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Original scientific article

CLONING AND MOLECULAR CHARACTERIZATION OF TWO NOVEL LMW-m TYPE GLUTENIN GENES FROM *Triticum spelta* L.

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Spelt wheat (*Triticum spelta* L., 2n=6x=42, AABBDD), as a hexaploid wheat species, is important sources of food and feed in Europe. It also serves as an important genetic resource for improvement of wheat quality and resistance. In this study, two novel m-type low molecular glutenin subunit (LMW-GS) genes, named as *TsLMW-m$_1$* and *TsLMW-m$_2$* were cloned by allelic specific polymerase chain reaction (AS-PCR) from German spelt wheat cultivars Rochbergers fruher Dinke and Schwabenkorn, respectively. The complete open reading frames (ORFs) of both genes contained 873 bp encoding 290 amino acid residues, and had typical LMW-GS structural features. Two same deletions with 24 bp at the position of 707-730 bp were present in both genes, while *TsLMW-m$_1$* had two nonsynonymous single-nucleotide polymorphism (SNP) variations at the positions of 434 bp (C-A transversion) and 857 bp (G-A transition). Phylogenic analysis revealed that both LMW-m genes were closely related to those from wheat A genome, suggesting that both subunits are encoded by the *Glu-A3* locus. Secondary structure prediction showed that TsLMW-m$_1$ and TsLMW-m$_2$ subunits had more α-helices than other wheat LMW-GS including superior quality subunit EU369717, which would benefit to form superior gluten structures and dough properties. The authenticity and

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expression activity of *TsLMW-m₁* and *TsLMW-m₂* genes were verified by prokaryotic expression in *E. coli*. Our results indicated that two newly cloned *TsLMW-m* genes could have potential values for wheat quality improvement.

Keywords: Spelt; LMW-GS; Molecular cloning; Phylogenetics; Gluten quality.

INTRODUCTION

Wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD), as a allohexaploid species and one of the most important food crops around the world, can be used to produce various foods such as bread, noodles, biscuits and cakes. These quality properties are mainly affected by wheat seed storage proteins that are rapidly synthesized and accumulated at about 10-30 days after anthesis (LIU *et al*., 2012; YU *et al*., 2016). The major storage proteins deposited in wheat endosperm include gliadins and glutenins, which determine dough extensibility and elasticity, respectively (PAYNE, 1987; XIE *et al*., 2010). Glutenins consist of high and low molecular weight glutenin subunits (HMW-GS and LMW-GS) accounting for about 40% of wheat storage proteins, and their composition and content are important factors affecting flour processing quality. LMW-GS account for 60% of glutenins and serve as important components of the gluten macropolymers (GMPs) that confer dough viscoelasticity (WRIGLEY *et al*., 1996; YAN *et al*., 2009; RASHEED *et al*., 2014; WU *et al*., 2017).

LMW-GS are controlled by the genes at *Glu-3* loci, including *Glu-A3*, *Glu-B3*, and *Glu-D3* on the short arm of the chromosomes 1A, 1B, and 1D, respectively (GUPTA and SHEPHERD, 1990). Most LMW-GS contain 250-300 amino acid residues and generally have four main structural regions: a signal, a short N-terminal, a repetitive domain, and a C-terminal (D'OVIDIO and MASCI, 2004). Based on the first N-terminal amino acid of the mature protein, LMW-GS are traditionally classified into LMW-m (methionine), LMW-s (serine) and LMW-i (isoleucine) types (LEW *et al*., 1992; CLOUTIER *et al*., 2001). The LMW-m type subunits have various N-terminal sequences containing METSHIPGL-, METSRIPGL-, and METSCIPGL-. The N-terminus of LMW-s is SHPGL- while the LMW-i type lacks the typical N-terminal sequence and starts directly with the repetitive region of ISQQQQ- after the signal peptide (LEW *et al*., 1992; MASCI *et al*., 1995).

