Characterization of novel LMW glutenin subunit genes at the *Glu-M3* locus from *Aegilops comosa*

Lin Huang¹ · Yu He¹ · Yarong Jin¹ · Fang Wang¹ · Jingshu He¹ · Lihua Feng¹ · Dengcai Liu¹ · Bihua Wu¹

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Abstract

We report the characterization of seven novel low-molecular-weight glutenin subunit (LMW-GS) genes from *Aegilops comosa* ($2n = 2x = 14$, MM). We found that all seven LMW-GS genes possessed the same primary structure shared by other known LMW-GSs. Three genes (*comosa-M1*, *comosa-M2*, and *comosa-M3*) encode LMW-m-type subunits, two (*comosa-I1* and *comosa-I2*) encode LWM-i-type subunits, and two (*comosa-L1* and *comosa-L2*) encode LWM-l-type subunits. The *comosa-M1* possessed seven cysteine residues, which resulted from a single-nucleotide polymorphism (SNP) of the G-A transition in the fifth position of the N-terminal sequence. Two l-type subunits, *comosa-L1* and *comosa-L2*, contained nine cysteine residues with an extra cysteine residue located in the signal peptide and repetitive domain. In addition, a long insertion of 13 residues (LGQQPQ8/LVQQPQ8) was present in the end of the C-terminal II. Phylogenetic analysis implied that the *comosa-L2* may be the intermediate type during the evolution of LMW-l and LMW-i-type genes. Our results demonstrated that the novel LMW-GSs, such as *comosa-M1*, *comosa-L1*, and *comosa-L2*, may have positive effects on dough properties.

Keywords *Aegilops comosa* · LMW-GS · M genome · Wheat quality improvement

Introduction

Glutenin proteins play a crucial role in determining the viscoelastic properties of wheat dough. According to their mobility on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) gel, the glutenins are divided into high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits (GSs), which are linked by inter- and intra-molecular disulphide bonds to form the glutenin macropolymer (Shewry and Tatham 1997). It is generally accepted that HMW-GSs are closely correlated with wheat dough elasticity, while LMW-GSs are essential in determining dough strength and extensibility (Anjum et al. 2007; Dong et al. 2010; Li et al. 2016; Ma et al. 2005; Tanaka et al. 2005). HMW-GS genes have been extensively studied in the past two decades. In contrast, the structure, organization, and roles of LMW-GS genes in grain processing quality have not been well investigated, partly because of the complexity

of the LMW-GS gene family and the abundance of allelic variants in wheat (Lee et al. 2016).

LMW-GSs are encoded by the multigene families at the loci of *Glu-A3*, *Glu-B3*, and *Glu-D3* on the short arms of the group 1 chromosomes (D'Ovidio and Masci 2004). The copy numbers of LMW-GS genes have been estimated to vary from 10–20 (Harberd et al. 1985) to 30–40 (Lee et al. 2016; Sabelli and Shewry 1991) in hexaploid wheat. The general structure of a typical LMW-GS consists of four domains: a conserved signal peptide of 20 amino acids, a short N-terminal domain, a central repetitive domain, and a C-terminal domain (Cassidy et al. 1998). Based on the first amino acid residue at the N-terminal region, LMW-GSs were traditionally classified into three types: LMW-serine (s), LMW-methionine (m), and LMW-isoleucine (i) (Cloutier et al. 2001; D'Ovidio and Masci 2004). Recently, a new class of LMW-GS genes from *Aegilops comosa*, designated as LMW-leucine (l) type subunits, was proposed by Wang et al. (2011).

Aegilops comosa ($2n = 2x = 14$, MM) is considered to be the progenitor of the M genome. It possesses significant candidate genes such as resistance genes against stripe rust that can be introgressed into bread wheat through interspecific hybridization (Riley et al. 1968). A previous study has

✉ Bihua Wu
wubihua2017@126.com

¹ Triticeae Research Institute, Sichuan Agricultural University, Wenjiang 611130, China

confirmed that a *Glu-M3* locus exists in the M genome. However, there are only limited studies on LMW-GSs cloned from *Ae. comosa*. In the current study, we report the characterization of seven novel LMW-GS genes from *Ae. comosa*, which would provide new candidate genes for wheat quality improvement and insights into the origin and evolution of LMW-GS gene family in wheat and related species.

