., 2016; Li *et al*., 2016; Xiang *et al*., 2019). The LMW-GSs encoding genes are located at loci of *Glu-A3*, *Glu-B3* and *Glu-D3* on the short arms of wheat chromosomes 1A, 1B and 1D, respectively (D'Ovidio and Masci, 2004). The copy numbers of LMW-GS genes in bread wheat are varied from 10–20 to 30–40 (Harberd *et al*., 1985; Lee *et al*., 2016). The copy numbers of active LMW-GS genes in single accession were estimated to be 9 to 13 (Zhang *et al*., 2013). Based on the first amino acid residue at the N-terminal domain of the mature protein, the LMW-GSs genes are traditionally categorized into LMW-methionine (m), LMW-serine (s) and LMW-isoleucine (i) types (D'Ovidio and Masci, 2004). A fourth type of LMW-GS gene, LMW-leucine (l), was characterized from *Aegilops comosa* (Wang *et al*., 2011*b*; Huang *et al*., 2018).

Aegilops umbellulata Zhuk. ($2n = 2x = 14$, UU) is a wild diploid relative of cultivated wheat that harbours great variability in such valuable traits as disease resistance (Gill *et al*., 1985; Chhuneja *et al*., 2008; Edae *et al*., 2016) and grain quality-related traits (Law and Payne, 1983; Dai *et al*., 2015; Wang *et al*., 2018). *Ae. umbellulata* has crossability with tetraploid wheat, and therefore agronomic desirable traits from *Ae. umbellulata* can be introgressed into common wheat using synthetic wheat hexaploids with the AABBUU genome as bridges (Bansal *et al*., 2017; Song *et al*., 2019). *PmY39* for powdery mildew resistance (Zhu *et al*., 2006) and *Lr9* for leaf rust resistance (Schachermayr *et al*., 1994) were successfully transferred from *Ae. umbellulata* to wheat cultivars. The introgression of 1U genome of *Ae. umbellulata* into hexaploid wheat showed significantly improved dough rheological properties and bread-making quality (Wang *et al*., 2018).

A pair of HMW-GS and their coding genes at *Glu-U1* locus was characterized and cloned (Liu *et al*., 2002). Some *Glu-U1* genes were transferred from *Ae. umbellulata* to the Chinese Spring wheat variety (Islam-Faridi, 1988). Characterization of LMW-GS genes from *Ae. umbellulata* was reported by limited studies (Li *et al*., 2010; Wang *et al*., 2011*a*). The numbers of LMW-GS genes reported in these studies varied from 1 to 5. In the present study, we isolated 35 novel LMW-GS genes (11–14 copies per accession) from three *Ae. umbellulata* accessions and their molecular characterization was investigated.

Materials and methods

Plant materials

Ae. umbellulata ($2n = 2x = 14$, UU) accessions (Fig. 1) numbered PI 554,396, AS3 and AS4 were used in this study. PI 554,396 was kindly provided by the National Plant Germplasm System of the USDA-ARS, USA. AS3 and AS4 were originally provided by Dr Sadao Sakamoto, Plant Germ-plasm Institute, Kyoto University, Kyoto, Japan. All these materials were kept at the Triticeae Research Institute, Sichuan Agricultural University, Chengdu, China.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from seedling leaves of *Ae. umbellulata* accessions using the cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich, 1985) with slight modification. One pair of allele-specific PCR (AS-PCR) primers (LMW-1: ATCATCACAAGCACAAGCATC and LMW-2: TTCTTATCAG TAGGCACCAAC) (Wang *et al.*, 2011*b*) was used to amplify LMW-GS genes. The PCR amplification was performed using the Veriti™ 96-Well Fast Thermal Cycler (Applied Biosystems, Foster City, CA, USA) in a 40 µl reaction volume as described by Huang *et al.* (2018). PCR products were separated using 1.5% agarose gel electrophoresis, stained with GelRed Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA). These fragments were purified by using Sureclean (Bioline Reagents, London), ligated to pMD19-T vector (Takara, Dalian, China) and used to transform *Escherichia coli* DH5α cells. The positive clones were sequenced by Sangon Biotechnology Company (Shanghai, China). To avoid possible error, the final sequence of each LMW-GS gene was determined from the sequencing results of at least three independent clones.