Compared to the *Glu-1* loci encoding HMW-GS, *Glu-3* loci exhibit more extensive allelic variations. GUPTA and SHEPHERD (1990) identified and named 20 alleles at *Glu-3* from 222 wheat cultivars, including six at *Glu-A3*, nine at *Glu-B3,* and five at *Glu-D3*. Through analysis of the micro-core collections (MCC) of Chinese wheat germplasm, ZHANG *et al*. (2013) identified more than 15 LMW-GS genes from individual MCC accessions, of which 4-6 were located at the *Glu-A3* locus, 3-5 at the *Glu-B3* locus, and eight at the *Glu-D3* locus. Considerable work has shown that the allelic variations at both *Glu-1* and *Glu-3* loci are closely related to dough strength and breadmaking quality. In particular, some glutenin subunits and alleles have shown a positive effect on dough properties and processing quality such as 1Dx5+1Dy10 encoded by *Glu-D1d* (PAYNE, 1987; WU *et al*., 2017), 1Bx17+1By18 encoded by *Glu-B1i* (GUO *et al*., 2019), *Glu-A3a* (ZHEN *et al*., 2014) and *Glu-B3h* (WANG *et al*. 2016). However, in addition to a few of HMW-GS, fewer superior genes at *Glu-3* loci in bread wheat have been identified and cloned. Therefore, it is highly important to discover new candidate genes from wheat related species.

Considerable work demonstrated that wheat related species had abundant glutenin variations and candidate superior subunits for gluten quality improvement. These related species included *Aegilops longissima* (HUANG *et al*., 2010; ZHU *et al*., 2015; HU *et al*., 2019;), *Ae. tauschii* (YAN *et al*., 2003a, 2004; PEI *et al*., 2007; ZHANG *et al*., 2008), *Ae. kotschyi* and *Ae. Juvenalis* (LI *et al*., 2008a), *T. dicoccum*, *T. dicoccoides* and *T. zhukovskyi* (YUE *et al*., 2005; LI *et al*., 2006, 2007a, 2008b; JIANG *et al*., 2008), *T. monococcum* (AN *et al*., 2006), *T. timopheevii* (LI *et al*., 2007b; ZHANG *et al*., 2010) and other wheat related species (WANG *et al*., 2008; 2011a,b; 2012b). Through molecular marker development, these glutenin subunits can be used for rapid improvement of wheat process quality (WANG *et al*., 2013; LIANG *et al*., 2015; CUI *et al*., 2019). Spelt wheat (*Triticum spelta* L., 2n=6x=42, AABBDD) possesses the same genomes as bread wheat, which is one of the major feed and food grains in ancient Europe (AN *et al*., 2005). The morphological character of spelt is evident: a narrow, lax and pyramidal spike with a brittle rachis and adherent glumes, generally long spike internodes and non-spherical seeds. Spelt wheat contains useful gene resources for wheat cultivar improvement such as stripe rust and powdery mildew resistance and higher amino acid and trace element content (CAMPBELL, 1997; PU *et al*., 2009). Spelt wheat also has extensive allelic variations at *Glu-1* and *Glu-3* loci (CABALLERO *et al*., 2001, 2003, 2004; YAN *et al*., 2003b; AN *et al*., 2005), which provide potential genetic resources for wheat quality improvement. However, the potential glutenin genes in spelt wheat, particular at *Glu-3* loci have not been cloned and utilized so far.

In this work, we cloned and identified two novel m-type LMW-GS genes from European spelt wheat varieties by allelic-specific polymerase chain reaction (AS-PCR). Analysis of their molecular structure features demonstrated that both genes have potential application value for wheat quality improvement.

MATERIALS AND METHODS

Plant materials

Two European spelt wheat cultivars Rochbergers fruher Dinke and Schwabenkorn from Plant Breeding Institute, Technical University of Munich, Germany, were used as materials in the work.

DNA extraction, AS-PCR cloning and sequencing

Genomic DNA from seedling leaves was extracted with CTAB protocol following the procedure of YAN *et al.* (2004). A pair of AS-PCR primers LMW-F (ATGAAGACCTTCCTCATCTTTGCC) and LMW-R (TCAGTAGGCACCAACTTG GCTG) were designed by Primer 5.0, and used to amplify the complete open reading frames (ORFs) of the LMW-GS. PCR was performed by CFX96 Real Time system (Bio-Rad Laboratories) with the following conditions: an initial denaturation of 95º for 3 min followed by 35 cycles of 95º for 15 s, 58º for 15 s, 72º for 60 s and finally extended at 72º for 5 min. The PCR products were separated in 1% agrose gel and the fragments of the expected size were collected and purified by using the Gel Extraction Kit (Omega), then the purified products were ligated into pMD18-T vector (TaKaRa Biotechnology, Dalian, China) and transformed into cells of *E. coli* strain DH5α according to LI *et al*. (2007b). To reduce the sequencing error, three positive clones were picked randomly and sequenced by TaKaRa Biotechnology (Dalian) Co. Ltd, China,

SNP and InDel identification and secondary structure prediction

Multiple sequences alignments of the cloned genes and other LMW-m genes were completed by using Bioedit 7.0. Single-nucleotide polymorphism (SNP) and insertions/deletions (InDel) variations in LMW-GS genes were identified. Prediction of secondary structure of deduced amino acid sequences of LMW-GS genes were carried out by PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/psiform.html) according to WANG *et al*. (2011a).

