

## Short Communication

# Cloning and characterization of high molecular weight glutenin gene from *Triticum aestivum* cultivar Dacke

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## ABSTRACT

**Background:** High molecular weight (HMW) glutenin protein plays a crucial role in determining dough viscoelastic properties that determines the quality of wheat flour. The aim of the present study was to isolate, clone and analyze (*in silico*) the HMW glutenin gene of *Triticum aestivum* cultivar Dacke.

**Methods:** Primers were designed to amplify a 2445 bp fragment of HMW glutenin gene. Ax type HMW glutenin gene from *Triticum aestivum* cultivar Dacke was isolated using PCR and it was sequenced by primer walking.

**Results:** Amplified HMW glutenin gene was designated as HMWG<sub>Ax</sub>. Sequence analysis revealed a complete open reading frame encoding 815 amino acid residues with N- and C terminal non-repetitive domain and a central repetitive domain. The calculated molecular weight of the deduced HMW glutenin protein was ~88 kDa and the number of cysteine residues in the HMWG<sub>Ax</sub> was four, in accordance with other x type HMW glutenin proteins. Phylogenetic analysis revealed 100% homology to the previously studied Ax2\* type HMW glutenin gene from cultivar Cheyenne. Predicted secondary structure results showed that it was similar to 1Ax1 type of common wheat (*Triticum aestivum*), having superior flour milling quality.

**Conclusions:** Sequence analysis suggests that HMWG<sub>Ax</sub> protein significantly and positively correlates with the properties of elasticity and extensibility of gluten.

**Keywords:** Dough, Dacke, HMW glutenin

## INTRODUCTION

The seed storage proteins of wheat are mainly composed of glutenins and gliadins. They are responsible for dough elasticity and extensibility of wheat flour.<sup>1,2</sup> The glutenins are divided into two types, HMW glutenin subunit (HMW-GS) and LMW glutenin subunit (LMW-GS) according to their molecular weight in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.<sup>3</sup> Even though HMW-GS represents only 5% to 10% of grain protein, it plays a crucial role in determining the viscoelastic properties and bread making quality of dough. The HMW-GSs are encoded by genes at Glu-1 loci on the long arms of chromosomes 1A, 1B

and 1D in bread wheat, and each locus consists of two tightly linked genes, designated as the x- and y-type HMW-GS. The two types can be distinguished from each other by their molecular weights.<sup>4</sup> The electrophoretic mobility of the y-type HMW-GS is faster than the x type on SDS-PAGE.<sup>5</sup> Differences in molecular weights are mainly due to differences in the lengths of their repetitive domains. Previous analysis of their coding sequences has revealed that the x- and y-type subunits have similar structures, consisting of a central repetitive region flanked by two highly conserved, terminal non-repetitive domains.<sup>3</sup> The length of the N-terminal domain is used as a reference to distinguish the x-type and y-type subunits. The N-terminal domain of x-type subunits comprises 81–

89 amino acid residues, and that of the y-type which usually comprises 104 or 105 residues. Central repetitive domain, that is composed of tri- (in the x-type subunit), hexa- and nanopeptide motifs.<sup>6</sup> The region which contains both x- and y-type subunits has repeated sequences of amino acids whose numbers range from 490 to 700. These regions are rich in glutamine, proline, and glycine and poor in sulfur (0 or 1 cysteine). The N-terminal region has no repetitive sequence and contains 3 to 5 cysteine residues. Cysteine residues provide intermolecular disulphide bonds between HMW-GS and LMW-GS to form protein polymers.<sup>7-10</sup> The disulphide-linked glutenin chains provide an “elastic backbone” to gluten. Furthermore, the high level of glutamine residues has a greater capacity to form both intra- and intermolecular hydrogen bonds. Thus, this feature may be involved in elasticity through the formation of intermolecular hydrogen bonds.<sup>2</sup> During the bread making process, some of these bonds break on stretching, giving rise to unbounded mobile regions (loops) and bonded regions (trains). The loops can be stretched and then reformed when the stress is removed, which accounts for the restoring elastic force.

Previous research reports the complete nucleotide and the derived amino acid sequences of six HMW-GS genes from cultivar Cheyenne: Ax2\*, Bx7, By9, Dx5, Dy10, and the silent Ay type subunit gene.<sup>11</sup> The quality of wheat flour depends on the types of HMW-GSs it contains. Generally, 1Ax1, 1Ax2\*, 1Bx7+1By9, 1Bx14+1By18 and 1Dx5+1Dy10 type HMW-GS from common wheat are combined to obtain superior quality flour.<sup>12,13</sup>

In the present study, we cloned and characterized HMW glutenin gene and their coding sequences from *Triticum aestivum* cultivar Dacke (HMWGx) and further investigated their evolutionary biology with phylogenetic analysis.

## METHODS

### *Plant material*

*Triticum aestivum* cultivar Dacke seeds were kindly provided by the late Professor S.R. Srimanne, Department of Biochemistry and Molecular Biology, Faculty of Medicine, and University of Colombo, Sri Lanka.