Sequence analysis of LMW-GS genes

The assembly of LMW-GS sequences was completed using Lasergene software (DNASTAR; http://www.dnastar.com/). The sequence alignment of LMW-GS genes was performed with BioEdit (Hall, 2007). The phylogenetic tree was constructed using the neighbour-joining method in the MEGA 6.0 software (Tamura *et al.*, 2013). Bootstrap values were calculated from 1000 replications.

Results

Cloning and characterization of LMW-GS genes

PCR products from three *Ae. umbellulata* accessions are shown in Fig. 2. A single candidate amplification product with approximately 900 bp was obtained using AS-PCR primers LMW-1/LMW2. After sequencing, a total of 35 LMW-GS sequences were obtained (online Supplementary Table S1). GenBank database comparison showed that these genes were different from reported LMW-GS genes in *Ae. umbellulata* (Li *et al.*, 2010; Wang *et al.*, 2011*a*) and other *Triticum* species; thus, all these 35 genes were novel and were deposited in GenBank with the accession numbers (MZ424047-MZ424081).

In total, 14, 12 and 11 LMW-GS genes were revealed by sequencing of 42, 24 and 22 clones from PI554396, AS3 and AS4, respectively. MZ424067, which present in three *Ae. umbellulata* accessions, was found to be the most common LMW-GS gene (>54%). MZ424080 and MZ424081 in PI554396 and MZ424079

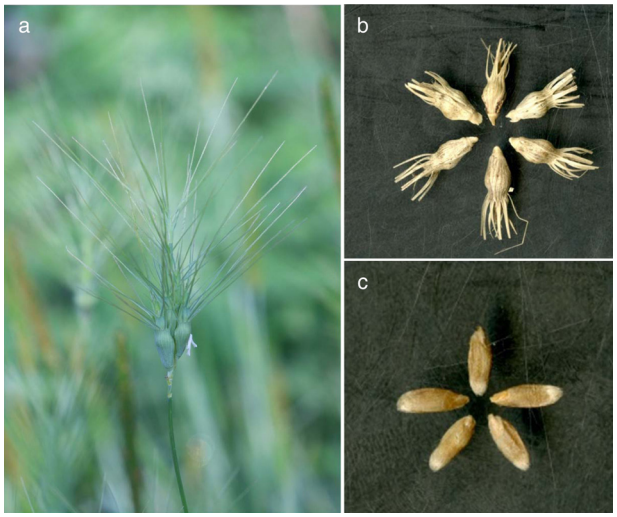

Fig. 1. Morphology of *Aegilops umbellulata* spike. (a), spike; (b), spikelets; (c), seeds.

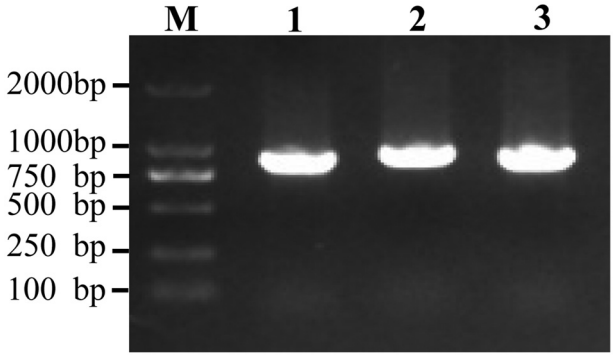

Fig. 2. PCR amplification of LMW-GS genes from *Aegilops umbellulata* accessions by the AS-PCR primers LMW-1/LMW2. Lanes 1, 2 and 3 PCR products were amplified from PI 554,396, AS3 and AS4, respectively. M, DL2000 DNA marker.

in AS3 are pseudogenes, the remaining 32 genes are complete active genes. The ORFs of the 32 active LMW-GS genes varied from 885 to 888 bp, which encoding 31 different LMW-GSs with 294 to 295 amino acid residues. The coding regions of these LMW-GS genes were all terminated by double stop codons.