Phylogenetic analysis

The ClustalW program was used to perform multiple sequences alignments of LMW-GS genes from different genomes with homologous nucleotide sequences, then the alignment file was used to constructed phylogenetic tree based on the complete coding regions by using software MEGA5.0. The related parameters used for phylogenetic tree construction were based on WANG *et al.* (2011a).

Heterologous expression in E. coli

The cloned LMW-GS genes were reamplified to remove the signal peptides by designing a pair of new primers LMW-ExF (5'-AAGCCATGG TGCCCTTCTAG CCGTTGTGGCGAC-3') and LMW-ExR (5'-AAACTCGAG TCAGTAGGCACCAAC TTGGCTGCC-3'). *NcoI* and *XhoI* sites (underlined) were added at the 5' ends of the LMW-ExF and LMW-ExR, respectively. After purification, the PCR products of the cloned genes were ligated into the expression vector pET-28α (Novagen) and transformed into *E. coli* BL21 (DE3) pLysS cells. The expressed protein extraction and separation were carried out by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to LI *et al*. (2007b). The gel electrophoresis was operated in 15 mA/gel and 1.5 hours, then stained with Coomassie Brilliant Blue R-250 and destained with the mixture of ethanol and acetic acid.

MALDI-TOF/TOF-MS

The expressed proteins in *E. coli* were collected, purified and detected by matrix-assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF-MS) according to the previously reported methods (AN *et al*., 2006; PEI *et al*., 2007). Tandem mass spectrometry was carried out using the 4800 MALDI-TOF/TOF™ Analyzer (Applied Biosystems, Carlsbad, USA). International two-dot calibration with standard sample Albumin-Aldrase at masses of 39212.88 Da and 66431.08 Da was used. The MS/MS spectra were searched against Viridiplantae (green plant) sequences in the nonredundant National Center for Biotechnology Information (NCBI) database and Triticum NCBI database by using Mascot software (version 2.1; Matrix Science, London). All searches were evaluated based on the significant scores obtained from Mascot. The Total Ions Score C.I.% was set to >95%, and a significance threshold of $p < 0.05$ was used.

RESULTS

AS-PCR amplification and molecular cloning of LMW-GS genes in spelt wheat

Two amplification fragments with about 1000 bp from both spelt cultivars were obtained by AS-PCR (Fig. 1), which were corresponding to the LMW-GS gene sizes. Both PCR amplification fragments were collected and sequenced, and two complete coding sequences with

typical structural characteristics of previously characterized LMW-GS genes were obtained and designated as *TsLMW-m₁* and *TsLMW-m₂*. Both gene sequences were deposited in GenBank with the accession number MK395160 and MK395161, respectively.

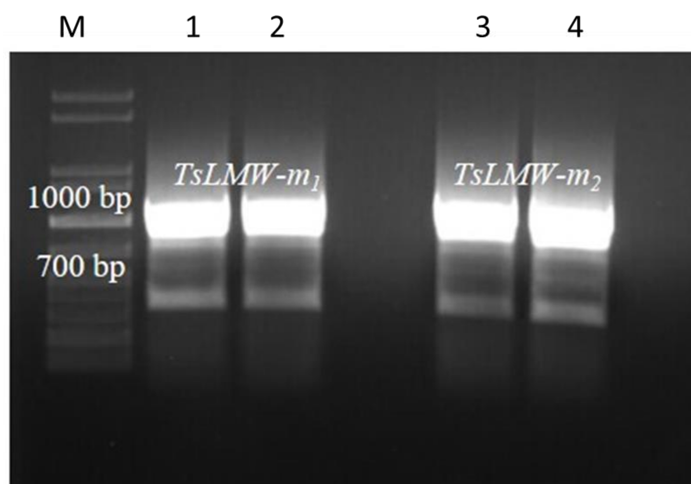

Fig 1. AS-PCR amplification of LMW-GS genes from German spelt wheat cultivars. 1-2: Rochbergers fruher Dinke. 3-4: Schwabenkorn. The amplified bands corresponding to *TsLMW-m_1* and *TsLMW-m_2* genes are indicated.

