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Short Communication

Characterization of 1Sty13, a novel high-molecular-weight glutenin subunit from *Elymus sibiricus* L.

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Abstract

High-molecular-weight glutenin subunit (HMW-GS) is a key factor affecting dough-processing quality. 1Sty13 is a novel HMW-GS found in the tetraploid species, *Elymus sibiricus* L. 1Sty13 has faster electrophoretic mobility than the 1Dy12 subunit on sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. The gene encoding the 1Sty13 subunit was composed of 1803 nucleotide base pairs with an open reading frame that was 599 amino acids in length. Analysis of the predicted amino acid sequence of 1Sty13 indicated that the N-terminal domain was similar to the y-type subunit, whereas the C-terminal domains were similar to the x-type subunit. Five cysteine residues were found in 1Sty13, which is one less than the published HMW-GS in the St genome. The 1Sty13 protein was purified at a scale sufficient for incorporation into flour for the SDS sedimentation test, which indicated that incorporating 1Sty13 improved dough quality.

Keywords: 1Sty13, *Elymus sibiricus* L, HMW-GS, molecular structure, processing quality, wheat

Introduction

High-molecular-weight glutenin subunit (HMW-GS) of wheat is a key protein component affecting dough-processing quality. HMW-GS is encoded by the *Glu-1* loci located on the long arm of homologous group 1 chromosomes in common wheat. Each locus consists of two closely linked genes, which encode a larger x-type subunit and a smaller y-type subunit (Payne *et al.*, 1980). The molecular structure of HMW-GS possesses three conserved domains, including a signal peptide, an N-terminal domain and a C-terminal domain. A non-conserved repetitive region is also present between the N-terminal and C-terminal domains (Wan *et al.*, 2002). The variation in HMW-GS depends on the composition of the motifs in the repetitive region.

Elymus sibiricus L., which belongs to *Triticeae*, is an important perennial herbaceous plant mainly distributed in the high-altitude regions of western and northern China (Zhang *et al.*, 2018). Previous studies have demonstrated that the genus *Elymus* contains unique HMW-GS alleles with the potential to improve wheat-processing quality (Jiang *et al.*, 2010; Sun *et al.*, 2014). However, the HMW-GS of *E. sibiricus* collected from the Yunnan-Guizhou Plateau of China has not been characterized. Here, a novel HMW-GS from *E. sibiricus* was cloned and its effect on sodium dodecyl sulphate (SDS)-sedimentation volume was analysed. This analysis revealed the potential utility of *E. sibiricus* genes to improve wheat quality.

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Fig. 1. Multiple alignments of deduced amino acid sequences of 1Sty13, 1Dy12, 1St2 and 1By15. Conservative cysteines have been marked by arrows and the extra cysteine in 1Sty13 has been marked by a triangle. The sequence deletion present in the N-terminal of 1Sty13 has been boxed.

Experimental

E. sibiricus (StStHH, $2n = 4X = 28$) was collected in the city of Guiyang, Guizhou Province, China (26°23′N, 106°37′E). The HMW-GS in hexaploid wheat cv. Chinese Spring and Guinong 19 were used as markers to assess the molecular weight of HMW-GS in *E. sibiricus*. Glutenins were extracted and detected according to a method reported previously (Wan *et al.*, 2002; Hou *et al.*, 2017). The results of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) indicated that *E. sibiricus* expressed one HMW-GS with faster electrophoretic mobility than 1Dy12 (online Supplementary Fig. 1a).

To clone the coding sequence of HMW-GS, a template was prepared according to Du *et al.* (2019). A pair of primers (P1 and P2, online Supplementary Table S1) was utilized to amplify the complete coding sequences of HMW-GSs according to Jiang *et al.* (2014). Polymerase chain reaction amplification resulted in a single fragment (online Supplementary Fig. 1b). The length of the *1Sty13* gene was 1803 bp. This sequence was deposited in the GenBank with accession number KY643874. Analysis of the predicted amino acid sequence indicated that the 1Sty13 protein possessed seven cysteine residues. Multiple sequence alignment indicated the presence of a deletion in the 1Sty13 N-terminal domain (Fig. 1).

To verify the functionality of the *1Sty13* open reading frame, *Escherichia coli* expression and western blot assays were carried out according to Hou *et al.* (2017). SDS-PAGE analysis of the protein extracts prepared from the induced bacterial cultures indicated that the electrophoretic mobility of the recombinant 1Sty13 protein was identical to that of the target subunit extracted from seeds (online Supplementary Fig. S1a). The overexpressed protein also reacted with a polyclonal antibody specific to HMW-GS (online Supplementary Fig. S1b), thus confirming its identity as an HMW-GS.