Amino acid sequence alignment indicated that all subunits shared four main structural domains, including a signal peptide, an N-terminal region, a repetitive domain and a C-terminal domain with three sub-regions (C-terminal I, II and III). Since the first amino acid residue of the mature protein was methionine, the 31 LMW-GS were regarded as typical LMW-m-type subunits (Fig. 3). All LMW-m-type proteins begin with METSCIPGL except MZ424060 begin with METSCILGL. As typical for LMW-GS genes, 30 subunits had eight highly conserved cysteine residues, while MZ424050 containing nine cysteine residues with an extra cysteine residue located at the last amino acid residue of the conserved C-terminal III domain in LMW-m subunit (online Supplementary Table S1).

Sequence variations of the LMW-GS genes

Multiple sequence alignment of the LMW-GS sequences was performed to identify the presence of single-nucleotide polymorphisms (SNPs) and insertions and deletions (InDels). As

https://doi.org/10.1017/S1479262122000016 Published online by Cambridge University Press

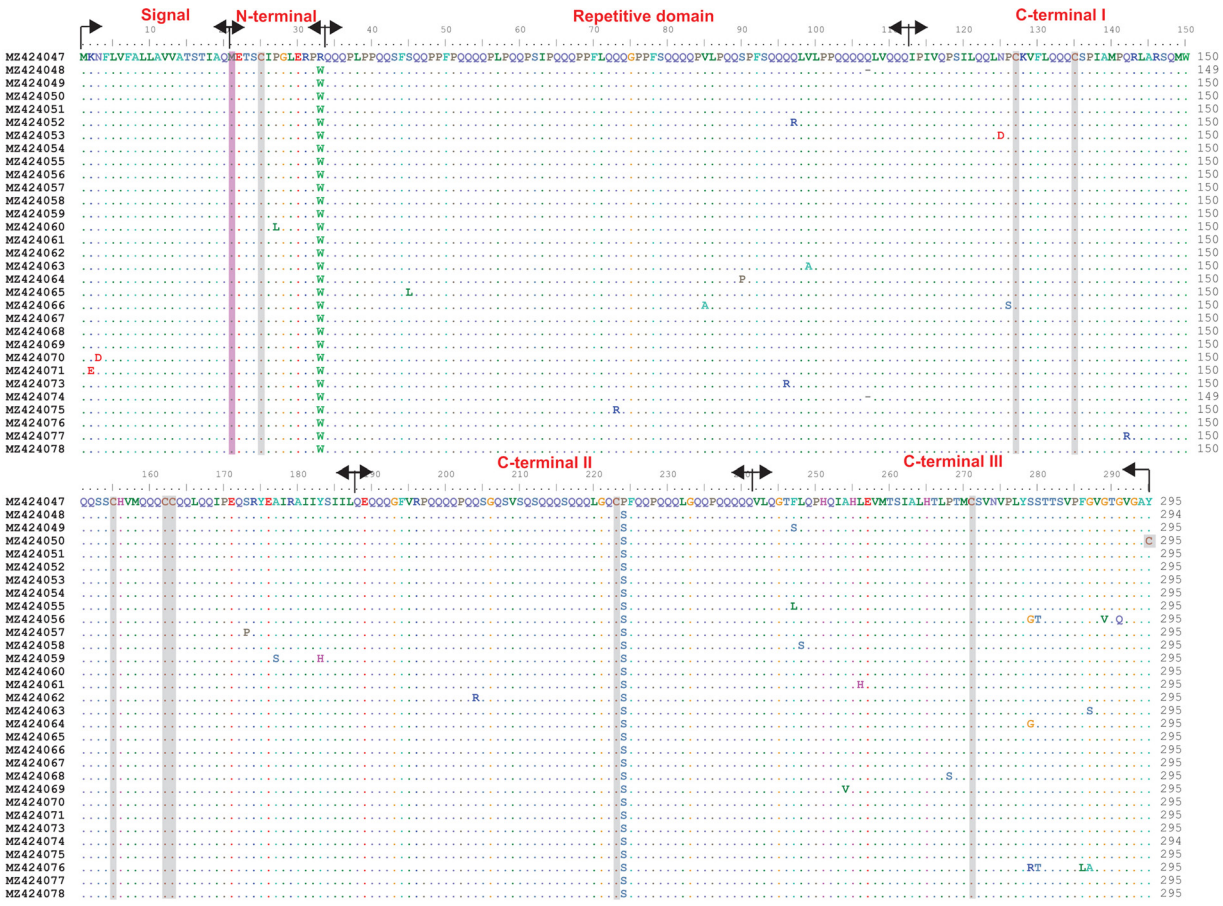