Molecular characterization of two novel LMW m-type glutenin genes

The nucleotide sequences of both *TsLMW-m_1* and *TsLMW-m_2* genes had 873 bp without introns as previously characterized *Glu-3* genes. The deduced amino acid sequences demonstrated that the first amino acid residue of their mature proteins was methionine, so both TsLMW-m_1 and TsLMW-m_2 belonged to the LMW-m type subunits. The deduced amino acid sequences were aligned with 15 other LMW-m type subunits from wheat and related species to compare their sequence characteristics (Fig. 2). The results showed that both *TsLMW-m_1* and *TsLMW-m_2* genes encoded 290 amino acid residues, and had a similar primary structure to LMW-m type subunits, including a signal peptide of 20 amino acid residues, a short N-terminal region of 13 amino acids containing the first cysteine residue, a repetitive domain rich in glutamine and proline residues and a C-terminal domain. The C-terminal domain could be further subdivided into three regions: a cysteine-rich region with five cysteine residues (I), a glutamine-rich region containing a cysteine residue and stretches of glutamine residues (II), and a C-terminal conserved sequence with the last cysteine residue (III). As shown in Fig. 2, all LMW-GS contained eight conserved cysteine residues at conserved positions. The first and seventh cysteines form the inter-molecular disulfide bond while the rest form three intra-molecular disulfide bonds (MASCI *et al*., 1998; D'OVIDIO *et al*., 1999).

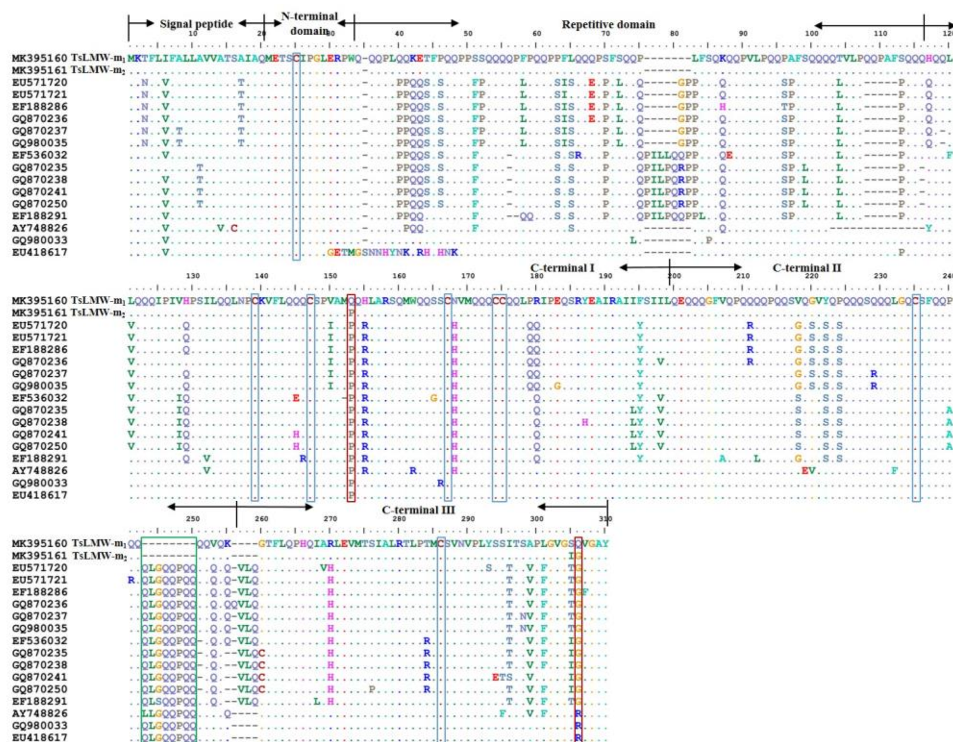

Fig 2. Multiple sequence alignment of the deduced amino acid sequences of *TsLMW-m₁* and *TsLMW-m₂* genes and other 15 LMW-m subunits from wheat and related species. The blue boxes indicate the cysteine residues, red boxes show the differences of amino acid residues and green box represents InDel. The typical structural domains of LMW-GS are showed. The 15 LMW-m subunits include EU571720 and GQ870250 from *Aegilops markgrafii*, EU571721 from *Triticum speltoides*, EF188286 from *Aegilops geniculata*, GQ870236, GQ870237, GQ870235 and GQ870238 from *Aegilops triuncialis*, GQ980035 and GQ870241 from *Aegilops umbellulata*, EF536032 from *Aegilops neglecta*, EF188291 from *Triticum zhukovskyi*, AY748826 from *Triticum dicoccoides*, and GQ980033 and EU418617 from *Triticum timopheevii*.