Materials and methods

Plant materials

Two *Ae. comosa* ($2n=2x=14$, MM) accessions numbered PI551034 and PI551038 (originated from Greece) were kindly provided by the National Plant Germplasm System of the USDA-ARS, USA.

DNA extraction and PCR amplification

Genomic DNA was isolated from seedling leaves using the CTAB method (Rogers and Bendich 1985) with minor modification. A pair of allele-specific PCR (AS-PCR) primers was designed to amplify the LMW-GS genes based on published LMW-GS gene sequences (Ikeda et al. 2002; Li et al. 2008; Wang et al. 2011). The primers were PF: ATC ATCACAAGCACAAGCATC; PR: TTCTTATCAGTAGGC ACCAAC. PCR was performed using the Veriti™ 96-Well Fast Thermal Cycler (Applied Biosystems, Foster City, CA, USA) in 40 µl reaction volume containing 1×PCR buffer, 60 ng of genomic DNA, 300 nM of each primer, 200 µM of each dNTP, and 1.5 U of Dream Taq™ DNA polymerase (Thermo Scientific, Waltham, MA, USA). The reaction was carried out as follows: 5 min at 94 °C; 38 cycles of 30 s at 94 °C, annealing 58 °C for 30 s, and 1 min at 72 °C; and a final extension of 6 min at 72 °C. The amplified PCR products were analyzed by 1.5% agarose gel electrophoresis, followed by staining with GelRed Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA).

Molecular cloning and sequencing of LMW-GS genes

The PCR fragments of the expected length were extracted from agarose gel using Zymoclean gel DNA recovery kit (Zymo Research, Orange, CA, USA). Purified products were then cloned into pMD19-T vector (Takara, Dalian, China) and transformed into cells of *Escherichia coli* DH5α. Positive clones were identified by colony PCR. The sequencing was performed by the Sangon Biotechnology Company (Shanghai, China). The final nucleotide sequence for each LMW-GS gene was determined from the sequencing results of five independent clones.

Sequence alignment and phylogenetic analysis

Multiple sequence alignment of LMW glutenin protein sequences was completed using BioEdit (Hall 2007). A phylogenetic tree was constructed based on the full-length coding sequences using the neighbor-joining method (Saitou and Nei 1987) and the MEGA software (Tamura et al. 2013). Bootstrap values were obtained from 1000 replications.

Results

Isolation and characterization of LWM-GS genes

In the current study, a total of seven complete coding genes of LMW-GS have been cloned. We designated them as *comosa-I1*, *comosa-I2*, *comosa-L1*, *comosa-L2*, *comosa-M1*, *comosa-M2*, and *comosa-M3* based on the sequencing results. GenBank database comparison indicated that the coding sequences of the seven genes were different from known LMW-GS genes in *Ae. comosa* (Wang et al. 2011) and other *Triticum* species; thus, all these seven genes were novel.

The complete nucleotide sequences of *comosa-I1*, *comosa-I2*, *comosa-L1*, *comosa-L2*, *comosa-M1*, *comosa-M2*, and *comosa-M3* were deposited in GenBank with the accession numbers MH119044, MH119045, MH119043, MH119046, MH119047, MH119048, and MH119049, respectively. The sizes of *comosa-I1*, *comosa-I2*, *comosa-L1*, and *comosa-L2* from PI551034 were 879, 882, 975, and 1038 bp, which encoded 292, 293, 324, and 345 amino acid residues, respectively. The genes *comosa-M1*, *comosa-M2*, and *comosa-M3* from PI551038 were 906, 810, and 939 bp long. They could be translated into 301, 269, and 312 amino acid residues, respectively (Table 1). Similar to other LMW-GS genes, the coding regions of the seven genes reported hereby were all terminated by double stop codons.

