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Isolation and characterization of a novel variant of HMW glutenin subunit gene from the St genome of *Pseudoroegneria stipifolia*

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Abstract

The x- and y-type high molecular weight (HMW) glutenin subunits are conserved seed storage proteins in wheat and related species. Here we describe investigations on the HMW glutenin subunits from several *Pseudoroegneria* accessions. The electrophoretic mobilities of the HMW glutenin subunits from *Pd. stipifolia*, *Pd. tauri* and *Pd. strigosa* were much faster than those of orthologous wheat subunits, indicating that their protein size may be smaller than that of wheat subunits. The coding sequence of the Glu-1St1 subunit (encoded by the *Pseudoroegneria stipifolia* accession PI325181) was isolated, and found to represent the native open reading frame (ORF) by *in vitro* expression. The deduced amino acid sequence of Glu-1St1 matched with that determined from the native subunit by mass spectrometric analysis. The domain organization in Glu-1St1 showed high similarity with that of typical HMW glutenin subunits. However, Glu-1St1 exhibited several distinct characteristics. First, the length of its repetitive domain was substantially smaller than that of conventional subunits, which explains its much faster electrophoretic mobility in SDS-PAGE. Second, although the N-terminal domain of Glu-1St1 resembled that of y-type subunit, its C-terminal domain was more similar to that of x-type subunit. Third, the N- and C-terminal domains of Glu-1St1 shared conserved features with those of barley D-hordein, but the repeat motifs and the organization of its repetitive domain were more similar to those of HMW glutenin subunits than to D-hordein. We conclude that Glu-1St1 is a novel variant of HMW glutenin subunits. The analysis of Glu-1St1 may provide new insight into the evolution of HMW glutenin subunits in *Triticeae* species.

Keywords: *Pseudoroegneria stipifolia*; HMW glutenin subunit; D-hordein; Wheat; Phylogenetic analysis; Evolution

1. Introduction

High molecular weight (HMW) glutenin subunits are important determinants of the baking quality of bread wheat (*Triticum aestivum* L.) (Payne, 1987; Shewry and Halford, 2002). There are two subfamilies of HMW glutenin subunits, known as x- and y-types arising from a gene duplication event that predates the divergence of the A, B, and D genomes of cultivated wheat (Gu et al., 2003). In hexaploid wheat, HMW glutenin subunits are encoded by genes contained in the *Glu-A1*, *Glu-B1* and *Glu-D1* loci located on the homoeologous group one chromosomes

Abbreviations: HMW-GS, high-molecular-weight glutenin subunit; IPTG, isopropyl b-D-thiogalactopyranoside; kDa, kilo Dalton; MALDI-TOF-MS, matrix assisted laser desorption ionization time-of-flight mass spectrometry; MYA, million years ago; ORF, open reading frame; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

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(Lawrence and Shepherd, 1981; Payne, 1987; Thompson et al., 1983). Theoretically, there should be six expressed HMW glutenin subunits in hexaploid wheat. However, the compositions of HMW glutenin subunits often vary among bread wheat cultivars due to gene silencing and allelic variation. Usually, three to five subunits are expressed in individual varieties (Payne and Lawrence, 1983). The primary structure of a HMW glutenin subunit is composed of four regions, the signal peptide (removed from mature protein), N- and C-terminal domains, and a central repetitive region (Shewry et al., 1995). In the N- and C-terminal regions, there exist cysteine residues that are highly conserved in both numbers and positions. The repetitive domain predominantly consists of repeats encoding tri-, hexa- and nonapeptides (Shewry et al., 1995). Both the conserved cysteine residues and the size of the repetitive domain contribute to the higher order structure of HMW glutenin subunits. The former are involved in the formation of disulphide bonds, the latter may initiate intermolecular interactions through hydrogen bonds (Shewry et al., 1995). Variations in the numbers of repeated peptide motifs often lead to changes in the length of the repetitive domains, which is the main cause for size differences among different subunits (Shewry et al., 1995). The open reading frames (ORFs) for HMW glutenin subunit genes are generally 1.8–2.5 kb, and the calculated molecular mass of these subunits ranges from 65 to 90 kDa (Shewry et al., 1995).