SNP and InDel variations in two novel LMW-m glutenin genes

The complete coding sequences of *TsLMW-m₁* and *TsLMW-m₂* genes were aligned with 15 other LMW-m genes to detect SNP and InDel variations, and the results are listed in Table 1. Two nonsynonymous SNP variations in *TsLMW-m₁* were detected: C-A transversion at the positions of 434 bp and G-A transition at 857 bp, which led to one amino acid residue change from proline to glutamine and glycine/arginine to glutamine, respectively. No any SNPs were found in *TsLMW-m₂*. In addition, two common deletions with 24 bases (TGG/AGTCAACAGCCTCAACAACAAC) at the position of 707-730 bp were present in both genes.

Table 1 Positions of SNPs and InDels identified in TsLMW-m1 and TsLMW-m2 genes

LMW-GS genes	707-730 (bp)	434 (bp)	857 (bp)
TsLMW-m1	–	A	A
TsLMW-m2	–	C	G
15 other LMW-m genes*	TGG/AGTCAACAGCCTCAACAACAAC	C	G

*15 other LMW-m genes include: EU571720 (*Ae. markgrafii*), EU571721 (*Ae. speltoides*), EF188286 (*Ae. geniculata*), GQ870236 (*Ae. triuncialis*), GQ870237 (*Ae. triuncialis*), GQ980035 (*Ae. umbellulata*), EF536032 (*Ae. neglecta*), GQ870235 (*Ae. triuncialis*), GQ870238 (*Ae. triuncialis*), GQ870241 (*Ae. umbellulata*), GQ870250 (*Ae. markgrafii*), EF188291 (*T. zhukovskyi*), AY748826 (*T. dicoccoides*), GQ980033 (*T. timopheevii* sub. *armeniacum*), and EU418617 (*T. timopheevii*).

Phylogenetic analysis of LMW-GS genes

A homology tree was constructed to reveal the phylogenetic relationships among two newly cloned LMW-m genes and 18 LMW-GS genes at *Glu-3* loci from different genomes through nucleotide sequence alignment using MEGA 5.0 software (Fig. 3). The homology tree was clustered into three clear clades, corresponding to genes encoded by A，B and D genomes, respectively. The LMW-GS genes from B genome were clustered into a separate clade, suggesting that they underwent greater divergence during the evolutionary process. *TsLMW-m1* and *TsLMW-m2* showed a close relationship with other LMW-GS genes encoded by A genome, suggesting that both genes were encoded by A genome.

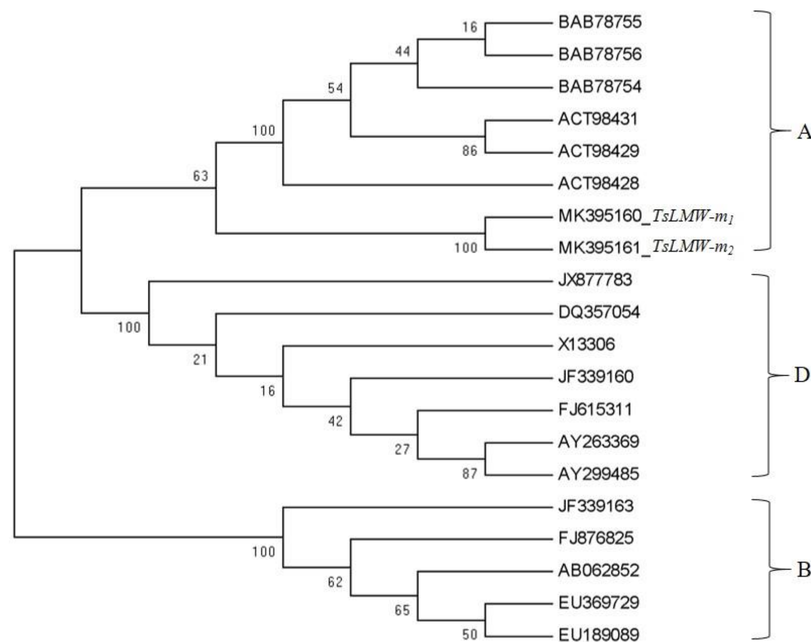

Fig 3. Phylogenetic tree of *TsLMW-m1* and *TsLMW-m2* genes and other 18 LMW-GS genes from A, B and D genomes of common wheat (*Triticum aestivum* L.).