Fig. 3. Multiple alignments of the deduced amino acid sequences of LMW glutenin genes. Signal, signal peptide. The mature protein sequences were divided into: N-terminal domain; repetitive domain; C-terminal domains (I–III). Magenta shows the first amino acid residue of the mature proteins. Grey shading indicates the cysteine residues. Identical sequences and deletions were presented by dots and dashes, respectively.

Table 1. SNPs and InDels identified among 35 LMW-GS genes from *Ae. umbellulata*

	SNP and InDel positions									
LMW-GS genes	4	7	80	97	105	117	134	150	218	226
Predominant MZ424067	A	A	C	T	G	A	C	T	A	C
Remaining 34 LMW-GS genes	G	G	T	C	A	T	T	C	G	-
	254	255	268	287	290	296	306–308	351	373	376
	T	T	T	A	A	T	ACA	G	A	C
	C	C	C	G	G	C	–	A	G	T
	423	425	453	514	517	529	547	564	611	636
	A	A	G	C	T	G	T	A	A	C
	G	G	A	T	C	T	C	G	G	A
	642	663	670	684	730	739	740	743	761	767
	G	A	T	T	C	T	T	T	C	T
	A	G	C	C	T	C	C	C	T	A
	802	834	835	837	838	843	846	852	856	859
	C	C	A	C	T	C	C	G	T	G
	T	T	G	A	A	T	T	A	C	A
	860	866	867	870	871	872	884			
	G	G	C	T	G	G	A			
	C	T	T	C	C	A	G			

https://doi.org/10.1017/S1479262122000016 Published online by Cambridge University Press