### *DNA extraction and PCR*

Genomic DNA was extracted from the young leaves of *Triticum aestivum* cultivar Dacke by the CTAB method.<sup>14</sup> Based on the characterized DNA sequences of wheat HMW glutenin genes available at NCBI database, the forward and the reverse primers were designed to amplify an approximately 2500 bp length of HMW glutenin gene. The forward primer: H2FP (5'GAATTCATGACTAAGCGGTTGGTTCTT 3')

contained the start codon of the HMW glutenin gene's ORF (open reading frame), and the reverse primer: H2RP (5'-GGTAACCTCACTGGCTGGCCAACAAY 3') was designed as a degenerate primer. PCR was carried out using GoTaq® DNA polymerase (Promega). The parameters for the PCR were 94°C for 5 min, followed by 35 cycles of 94°C for 1 min. The annealing temperature for gradient PCR amplification ranged from 65 to 69°C for 40 sec and 72°C for 3 min, and a final extension step at 72°C for 15 min. The optimum temperature was found to be 65°C.

### *Cloning of HMWGx gene*

PCR products were separated on 0.8% agarose gel. DNA fragments of expected sizes were eluted from the gel and purified prior to ligation into the pGEM-T vector (Promega). *Escherichia coli* JM 109 competent cells were transformed with the ligation reactions following the standard procedure (Promega). Recombinant positive clones were selected, then the complete DNA sequence was obtained by primer walking (Macrogen Korea).

### *Phylogenetic analysis of HMW glutenin genes*

The phylogenetic relationship was analyzed between the isolated HMWGx gene from *Triticum aestivum* cultivar Dacke and different HMW glutenin genes extracted from GenBank, namely: 1Ax2\* (M22208), 1Bx7 (X13927), 1Dx5 (X12928), 1By9 (X61026) 1Dy10 (X12929) and 1Ay (X03042) from *Triticum aestivum* cultivar Cheyenne. The phylogenetic tree was generated based on the distance method (Tamura-Nei model) with 1000 bootstrap replications using MEGA7.<sup>15</sup> The secondary structure of HMWGx protein was predicted by PSIPRED server.<sup>16,17</sup>

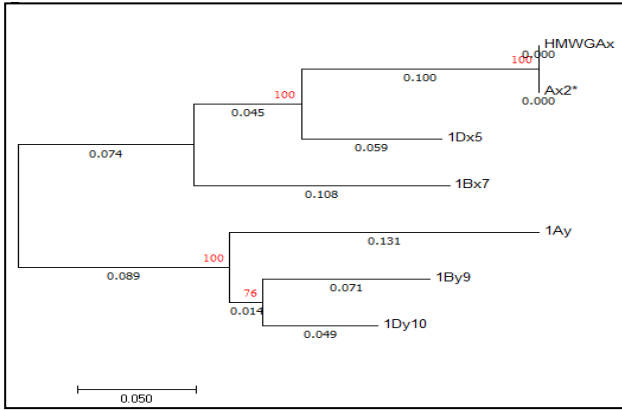
## RESULTS

### *Molecular characterization of the HMWGx gene*

The cloned HMWGx gene was deposited in GenBank (accession number KJ939340). The open reading frame of the cloned sequence, encoded a 815 amino acid residues and possessed the typical structural characteristics of the HMW glutenin genes. Blastx analysis of the HMWGx revealed 100% identity to a previously published *Triticum aestivum* HMW-GS. The four distinct domains common to all HMW-GSs were present in HMWGx: 1) signal peptide of 21 amino acids; 2) a non-repetitive N-terminal domain; 3) a central repetitive domain, and 4) a non-repetitive C-terminal domain. Most of the reported HMW glutenin genes are highly conserved at N and C terminal repetitive domains. The difference among the HMW glutenin genes were a result of the amino acid composition in the repetitive domain.

The composition of repetitive domains of the deduced HMWGx protein was 44 tripeptides (GQQ), 6 hexapeptides (PGQQQ) and a nanopeptide

(GYYPSTLQQ), indicating it to be a typical x-typed HMW glutenin protein which is similar to Ax2\* reported by Anderson and Greene.<sup>11</sup> Calculated molecular weight of the HMWG<sub>Ax</sub> protein was ~88 kDa and three cysteine residues were located in the N terminal domain, as in the other x-type encoded by most of the *Triticum spp.* The remaining cysteine residue was present in the C-terminus of the non repetitive domain.