Heterologous expression of two novel LMW-m glutenin genes and MALDI-TOF/TOF-MS identification

To confirm the authenticity of the two cloned genes, their open reading frames (ORFs) were amplified and ligated into the expression vector pET-28α, and then transformed into expression host *E. coli* strain BL21 (DE3) pLysS. The heterologous expression of *TsLMW-m₁* and *TsLMW-m₂* genes in *E. coli* was induced by IPTG and the expressed proteins were identified by SDS-PAGE. As shown in Fig. 4, two specific expressed protein bands indicated by the red frame, corresponding to *TsLMW-m₁* and *TsLMW-m₂*, respectively, were separated after IPTG induction. To further verify the *E. coli* expression results, two expressed glutenin subunits were identified by MALDI-TOF/TOF-MS (Table 2). One peptide (GTFLQPHQIARLEVMTSIALR) specifically present at the position of 248-268 bp of *TsLMW-m₁* gene was identified in the expressed protein. For the *TsLMW-m₂* gene, three specific peptides were present in the its expressed protein, including VFLQQQCSPVAMPQHLAR (133-150 bp), SSCNVMQQQCCQQLPR (157-172 bp) and GTFLQPHQIARLEVMTSIALR (248-268). All identifications by tandem mass spectrometry had a high protein score and credibility, validating the authenticity of two newly cloned LMW-GS genes.

Table 2. Expressed proteins of the TsLMW-m₁ and TsLMW-m₂ genes identified by MALDI-TOF/TOF-MS

Expressed proteins	Identified proteins	Accession no	Tryptic fragments identified by MS data	Position (bp)	Matched peptides	Protein score C. I.%
TsLMW-m₁	LMW-GS	ABG76007	GTFLQPHQIARLEVMTSIALR	248-268	1	99.949
			VFLQQQCSPVAMPQHLAR	133-150		
TsLMW-m₂	LMW-GS	ACX46516	SSCNVMQQQCCQQLPR	157-172	3	100
			GTFLQPHQIARLEVMTSIALR	248-268		

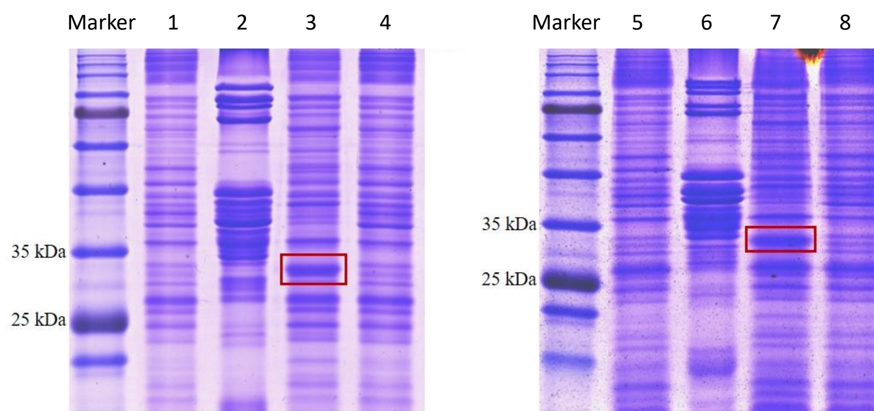

Fig 4. Heterologous expression of *TsLMW-m₁* and *TsLMW-m₂* genes in *E. coli*. 1, 5: Empty vector pET-28α. 2: Rochbergers fruher Dinke. 3: *TsLMW-m₁* expression induced by isopropyl-β-d-thiogalactoside (IPTG). 4: *TsLMW-m₁* expression without IPTG induction. 6: Schwabenkorn. 7: *TsLMW-m₂* expression induced by IPTG. 8: *TsLMW-m₂* expression without IPTG induction. The red boxes indicate the expressed proteins of *TsLMW-m₁* and *TsLMW-m₂* genes.

Secondary structure analysis of two novel LMW-m glutenin genes

The secondary structures of the TsLMW-m_1 (MK395160) and TsLMW-m_2 (MK395161) glutenin subunits and other six LMW-s, LMW-m and LMW-i type LMW-GS (EU369717, EU189088, FJ876824, FJ876825, AY724436 and AY724437) were predicted by the PSIPRED server, and a comparative analysis was performed (Table 3). The results showed that the α-helices and β-strands were dispersed in the normal configuration in the C-terminal I and had high conservation in the C-terminal III. The *TsLMW-m_1* encoded subunit contained nine α-helices mainly located at the C-terminal I while the *TsLMW-m_2* encoded subunit had eight α-helices and one β-strand in the conserved C-terminal region. Thus, the number and percentage of α-helices in both TsLMW-m_1 and TsLMW-m_2 subunits were much higher than those of other six subunits, including EU369717 that was confirmed to have positive effects on wheat bread quality (WANG *et al*., 2016).