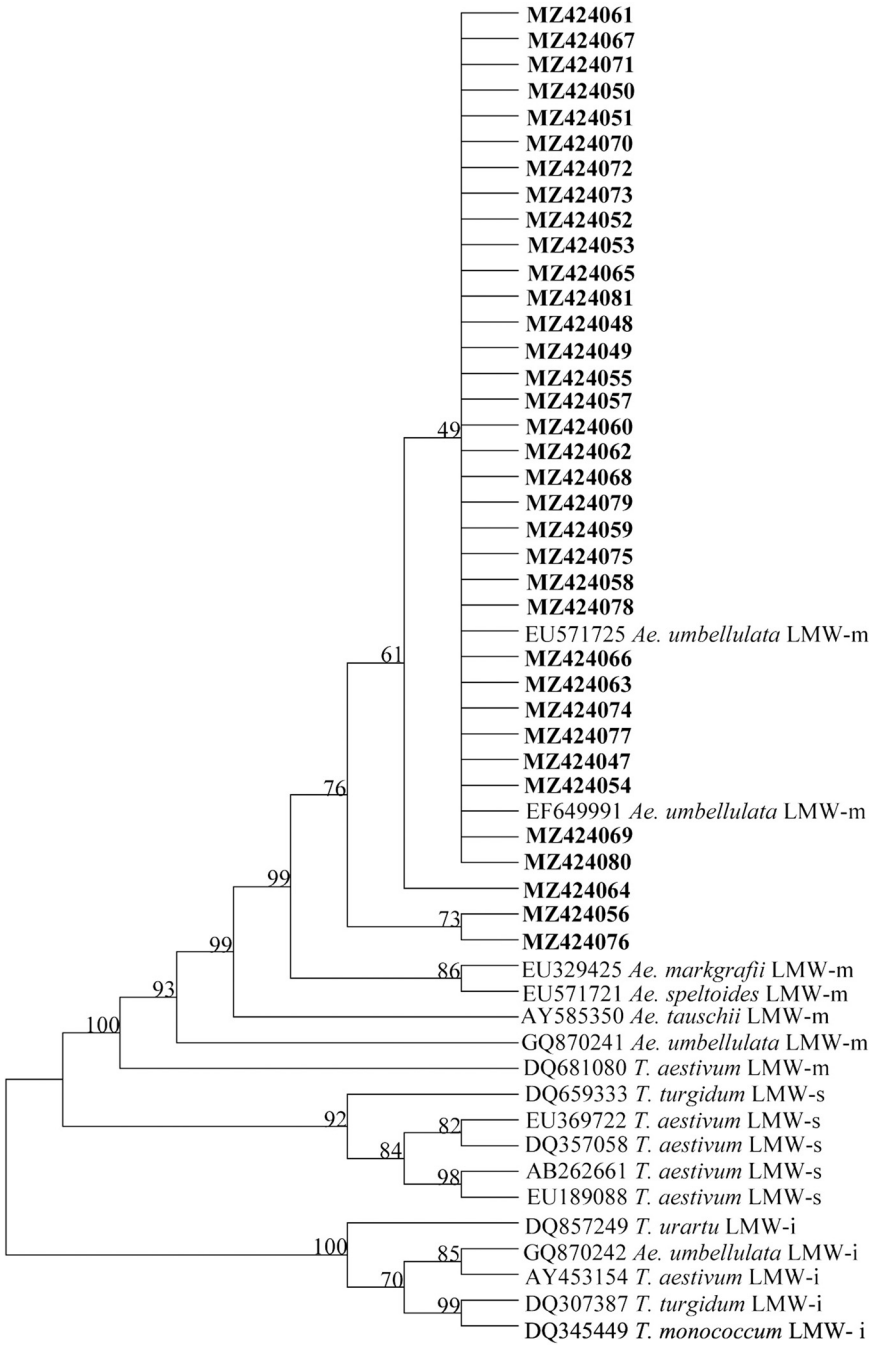

Fig. 4. Phylogenetic relationships of the 35 newly isolated and LMW-GS sequences retrieved from GenBank. The nucleotide sequences were specified by their corresponding GenBank accession numbers. The LMW-GS gene types and species are indicated. Bold, the 35 LMW-GSs identified in this study.

compared to the predominant MZ424067 in three *Ae. umbellulata* accessions, a total of 55 SNPs were detected at different positions (Table 1). One-base deletion (C) was found at the position 226 in MZ424081 and a three-base deletion (ACA) at 306–308 were detected in MZ424048 and MZ424074.

Phylogenetic analysis of LMW-GS genes

To further investigate the phylogenetic relationships among the LMW-GS genes at the *Glu-3* loci, a phylogenetic tree was constructed based on 35 LMW-GS genes in this study and other 17 LMW-GS genes retrieved from GenBank. The results are shown in Fig. 4.

The phylogenetic tree was divided into three clear branches, with alleles of LMW-m-type genes at the top, LMW-s-type genes at the middle and LMW-i-type genes at the bottom. The 35 LMW-GS genes obtained in the current study were located in the LMW-m-type branch, which clustered with two published LMW-m-type genes EU571725 and EF649991 from *Ae. umbellulata*, further confirming that they are LMW-m-type genes.

Discussion

The LMW-GS genes are widely presented in genomes of wheat and related cereals (Ikeda *et al.*, 2002; Henkrar *et al.*, 2017; Huang *et al.*, 2018; Xiang *et al.*, 2019). In the current study, we have isolated 32 novel active LMW-GS genes and three pseudogenes from the *Ae. umbellulata* genome using AS-PCR primers. We have found extensive allelic variations at *Glu-3U* that were

https://doi.org/10.1017/S1479262122000016 Published online by Cambridge University Press

mainly resulted from SNPs and InDels presented in the LMW-GS genes. A phylogenetic tree was constructed to understand the phylogenetic relationships within these LMW-GS genes.