Amino acid sequence analysis revealed that all seven LMW-GSs shared four main structural domains with previously characterized LMW-GSs (Cassidy et al. 1998), including a signal peptide of 20 amino acid residues, a short N-terminal (I), a repetitive domain (II), and a C-terminal domain. The C-terminal domain can be further divided into three sub-regions, including a cysteine-rich region (C-terminal I), a glutamine-rich region (C-terminal II), and a C-terminal conserved region (C-terminal III) (Fig. 1; Wang et al. 2011). Since the first amino acid residue of the mature protein was methionine, the *comosa-M1*, *comosa-M2*, and *comosa-M3* were defined

Table 1 Comparison of the mature protein sequences of seven novel LMW-GSs

Subunit types	GenBank accession	Nucleotide length (bp)	Amino acid number	N-terminal sequence	Number of cysteine residues					Total
					Signal	N-terminal domain	Repetitive domain	C-ter domain I	C-ter domain II	C-ter domain III
LMW-i	MH119044	879	292	ISQQQPP	0	0	0	6	1	1
LMW-i	MH119045	882	293	ISQQQPP	0	0	0	6	1	1
LMW-l	MH119043	975	324	LPISQQQQ	1	0	1	5	1	1
LMW-l	MH119046	1038	345	LPISQQQQ	1	0	1	5	1	1
LMW-m	MH119047	906	301	METSYIPGL	0	0	0	5	1	1
LMW-m	MH119048	810	269	METSCIPGL	0	1	0	5	1	1
LMW-m	MH119049	939	312	METSCIPGL	0	1	0	5	1	1

as typical LMW-m-type subunits. The number and position of cysteine residues were as expected and conserved as LMW-m-type subunits except comosa-M1 containing only seven cysteine residues because of one amino acid residue substitution in the fifth position of the N-terminal sequence (Fig. 1). Comosa-I1 and comosa-I2 were characterized by isoleucine start amino acid which is typical for mature protein of the LMW-i-type subunits. All eight cysteine residues of the two LMW-i-type subunits were located in the highly conserved C terminus, consistent with earlier reports (D'Ovidio and Masci 2004; Wang et al. 2011). Comosa-L1 and Comosa-L2 had the same N-terminal sequence of LPISQQQQ, and therefore defined as LMW-l-type subunits as proposed by Wang et al. (2011). The two LMW-l-type subunits possessed nine cysteine residues with an extra cysteine residue located in the signal peptide and repetitive domain (Table 1; Fig. 1). A long insertion of 13 residues (LGQQPQ8 for comosa-L1, LVQQPQ8 for comosa-L2) was present in the end of the C-terminal II as shown in Fig. 1.

Phylogenetic analysis of LMW-GS genes

We have constructed a phylogenetic tree using the seven new LMW-GS genes obtained in this study and other 24 representative LMW-GS genes retrieved from GenBank to further investigate the evolutionary relationships between LMW-GS genes in *Ae. comosa* and other Triticeae species. These genes included AB062872 (Ikeda et al. 2002), AY263369 (Zhao et al. 2004), AB164416, DQ357057, DQ357058 (Zhao et al. 2006), and AY542896 (Cloutier et al. 2001) from *T. aestivum*; KF562511, KF562512, KF562513, KF562514 (Qin et al. 2015), and DQ217663 from *T. dicoccoides*; EU594332, EU594333, EU594334, EU594335, EU594336, EU594338, and EU594339 (Wang et al. 2011) from *Ae. comosa*; AY606257 and AY646285 (Luo et al. 2005) from *Agropyron elongatum*; DQ345449 and DQ307389 from *T. monococcum*; AY585350 (Johal et al. 2004) and AY841014 from *Ae. tauschii*. The results are presented in Fig. 2.