Table 3. Secondary structure prediction of the subunits encoded by TsLMW-m_1 and TsLMW-m_2 and other six LMW-GS from wheat related species

LMW-GS	Species	Type	Structure motifs	Content (%)	Total	N-terminal domain	Repetitive domain	C-ter domain I	C-ter domain II	C-ter domain III
TsLMW-m_1 (MK395160)	*Triticum spelta*	LMW-m	α-helix	25.86	9	0	0	6	2	1
			β-strand	0	0	0	0	0	0	0
TsLMW-m_2 (MK395161)	*Triticum spelta*	LMW-m	α-helix	24.14	8	0	0	5	2	1
			β-strand	1.03	1	0	0	0	0	1
EU369717	*Triticum aestivum*	LMW-s	α-helix	9.95	4	0	0	3	1	0
			β-strand	0.54	1	0	0	0	0	1
EU189088	*Triticum aestivum*	LMW-s	α-helix	12.54	5	0	0	4	0	1
			β-strand	0.58	1	0	0	0	0	1
FJ876824	*Triticum aestivum*	LMW-m	α-helix	14.00	7	0	0	4	2	1
			β-strand	0	0	0	0	0	0	0
FJ876825	*Triticum aestivum*	LMW-m	α-helix	14.57	5	0	0	3	1	1
			β-strand	0.57	1	0	0	0	0	1
AY724436	*Elymus elongatus*	LMW-i	α-helix	0	0	0	0	0	0	0
			β-strand	1.32	2	0	0	0	0	2
AY724437	*Elymus elongatus*	LMW-i	α-helix	10.51	3	0	0	2	0	1
			β-strand	0.68	1	0	0	0	0	1

DISCUSSION

Spelt wheat includes two large geographic groups: European (supraconvar. *spelta*) and Asian (supraconvar. *kuckuckianum* Gökg. ex Dorof.) (KOZUB *et al*., 2014). Compared to European spelt, Asian spelt showed very few anatomical differences. Compositions of glutenin subunits of Iranian spelt were more similar to common bread wheat and no special alleles were found (YAN *et al*., 2003b). Meanwhile, Iranian spelt is generally of the awn spike while the ancient European spelt is typically of the awnless type (OHTSUKA, 1998). These support the

hypothesis that European spelt may have a different, independent origin than Asian spelt, which probably originated from hybridization between cultivated emmer (*T. dicoccum*, AABB) and club wheat (*T. aestivum* ssp. *compactum*, AABBDD) (OHTSUKA, 1998; YAN *et al*., 2003b).

Although spelt wheat is now considered as a minor crop, recent interest in its use for ecologically grown foods has led to resurgence in its cultivation. As a low-input plant, spelt wheat possesses outstanding stress resistance, suitable for growing in harsh ecological conditions and marginal areas of cultivation without the use of pesticides (BONAFACCIA *et al*., 2000). Meanwhile, spelt wheat also has superior quality performance and can be used to make unconventional foods. The starch in spelt wheat undergoes hydrolysis more rapidly in the first period, thus its food products could be used in diets (CAMPBELL, 1997). The final spelt wheat product also has a great proportion of soluble fiber and high nutritional value (BONAFACCIA *et al*., 2000). In addition, analysis from a large number of spelt cultivars and accessions showed an extensive allelic variation at both *Glu-1* and *Glu-3* loci (CABALLERO *et al*., 2001, 2003, 2004; AN *et al*., 2005). In particular, some new variations of glutenin subunits originated from cultivated emmer and club wheat were found in spelt wheat (YAN *et al*., 2003a). These new allelic variants provide potential gene resources for wheat gluten quality improvement. However, these novel glutenin genes have not been cloned and applied in wheat quality improvement program so far.