To date, a few LMW-GS genes have been cloned from *Ae. umbellulata* accessions. For example, one LMW-GS gene EU571725 from *Ae. umbellulata* accession PI222762 (Li *et al.*, 2010); five genes GQ980034, GQ980035, GQ870240-GQ870242 from PI573516 (Wang *et al.*, 2011*a*). Previous studies showed that the copies of *Glu-3* genes in common wheat are varied from 10–20 to 30–40 (Harberd *et al.*, 1985; Lee *et al.*, 2016) and active LMW-GS genes in single accession were estimated to be 9 to 13 (Zhang *et al.*, 2013). Wang *et al.* (2018) identified 10 abundant 1U-encoded LMW-GS subunits in CNU609 derived from crosses between Chinese Spring and the wheat-*Ae. umbellulata* 1U(1B) substitution line and cloned two Glu-U3-encoded LMW-m subunit genes. In the present study, we have isolated 14 (12 active), 12 (11 active) and 11 (11 active) LMW-GS genes from three *Ae. umbellulata* accessions, respectively. These results suggest that almost all active genes at *Glu-3U* loci were cloned and the *Ae. umbellulata* genome may have the similar *Glu-3* gene copies to those of wheat.

Previous studies have shown that the cysteine residues were involved in the formation of inter- and intra-molecular disulphide bonds, and any modification in the number or location of cysteine residues could lead to functional variations (D'Ovidio and Masci, 2004). The most mature Glu-3 proteins contain eight highly conserved cysteine residues. The first and seventh cysteine residues are involved in inter-molecular disulphide bonds, while the remaining six cysteine residues are participated in forming intra-molecular disulphide bonds. In this study, 30 subunits had eight cysteine residues, and the position of these cysteine residues was similar to that of the typical LMW-m-type subunits, whereas MZ424050 subunit had nine cysteine residues with an extra cysteine residue located in the conserved C-terminal III domain. The GQ870241 subunit from U genome with an extra cysteine residue at the C-terminal III was predicted to have positive effects on dough properties. The same number of cysteine residues was found in a LMW-m-type subunit AY263369, which was likely associated with good bread-making quality (Zhao *et al.*, 2004; Xu *et al.*, 2006). It has been suggested that the extra cysteine residue might benefit the formation of larger glutenin polymers and contributed to superior gluten quality (Lan *et al.*, 2013). Therefore, the MZ424050 subunit could contribute to good dough properties and may be new candidate gene for wheat quality improvement.

In the present study, all LMW-GS proteins belonged to typical LMW-m-type subunits. Wang *et al.* (2011*a*) identified a LMW-i-type gene (GQ870242) from *Ae. umbellulata* PI573516, while this gene is not active due to the presence of premature stop codon in the ORFs. To our best knowledge, the active LMW-GS genes cloned in *Ae. umbellulata* so far all belonged to LMW-m-type subunits. We have found considerable SNPs variations at the *Glu-3U* loci, which could be produced by unequal crossing over, point mutations and illegitimate recombination (Anderson and Greene, 1989; An *et al.*, 2006; Zhang *et al.*, 2006; Li *et al.*, 2008). The extensive allelic variations at *Glu-3U* could facilitate the development of genome-specific molecular markers and marker-assisted selection (MAS) in wheat quality breeding. The novel LMW-GS genes could be served as valuable genetic sources for wheat quality improvement.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262122000016

Acknowledgements. This work was financially supported by grants from the National Natural Science Foundation of China (31901493), the Science & Technology Department of Sichuan Province (2021YJ0505 and 2021YFH0110).

References

An X, Zhang Q, Yan Y, Li Q, Zhang Y, Wang A, Pei Y, Tian J, Wang H, Hsam SLK and Zeller FJ (2006) Cloning and molecular characterization of three novel LMW-i glutenin subunit genes from cultivated einkorn (*Triticum monococcum* L.). *Theoretical and Applied Genetics* **113**, 383–395.

Anderson OD and Greene FC (1989) The characterization and comparative analysis of high-molecular-weight glutenin genes from genomes A and B of a hexaploid bread wheat. *Theoretical and Applied Genetics* **77**, 689–700.

Bansal M, Kaur S, Dhaliwal HS, Bains NS, Bariana HS, Chhuneja P and Bansal UK (2017) Mapping of *Aegilops umbellulata*-derived leaf rust and stripe rust resistance loci in wheat. *Plant Pathology* **66**, 38–44.