The phylogenetic tree was divided into two clear branches, with alleles of LMW-i- and LMW-l-type genes at the top and LMW-m- and LMW-s-type genes at the bottom. For each cluster, two different subgroups were apparently separated, namely LMW-i- and LMW-l-type genes, and LMW-m- and LMW-s-type genes, respectively. The LMW-l-type genes obtained in the current study appeared to be more homologous to LMW-i than to LMW-m-type genes. These results were consistent with former studies (Wang et al. 2011). In the current study, the two genes *comosa-I1* and *comosa-I2* were clustered with the LMW-i-type genes and closely related to EU594336 from *Ae. comosa*. The genes *comosa-L1* and *comosa-L2* were located in the LMW-l-type group. Interestingly, *comosa-L2* was closely related to

Fig. 1 Multiple alignments of the deduced amino acid sequences of 18 LMW glutenin genes including LMW-m, LMW-i, and LMW-l-type subunits. Signal represents signal peptide. The amino acid sequences of mature proteins were divided into five regions: N-terminal domain (I), repetitive domain (II), and three sub-regions of C-terminal (I–III) domains. The first amino acid residue of the mature proteins and cysteine residues were highlighted by magenta and gray shading, respectively. Identical sequences and deletions were indicated by dots and dashes, respectively. The box indicated a long insertion

the previous reported LMW-i-type gene EU594335 of *Ae. comosa* (Wang et al. 2011). The genes *comosa-M1*, *comosa-M2*, and *comosa-M3* were all located in the LMW-m-type group, further confirming that they are LMW-m-type subunit genes.

Discussion

It is known that LMW-GS genes are widely presented in different genomes of wheat and related cereals (D'Ovidio and Masci 2004; Henkrar et al. 2017; Jiang et al. 2008; Luo et al. 2015; Wang et al. 2011). In this study, we have identified seven novel LMW-GS genes from the M genome of *Ae. comosa*. A phylogenetic tree was constructed to analyze the evolutionary relationships within the LMW-GS gene family. Our work provides useful information for further analyzing the structure, function, and evolution of the LMW-GS gene family. These novel genes are expected to be served as candidate gene sources for quality improvement of wheat.

Previous studies have demonstrated that the cysteine residues were likely to form inter- and intra-molecular disulphide bonds which are of great importance for the formation of glutenin polymers (D'Ovidio and Masci 2004). The number and location of cysteine residues could lead to functional variations. The most mature *Glu-3* gene products contain eight cysteine residues. In this study, the *comosa-I1*, *comosa-I2*, *comosa-M2*, and *comosa-M3* contained eight cysteine residues, and the location of these cysteine residues was similar to that of the typical LMW-m- or LMW-i-type subunits. However, two LMW-l-type subunits (*comosa-L1* and *comosa-L2*) were found to contain nine cysteine residues with five in the C-terminal domain I and one in each of the signal peptide, repetitive, C-terminal II and C-terminal III domains. This was consistent with the LMW-l-type gene reported by Wang et al. (2011). The AcLMW-L1 subunit with nine cysteine residues was predicted to have positive effects on dough properties. The same number of cysteine residues was present in the LMW-m gene AY263369, which was probably associated with good properties of Chinese wheat cultivar Xiaoyan 6 (Zhao et al. 2004). It has been suggested that the additional cysteine residue in LMW subunits might

lead to a higher cross linkage with other glutenin subunits, which contributes to enhanced dough properties (Lan et al. 2013). *Comosa-M1* possessed seven cysteine residues, with the first cysteine residue replaced by tyrosine. The first cysteine residue was found to form a lower amount of polymers than the wild-type LMW-GS (D'Ovidio and Masci 2004). Moreover, previous report demonstrated that the LMW-GS subunit Ee34, with seven cysteine residues (six conserved and one free), had a positive effect on flour quality. Based on these results, the *comosa-L1*, *comosa-L2*, and *comosa-M1* may be new candidate LMW-GS genes with potential value for wheat quality improvement.

The LMW-m-, LMW-i-, and LMW-s-type subunits are extensively characterized in the *Triticum* species (Lee et al. 2016; Luo et al. 2015; Qin et al. 2015). However, to our knowledge, the LMW-l-type genes were only identified in the wild source *Ae. comosa* (Wang et al. 2011) and *Agropyron elongatum* (StStE^aE^bE^x, 2n = 70) (Luo et al. 2005). In the present study, two novel LMW-l-type genes were isolated and characterized from *Ae. comosa*, which had the N-terminal sequence of LPISQQQQ and possessed similar structural characteristics to the reported LMW-l-type genes. The coding sequences of the current LMW-l-type genes shared high similarity to the reported LMW-l-type subunit genes from *Ae. comosa*, and the variations were mainly resulted from SNPs and InDels presented in the LMW-GS genes (data not shown). It has further been confirmed that the LMW-l-type genes are novel by the presence of specific cysteine residues and phylogenetic analysis.