LMW-GS with 30 to 50 kDa are closely related to dough resistance and extensibility, and play an important role in determining wheat flour properties and processing quality (CIAFFI *et al*., 1999; AN *et al*., 2006). It is known that LMW-GS contain eight highly conserved cysteine residues, higher than HMW-GS, which contribute to the formation of gluten macropolymers (LI *et al*., 2008b). Among them, seven cysteine residues are present in the C-terminal domain and one in the N-terminus. In general, the first and the seventh cysteines form the inter-molecular disulfide bond while the remaining cysteines form three intra-molecular disulfide bonds (LEW *et al*., 1992), which are related to the formation of the protein secondary structure and dough quality. In this work, we cloned and characterized, for the first time, two novel LMW-m type glutenin genes ($TsLMW\text{-}m_1$ and $TsLMW\text{-}m_2$) from European spelt wheat. In comparison with the LMW-GS (EU369717) that has positive effects on quality properties (WANG *et al*., 2016), both TsLMW-m_1 and TsLMW-m_2 subunits had more α-helices (Table 3). The previous study showed that the *M*r 42 K LMW-GS is associated with good quality properties in durum and bread wheat (MASCI *et al*., 2000). All the predicted α-helices in the *M*r 42 K LMW-GS seem to be located near the intra-molecular disulfide bonds, suggesting that helix-helix interactions are involved in guiding the formation of the intra-molecular disulfide bonds (MASCI *et al*., 1998). Therefore, higher α-helix content may contribute to better quality of the dough (WANG *et al*., 2012a; WANG *et al*., 2018). In addition, the β-strands are generally considered to endow the protein with high elasticity and to improve the capability to resist distortion (SHEWRY *et al*., 2002). Similar to the superior subunit EU369717, $TsLMW\text{-}m_2$ also contained one β-strand in the C-terminal domain III (Table 3), which could benefit to form a superior elasticity structure of dough. The secondary structure including α-helices, β-strands and random coils is the foundation for a highly complex spatial conformation (ZHEN *et al*., 2014). The compositions of α-helices and β-strands suggest that both TsLMW-m_1 and TsLMW-m_2 subunits have potential application values for wheat gluten quality improvement. In addition, the SNP and InDel variations identified in both genes

(Table 1) provide a possibility for developing AS-PCR based molecular markers used for marker-assisted selection in wheat quality improvement program.

CONCLUSION

In this study, two novel LMW-m type glutenin genes (*TsLMW-m_1* and *TsLMW-m_2*) from German spelt wheat cultivars Rochbergers fruher Dinke and Schwabenkorn were cloned and characterized. Compared to other LMW-m type genes from different wheat and related genomes, both *TsLMW-m_1* and *TsLMW-m_2* genes had a 24 bp deletion at position of 707-730 bp while *TsLMW-m_1* contained two nonsynonymous SNP variations. Phylogenetic analysis indicated that both genes had closer relationship with LMW-GS genes encoded by A genome in common wheat, suggesting that they are encoded by the A genome of spelt wheat. Secondary structure prediction demonstrated that both TsLMW-m_1 and TsLMW-m_2 subunits contained a high percentage of α-helices that benefit to form a superior gluten structure, suggesting that both genes have potential application values for wheat quality improvement.

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KLONIRANJE I MOLEKULARNA KARAKTERIZACIJA DVA NOVA GENA GLUTENINA LMV-m TIPA IZ *Triticum spelta* L.

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Izvod

Spelta (Triticum spelta L., 2n = 6k = 42, AABBDD), kao heksaploidna vrsta pšenice, jedan je od bitnih resursa hrane i krme u Evropi. Takođe služi kao važan genetički resurs za poboljšanje kvaliteta i otpornosti pšenice. U ovoj studiji, dva nova gena niskomolekularnih gluteninskih subjedinica (LMV-GS) m-tipa, nazvana TsLMV-m1 i TsLMV-m2 klonirana su alelno specifičnom lančanom reakcijom polimeraze (AS-PCR) iz nemačkih sorti pšenice Rochbergers fruher Dinke i Schwabenkorn. Kompletni otvoreni fragmenti čitanja (ORF) oba gena su sadržavali 873 bp, koji kodiraju 290 aminokiselinskih ostataka, i imali su tipične karakteristike LMV-GS. Dve iste delecije sa 24 bp na položaju 707-730 bp bile su prisutne u oba gena, dok je TsLMV-m1 imao dve nesinonimne varijacije jednonukleotidnog polimorfizma (SNP), na pozicijama 434 bp (C-A transverziju) i 857 bp (G-A tranziciju). Filogenetska analiza otkrila je da su oba LMV-m gena usko povezana s genima iz pšeničnog A genoma, što sugeriše da su obe podjedinice kodirane lokusom Glu-A3. Predviđanje sekundarne strukture pokazalo je da TsLMV-m1 i TsLMV-m2 podjedinice imaju više α-heliksa od ostalih pšeničnih LMV-GS, uključujući podjedinicu vrhunskog kvaliteta EU369717, što će biti od koristi da se formiraju superiorne glutenske strukture i svojstava testa. Autentičnost i aktivnost ekspresije gena TsLMV-m1 i TsLMV-m2 verifikovani su prokariotskom ekspresijom u *E. coli*. Naši rezultati pokazali su da dva novo klonirana TsLMV-m gena mogu imati potencijalne vrednosti za poboljšanje kvaliteta pšenice.

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