Chhuneja P, Kaur S, Goel RK, Aghaee-Sarbaezeh M, Parashar M and Dhaliwal HS (2008) Transfer of leaf rust and stripe rust resistance from *Aegilops umbellulata* Zhuk. to bread wheat (*Triticum aestivum* L.). *Genetic Resources and Crop Evolution* **55**, 849–859.

Dai SF, Zhao L, Xue XF, Jia YN, Liu DC, Pu ZJ, Zheng YL and Yan ZH (2015) Analysis of high-molecular-weight glutenin subunits in five amphidiploids and their parental diploid species *Aegilops umbellulata* and *Aegilops uniaristata*. *Plant Genetic Resources* **13**, 186–189.

D'Ovidio R and Masci S (2004) The low-molecular-weight glutenin subunits of wheat gluten. *Journal of Cereal Science* **39**, 321–339.

Edae EA, Olivera PD, Jin Y, Poland JA and Rouse MN (2016) Genotype-by-sequencing facilitates genetic mapping of a stem rust resistance locus in *Aegilops umbellulata*, a wild relative of cultivated wheat. *BMC Genomics* **17**, 1039.

Gill BS, Sharma C, Raupp WJ, Browder LE, Heachett JH, Harvey TL, Moseman JG and Waines JG (1985) Evaluation of *Aegilops* species for resistance to wheat powdery mildew, wheat leaf rust, Hessian fly, and greenbug. *Plant Disease* **69**, 314–316.

Hall T (2007) *BioEdit, Version 7.0.9*. Carlsbad, CA: Computer Program and Documentation, lbis Biosciences.

Harberd NP, Bartels D and Thompson RDM (1985) Analysis of the gliadin multigene loci in bread wheat using nullisomic-tetrasomic lines. *Molecular Genetics and Genomics* **198**, 234–242.

Henkrar F, El-Haddoury J, Iraqi D, Bendaou N and Udupa SM (2017) Allelic variation at high-molecular weight and low-molecular weight glutenin subunit genes in Moroccan bread wheat and durum wheat cultivars. 3 *Biotech* **7**, 287.

Huang L, He Y, Jin YR, Wang F, He JS, Feng LH, Liu DC and Wu BH (2018) Characterization of novel LMW glutenin subunit genes at the *Glu-M3* locus from *Aegilops comosa*. *3 Biotech* **8**, 379.

Ikeda TM, Nagamine T, Fukuoka H and Yano H (2002) Identification of new low-molecular-weight glutenin subunit genes in wheat. *Theoretical and Applied Genetics* **104**, 680–687.

Islam-Faridi MN (1988) *Genetical Studies of Grain Protein and Developmental Characters in Wheat* (PhD Thesis). University of Cambridge, Cambridge.

Lan QX, Feng B, Xu ZB, Zhao GJ and Wang T (2013) Molecular cloning and characterization of five novel low molecular weight glutenin subunit genes from Tibetan wheat landraces (*Triticum aestivum* L.). *Genetic Resources and Crop Evolution* **60**, 799–806.

Law CN and Payne PI (1983) Genetical aspects of breeding for improved grain protein content and type in wheat. *Journal of Cereal Science* **1**, 79–93.

Lee JY, Beom HR, Altenbach SB, Lim SH, Kim YT, Kang CS, Yoon UH, Gupta R, Kim ST, Ahn SN and Kim YM (2016) Comprehensive identification of LMW-GS genes and their protein products in a common wheat variety. *Functional & Integrative Genomics* **16**, 269–279.

Li XH, Ma W, Gao LY, Zhang YZ, Wang AL, Ji KM, Wang K, Appels R and Yan Y (2008) A novel chimeric LMW-GS gene from the wild relatives of wheat *Ae. kotschyi* and *Ae. juvenalis*: evolution at the *Glu*-3 loci. *Genetics* **180**, 93–101.

Li XH, Wang K, Wang SL, Gao LY, Xie XX, Hsam SLK, Zeller FJ and Yan YM (2010) Molecular characterization and comparative transcriptional

analysis of LMW-m-type genes from wheat (*Triticum aestivum* L.) and *Aegilops* species. *Theoretical and Applied Genetics* **121**, 845–856.