The contribution of 1Sty13 to dough quality was analysed using SDS sedimentation test according to Hou *et al.* (2017). Flour prepared from Guinong 19, an important wheat variety in Guizhou Province of China, has poor processing quality and was chosen as the base flour (control). HMW-GS 1Dy12 and 1Dy12** of wheat were purified and used to compare the SDS sedimentation volume to 1Sty13. 1Dy12 is a common HMW-GS in wheat that confers a negative effect on wheat-processing quality (Rasheed

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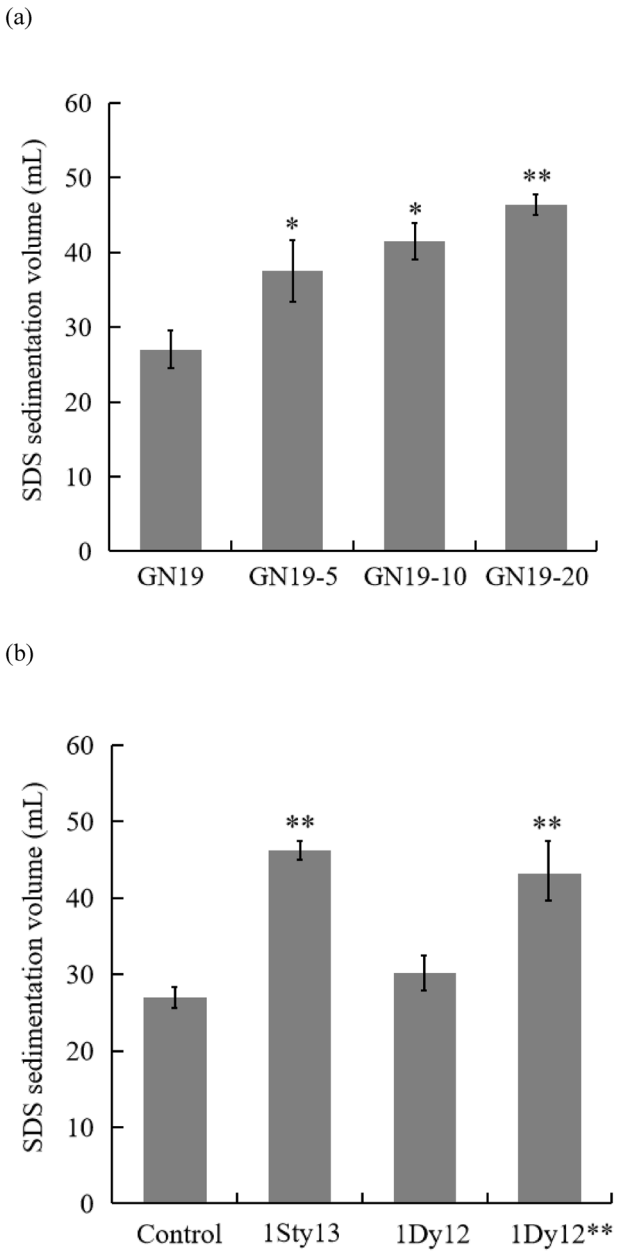

Fig. 2. Function of 1Sty13 investigated by *in vitro* SDS sedimentation test. (a) SDS sedimentation volumes of base flour incorporated by purified 1Sty13. GN19-5, GN19-10 and GN19-20 indicate that the additive amount of purified 1Sty13 to base flour of Guinong 19 were 5, 10 and 20 mg, respectively. (b) SDS sedimentation volumes of base flour incorporated by 20 mg purified 1Sty13, 1Dy12 or 1Dy12**. Control means base flour of Guinong 19. * and ** above the histogram indicate significant difference compared with control at $P = 0.01$ and 0.05, respectively.

et al., 2012). 1Dy12**, which provides strong dough stress, was identified in Yunnan-hulled wheat (Du *et al.*, 2019). The SDS sedimentation test was performed three times. SDS sedimentation volume is an important index to evaluate the processing quality of flour, which was improved after the purified 1Sty13 protein was added (Fig. 2a). In addition, the SDS sedimentation volumes were significantly higher after incorporating 1Sty13 and 1Dy12** than 1Dy12, indicating that incorporating 1Sty13 into the base flour of Guinong 19 significantly increased SDS sedimentation volume. The difference in SDS sedimentation volume was significant when different amounts and types of proteins were incorporated into the flour.

Discussion

Tetraploid *E. sibiricus* should theoretically possess four HMW-GS, but only one HMW-GS was detected. A frame-shift mutation occurs frequently in HMW-GS genes of wheat and related species, which may explain the detection of only a single HMW-GS gene in *E. sibiricus*. Yuan *et al.* (2009) reported that 1By subunit genes in two wheat lines were silenced due to base deletions that produced premature stop codons. Sun *et al.* (2014) analysed HMW-GS in *Leymus* and suggested that *Chiy2* silencing was due to a two-base deletion in its N-terminal, which resulted in a frame-shift mutation.

Variations in HMW-GS are strongly associated with wheat-processing quality. For example, previous studies have demonstrated that use of HMW-GS, which has a longer repetitive region and more cysteine residues, results in superior dough strength and bread-making quality (Ma *et al.*, 2013; Garg *et al.*, 2014). Thus far, many HMW-GS genes have been cloned and characterized from different wheat varieties. However, favourable variations in HMW-GS genes of common wheat are limited, with few varieties possessing the most favourable alleles. Relatives of wheat occasionally have superior glutenin genes with the potential for improving wheat quality (Garg *et al.*, 2014; Du *et al.*, 2020). An increase in the number of cysteine residues in HMW-GS resulted in a positive effect on dough strength by forming more intermolecular disulphide bonds. The newly identified 1Sty13 possesses an extra cysteine compared with the x-type subunit and may therefore represent a superior HMW-GS.

In conclusion, the 1Sty13 subunit cloned in this study improved dough quality, indicating that transferring 1Sty13 by genetic modification or interspecific hybridization may be a useful strategy to improve wheat quality.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262121000344.

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