In the current study, the phylogenetic tree was clustered into two clear branches that represent the LMW-i/LMW-l and LMW-m/LMW-s-type genes. Each type of genes was further clustered into a separate subgroup. These results supported the new LMW-GS gene classification system as proposed by Wang et al. (2011) according to which the LMW-GS genes were divided into two major classes: LMW-M and LMW-I, with each containing two subclasses, namely LMW-m and LMW-s in the LMW-M type and LMW-l and LMW-i in the LMW-I type. According to Wang et al. (2011), the LMW-m-type genes were the progenitors of other LMW-GS genes, and LMW-s genes were a variant form of group 1 of the LMW-m type. The LMW-l genes diverged from group 2 of the LMW-m type, while LMW-i type evolved from the LMW-l type. Interestingly, within the LMW-l subclass, *comosa-L2* clustered closely with the LMW-i-type subunit gene EU594335. This implies that *comosa-L2* and EU594335 may be the intermediate types during the evolution of LMW-l and LMW-i.

In conclusion, we have characterized seven novel LMW-GS genes from *Ae. comosa*. These new LMW-GS genes would enrich the genetic resources in wheat quality breeding and accelerate their application to improve bread-making quality of common wheat. Their molecular

Fig. 2 Phylogenetic relationships of the seven newly cloned and LMW-GS sequences retrieved from GenBank. The nucleotide sequences were specified by their corresponding GenBank accession numbers. The chromosomal assignments and the types of LMW-GS are shown in the brackets. The arrows indicate the seven novel LMW-GSs identified in this paper

characteristics could provide insights into the origin and evolution of the LMW-GS gene family.

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Compliance with ethical standards

Conflict of interest The authors state that there is no conflict of interest.