Li XJ, Liu TH, Song LJ, Zhang H, Li LQ and Gao X (2016) Influence of high-molecular-weight glutenin subunit composition at *Glu-A*1 and *Glu-D*1 loci on secondary and micro structures of gluten in wheat (*Triticum aestivum* L.). *Food Chemistry* **213**, 728–734.

Liu ZJ, Zhang XM, Wan YF, Liu KF and Wang DW (2002) Characterization of high-molecular-weight glutenin subunits and their coding genes from *Aegilops umbellulata*. *Acta Botanica Sinica* **44**, 809–814.

Rogers SO and Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Molecular Biology* **5**, 69–76.

Schachermayr GM, Siedler H, Gale MD, Winzeller H, Winzeller M and Keller B (1994) Identification and localization of molecular markers linked to the *Lr*9 leaf rust resistance gene of wheat. *Theoretical and Applied Genetics* **88**, 110–115.

Song ZP, Dai SF, Jia YN, Zhao L, Kang LZ, Liu DC, Wei YM, Zheng YL and Yan ZH (2019) Development and characterization of *Triticum turgidum–Aegilops umbellulata* amphidiploids. *Plant Genetic Resources* **17**, 24–32.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725–2729.

Wang SL, Li XH, Wang K, Wang XZ, Li SS, Zhang YZ, Guo GF, Zeller FJ, Hsam SLK and Yan YM (2011*a*) Phylogenetic analysis of C, M, N and U genomes and their relationships with *Triticum* and other related genomes as revealed by LMW-GS genes at *Glu*-3 loci. *Genome* **54**, 273–284.

Wang K, Gao L, Wang S, Zhang Y, Li X, Zhang M, Xie Z, Yan Y, Belgard M and Ma W (2011*b*) Phylogenetic relationship of a new class of LMW-GS genes in the M genome of *Aegilops comosa*. *Theoretical and Applied Genetics* **122**, 1411–1425.

Wang J, Wang C, Zhen S, Li X and Yan Y (2018) Low-molecular-weight glutenin subunits from the 1U genome of *Aegilops umbellulata* confer superior dough rheological properties and improve bread making quality of bread wheat. *Journal of the Science of Food and Agriculture* **98**, 2156–2167.

Xiang L, Huang L, Gong FY, Liu J, Wang YF, Jin YR, He Y, He JS, Jiang QT, Zheng YL, Liu DC and Wu BH (2019) Enriching LMW-GS alleles and strengthening gluten properties of common wheat through wide hybridization with wild emmer. *3 Biotech* **9**, 355.

Xu H, Wang RJ, Shen X, Zhao YL, Sun GL, Zhao HX and Guo AG (2006) Functional properties of a new low-molecular-weight glutenin subunit gene from a bread wheat cultivar. *Theoretical and Applied Genetics* **113**, 1295–1303.

Zhang Y, Li Q, Yan Y, Zheng J, An X, Xiao Y, Wang A, Wang H, Hsam SLK and Zeller FJ (2006) Molecular characterization and phylogenetic analysis of a novel glutenin gene (*Dy*10.1*t*) from *Aegilops tauschii*. *Genome* **49**, 735–745.

Zhang XF, Liu DC, Zhang JH, Jiang W, Luo GB, Yang WL, Sun JZ, Tong YP, Cui DQ and Zhang AM (2013) Novel insights into the composition, variation, organization, and expression of the low-molecular-weight glutenin subunit gene family in common wheat. *Journal of Experimental Botany* **64**, 2027–2040.

Zhao H, Wang R, Guo A, Hu S and Sun G (2004) Development of primers specific for LMW-GS genes located on chromosome 1D and molecular characterization of a gene from *Glu-D*3 complex locus in bread wheat. *Hereditas* **141**, 193–198.

Zhu ZD, Zhou RH, Kong XY, Dong YC and Jia JZ (2006) Microsatellite marker identification of a *Triticum aestivum-Aegilops umbellulata* substitution line with powdery mildew resistance. *Euphytica* **150**, 149–153.

https://doi.org/10.1017/S1479262122000016 Published online by Cambridge University Press