**Figure 1: Phylogenetic tree derived from the complete amino acid sequence alignment of HMWG<sub>Ax</sub> and six different HMW glutenin from cultivar Cheyenne.**

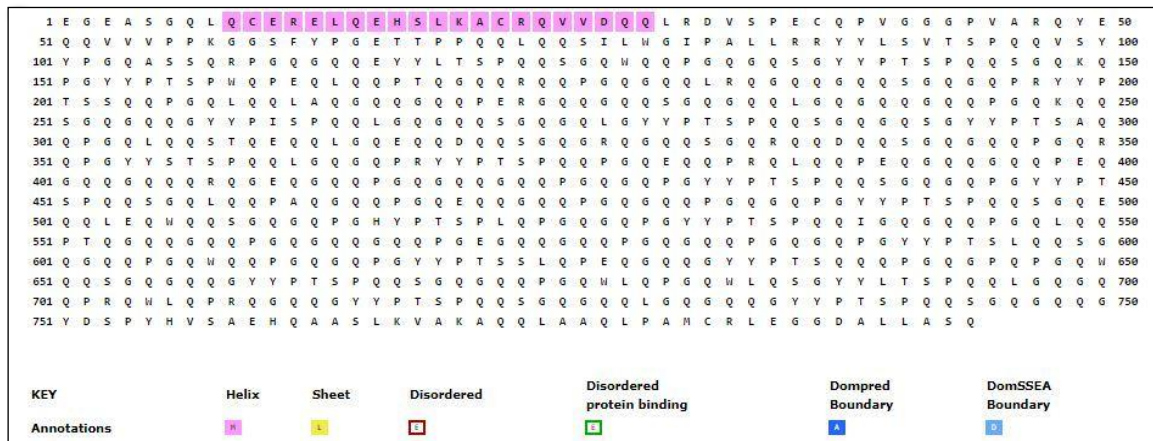
**Phylogenetic analysis of HMW glutenin genes**

The homology tree was constructed to analyze the phylogenetic relationships of HMWG<sub>Ax</sub> among six different HMW glutenins from *Triticum aestivum* (Figure 1).

The x and y type HMW glutenin proteins clearly separated into two groups. Within the x type subgroup, the Ax, Bx and Dx proteins were clustered in 3 separate clades. HMW glutenin protein from *Triticum aestivum* cultivar Dacke is closely related with the A genome of *Triticum aestivum* cultivar Cheyenne.

**Predicted secondary structure of the deduced HMWG**

The secondary structure of mature HMWG<sub>Ax</sub> protein was predicted by the PSIPRED protein structure prediction servers (Figure 2) and compared with previously reported 1Ax1 (GenBank accession number: X61009) of common wheat, identified as having a superior flour milling quality.<sup>18,19</sup> The predicted result indicated the presence of one α-helix in N-terminal domain, and the absence of β-strands in both HMWG<sub>Ax</sub> from the Dacke variety and 1Ax1 from common wheat.



**Figure 2: Predicted secondary structure of mature HMWG<sub>Ax</sub> protein of Dacke.**

**DISCUSSION**

The HMWG<sub>Ax</sub> gene was comprehensively analyzed in this study. Due to the variation in the nucleotide sequence of the HMW glutenin genes among the *Triticum aestivum* different cultivars a degenerate primer (H2RP) was used to cover all possible nucleotide combinations for HMW glutenin gene sequences in different cultivars. The designed H2FP and H2RP primers were employed to amplify the HMWG<sub>Ax</sub> gene using gradient PCR to determine the optimal annealing temperature of the primers.

In the past, many researchers have been focused on HMW glutenin genes of different cultivars of wheat and wheat related species.<sup>20-24</sup> Dacke is reported as a Swedish spring variety. It has a high protein content in the kernel, especially the gluten protein (37%).<sup>25</sup> Here we report the *in silico* analysis of the HMW glutenin from the Swedish spring wheat variety Dacke.

Gilbert et al, reported that the secondary structure of the α-helices of HMW glutenin are present at C and N terminal domains where as β turns and intermolecular β sheets are present in the central repetitive domain.<sup>26</sup> In contrast, Pandey et al, indicated that Ax1, Dx5 and Dy10 HMW glutenin of indigenous hexaploidy wheat cultivar

PBW-343 contains  $\alpha$ -helices in the central repetitive domain.<sup>27</sup> The content of  $\beta$  turns and intermolecular  $\beta$  sheets are positive indicators to determine the wheat quality for bread making. However, positive as well as negative contributions are predicted for  $\alpha$  helices.<sup>28,29</sup> In addition, research has revealed that increased length of repetitive domain, a number of cysteine residues and coils would benefit the formation of gluten polymer.<sup>22,29-31</sup>

Protein secondary structure prediction of HMWG<sub>Ax</sub> demonstrates that the number of the  $\alpha$ -helices and their position is similar to the good flour quality Ax1 type HMW glutenin subunit. Moreover, HMWG<sub>Ax</sub> protein contained 34% of glutamine residues, indicating the capacity to form inter-chain hydrogen bonds that could provide the elasticity for the dough.<sup>2</sup> Therefore, HMWG<sub>Ax</sub> protein is expected to be a useful gene to improve the dough functionality.

The HMW<sub>Ax</sub> protein displayed four cysteine residues distributed as in other typical Ax type HMW glutenin protein so far which may be important for forming the normal gluten polymers. However, the extra cysteine residue in the central repetitive domain as in 1Dx5, 1By9, 1Dy10, and 1Dy12 type HMW glutenin, which is considered to be responsible for its positive effect on quality of the flour was absent in HMW<sub>Ax</sub>.<sup>6,11,32-34</sup>

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