References

- Anjum FM, Khan MR, Din A, Saeed M, Pasha I, Arshad MU (2007) Wheat gluten: high molecular weight glutenin subunits-structure, genetics, and relation to dough elasticity. *J Food Sci* 72:R56–R63
- Cassidy BG, Dvorak J, Anderson OD (1998) The wheat low molecular weight glutenin genes: characterization of six new genes and progress in understanding gene family structure. *Theor Appl Genet* 96:743–750
- Cloutier S, Rampitsch C, Penner G, Lukow O (2001) Cloning and expression of a LMW-i glutenin gene. *J Cereal Sci* 33:143–154
- D'Ovidio R, Masci S (2004) The low-molecular-weight glutenin subunits of wheat gluten. *J Cereal Sci* 39:321–339
- Dong LL, Zhang XF, Liu DC, Fan HJ, Sun JZ, Zhang ZJ, Qin HJ, Li B, Hao ST, Li ZS, Wang DW, Zhang AM, Ling HQ (2010) New insights into the organization, recombination, expression and functional mechanism of low molecular weight glutenin subunit genes in bread wheat. *PLoS One* 5:e13548
- Hall T (2007) BioEdit, version 7.0.9. Computer program and documentation. Ibis Biosciences, Carlsbad
- Harberd NP, Bartels D, Thompson RD (1985) Analysis of the gliadin multigene loci in bread wheat using nullisomic-tetrasomic lines. *Mol Gen Genet* 198:234–242
- Henkrar F, El-Haddoury J, Iraqi D, Bendaou N, 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
- Ikeda TM, Nagamine T, Fukuoka H, Yano H (2002) Identification of new low-molecular-weight glutenin subunit genes in wheat. *Theor Appl Genet* 104:680–687
- Jiang C, Pei Y, Zhang Y, Li X, Yao D, Yan Y, Ma W, Hsam SL, Zeller FJ (2008) Molecular cloning and characterization of four novel LMW glutenin subunit genes from *Aegilops longissima*, *Triticum dicoccoides* and *T. zhukovskyi*. *Hereditas* 145:92–98
- Johal J, Gianibelli MC, Rahman S, Morell MK, Gale KR (2004) Characterization of low-molecular-weight glutenin genes in *Aegilops tauschii*. *Theor Appl Genet* 109(5):1028–1040
- Lan QX, Feng B, Xu ZB, Zhao GJ, Wang T (2013) Molecular cloning and characterization of five novel low molecular weight glutenin subunit genes from Tibetan wheat landraces (*Triticum aestivum* L.). *Genet Resour Crop Ev* 60:799–806
- Lee JY, Beom HR, Altenbach SB, Lim SH, Kim YT, Kang CS, Yoon UH, Gupta R, Kim ST, Ahn SN, Kim YM (2016) Comprehensive identification of LMW-GS genes and their protein products in a common wheat variety. *Funct Integr Genom* 16:269–279
- Li XH, Wang AL, Xiao YH, Yan YM, He ZH, Appels R, Ma WJ, Hsam SLK, Zeller FJ (2008) Cloning and characterization of a novel low molecular weight glutenin subunit gene at the *Glu-A3* locus from wild emmer wheat (*Triticum turgidum* L. var. *dicoccoides*). *Euphytica* 159:181–190
- Li XJ, Liu TH, Song LJ, Zhang H, Li LQ, Gao X (2016) Influence of high-molecular-weight glutenin subunit composition at *Glu-A1* and *Glu-D1* loci on secondary and micro structures of gluten in wheat (*Triticum aestivum* L.). *Food Chem* 213:728–734
- Luo Z, Chen F, Feng D, Xia G (2005) LMW-GS genes in *Agropyron elongatum* and their potential value in wheat breeding. *Theor Appl Genet* 111:272–280
- Luo G, Zhang X, Zhang Y, Yang W, Li Y, Sun J, Zhan K, Zhang A, Liu D (2015) Composition, variation, expression and evolution of low-molecular-weight glutenin subunit genes in *Triticum urartu*. *BMC Plant Biol* 15:68
- Ma W, Appels R, Bekes F, Larroque O, Morell MK, Gale KR (2005) Genetic characterisation of dough rheological properties in a wheat doubled haploid population: additive genetic effects and epistatic interactions. *Theor Appl Genet* 111:410–422
- Qin L, Liang Y, Yang D, Sun L, Xia G, Liu S (2015) Novel LMW glutenin subunit genes from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) in relation to *Glu-3* evolution. *Dev Genes Evol* 225:31–37
- Riley R, Chapman V, Johnson R (1968) Introduction of yellow rust resistance of *Aegilops comosa* into wheat by genetically induced homoeologous recombination. *Nature* 217:383–384
- Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol Biol* 5:69–76
- Sabelli PA, Shewry PR (1991) Characterization and organization of gene families at the *Gli-1* loci of bread and durum wheats by restriction fragment analysis. *Theor Appl Genet* 83:209–216
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Shewry PR, Tatham AS (1997) Disulphide bonds in wheat gluten proteins. *J Cereal Sci* 25:207–227
- Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- Tanaka H, Shimizu R, Tsujimoto H (2005) Genetical analysis of contribution of low-molecular-weight glutenin subunits to dough strength in common wheat (*Triticum aestivum* L.). *Euphytica* 141:157–162
- Wang K, Gao L, Wang S, Zhang Y, Li X, Zhang M, Xie Z, Yan Y, Belgard M, Ma W (2011) Phylogenetic relationship of a new class of LMW-GS genes in the M genome of *Aegilops comosa*. *Theor Appl Genet* 122:1411–1425
- Zhao H, Wang R, Guo A, Hu S, Sun G (2004) Development of primers specific for LMW-GS genes located on chromosome 1D and molecular characterization of a gene from *Glu-D3* complex locus in bread wheat. *Hereditas* 141:193–198
- Zhao XL, Xia XC, He ZH, Gale KR, Lei ZS, Appels R, Ma W (2006) Characterization of three low-molecular-weight *Glu-D3* subunit genes in common wheat. *Theor Appl Genet* 113:1247–1259