Orthologous HMW glutenin subunits have been found in many *Triticeae* grasses including various *Aegilops* species and rye (De Bustos et al., 2001; De Bustos and Jouve, 2003; Liu et al., 2003; Wan et al., 2002; William et al., 1993). In barley (*Hordeum vulgare* L.), the seed storage protein D-hordein is structurally related to HMW glutenin subunits of wheat (Halford et al., 1992). The N-terminal domain of D-hordein is more similar to that of typical y-type HMW glutenin subunits, whereas its C-terminal domain exhibits higher resemblance to that of typical x-type HMW glutenin subunits (Gu et al., 2003). However, the central repetitive region of D-hordein shows multiple differences from that of typical HMW glutenin subunits in both domain organization and repeat motifs (Gu et al., 2003). The D-hordein central domain is composed of two repetitive regions separated by a non-repetitive and cysteine-containing fragment that is unique to D-hordein. The repeated motifs contained in the first repetitive region (PGQGQQ, PGQGQQGYYPSATSPQQ) bear high resemblance to those found in the repetitive domain of HMW glutenin subunits. However, the main repeated motif in the second repetitive region of D-hordein (PHQGQQTTVS) has no equivalent in the HMW glutenin subunits characterized thus far (Gu et al., 2003).

Since HMW glutenin subunit genes display similar temporal and spatial patterns of expression (Thomas and Flavell, 1990), they must have conserved 5′ flanking regulatory elements. Previous studies have indicated that HMW glutenin subunit genes contain a major regulatory element 5′-GTTTTGCAAAGCTCCAATTGCTCCTTG-CTTATCCAATATT-3′ in their promoter regions (Thomas and Flavell, 1990). The location of this element is highly conserved in all HMW glutenin gene promoters reported to date, beginning at positions −185 to −189 (Shewry et al., 1999). In addition, HMW glutenin subunit genes also have some common *cis*-acting elements in their promoter regions, such as E box (TGTAAA), N box (TGAGTCA), G box (TTACGTGG) and TATA box (TATAAAA) (Thomas and Flavell, 1990).

In contrast to the studies described above, there is still little information on the HMW glutenin subunits and their coding genes in the St genome, which is contained in a wide range of *Triticeae* species (McMillan and Sun, 2004; Xu and Ban, 2004). The diploid and tetraploid *Pseudoroegneria* species have been considered to be the donor of St genome in many natural intergeneric *Triticeae* hybrids (Jensen et al., 1990; Lu, 1994; McMillan and Sun, 2004; Xu and Ban, 2004). In this work, we have investigated the HMW glutenin subunits in several diploid and tetraploid *Pseudoroegneria* accessions, with the Glu-1St1 subunit (expressed in the diploid *Pd. stipifolia* accession PI325181) being analyzed in more detail. The results of our investigations are reported in the following sections, together with the discussions on the unusual structural features of Glu-1St1 and the new insight gained into the evolution of HMW glutenin subunits based on the analysis of Glu-1St1.

2. Materials and methods

2.1. Plant materials

Seeds of two diploid *Pd. stipifolia* accessions (PI531750 and PI325181, $2n = 14$, StSt), one tetraploid *Pd. tauri* accession (PI380650, $2n = 28$, StStPP) and one tetraploid *Pd. strigosa* accession (PI531752, $2n = 28$, StStStSt) were obtained from the Institute of Crop Sciences of the Chinese Academy of Agricultural Sciences, Beijing, China. These accessions were originally obtained from the United States Department of Agriculture (USDA). The HMW glutenin subunits from the bread wheat variety Chinese Spring (1Bx7 + 1By8, 1Dx2 + 1Dy12) were used as electrophoretic mobility standards in SDS-PAGE experiments.

2.2. SDS-page and protein blot analysis

HMW glutenin subunits were extracted from the seeds and were separated using SDS-PAGE as previously described (Deng and Zhang, 2004). At least five individual seeds were examined to ascertain the composition of HMW glutenin subunits in a given accession. A polyclonal antibody recognizing both x- and y-type HMW glutenin subunits of wheat was used in protein blot analysis according to the method published previously (Wan et al., 2002).