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Cloning and characterization of novel γ-gliadin genes from *Aegilops markgrafii* in relation to evolution and wheat breeding

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ABSTRACT

Gliadins are the major components of storage proteins in wheat and play an important role in determining the extensibility properties of dough. In the present work, six novel full-length γ-gliadin genes were cloned from the C genome of *Aegilops markgrafii* using a PCR-based strategy. Analysis of the deduced amino acid sequences showed that the cloned genes had primary structures that were similar, but not identical, to published γ-gliadins from other wheat-related species. The lengths of the open reading frames (ORFs) ranged from 909 to 963 bp, and the repetitive and glutamine-rich domains were mainly responsible for the size of the proteins. An extra cysteine residue was present in the repetitive domain of sequence JX566513. All amino acid sequences of γ-gliadin genes from *Ae. markgrafii* were searched for the five peptides identified as T cell stimulatory epitopes in celiac disease (CD) patients. Peptide Gliγ-3 was present in sequences JX566513 and JX566514. Peptide Gliγ-5 was present only in JX566513. The other γ-gliadins contained no toxic epitopes. These results provide information to better understand the use of *Ae. markgrafii* in wheat breeding and the evolutionary relationship of the γ-gliadin genes in *Ae. markgrafii* and other Triticeae species.

1. Introduction

The processing quality of wheat flour is largely determined by seed storage proteins present in the grain endosperm [1]. Wheat storage proteins are traditionally classified into glutenins and gliadins [1,2]. Glutenins are polymeric proteins, subdivided into high-molecular-weight (HMW-GS) and low-molecular-weight (LMW-GS) glutenin subunits, whereas gliadins are normally monomeric proteins with molecular weights ranging from 30 to 100 kDa [3,4]. Gliadins are classified into α, β, γ, and ω types, based on electrophoretic mobility in acid polyacrylamide gel electrophoresis (A-PAGE)

Abbreviations: CD, Celiac disease; ORFs, Open reading frames; HMW-GS, High-molecular-weight glutenin subunits; LMW-GS, Low-molecular-weight glutenin subunits; A-PAGE, Acid polyacrylamide gel electrophoresis

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[3]. Because α- and β-gliadins possess the same molecular structures, they are always referred to as α-gliadins [2]. Previous studies indicated that the gliadin genes were linked and mainly located on a few chromosomes. The α-gliadins are located at the *Gli-A2*, *Gli-B2*, and *Gli-D2* loci on the short arms of homoeologous group 6 chromosomes [5], whereas ω-gliadins and most of the γ-gliadins are located at the *Gli-A1*, *Gli-B1*, and *Gli-D1* loci on the short arms of homoeologous group 1 chromosomes [4].

Although the contributions of gliadins to dough quality have been studied, there is a large divergence in the findings. The addition of single pure gliadins to flour had different effects on dough quality [6,7]. However, recent studies showed that down-regulation of γ-gliadins has a minor effect on dough quality [8–10], suggesting that γ-gliadins are not major determinants of mixing properties of wheat flour.

A food-sensitive enteropathy named celiac disease (CD) in Western countries is caused by ingestion of gliadins and LMW-GS [11]. Morbidity due to CD amounts to 0.7% and 1.0% in non-Hispanic whites and the overall United States population, respectively [12]. In the last decade, CD symptoms have also begun to appear in Chinese children [12,13]. CD patients are limited to a lifelong gluten-free diet [13]. Among the storage proteins in wheat, γ-gliadins containing toxic peptides that are present in proline rich regions trigger CD in susceptible individuals [5,13]. Several toxic peptides associated with CD have been identified in γ-gliadins [5]. Thus screening for epitopes in gliadin genes from specific genomes should be helpful for wheat quality improvement programs.

Aegilops markgrafii is an important source of genetic material for enlarging genetic variability in cultivated bread wheat. It carries genes for high protein content and disease resistance potentially useful in wheat improvement [14]. In the present study, six novel γ-gliadin genes were cloned from the C genome of *Ae. markgrafii*, and variation of their molecular structures, along with potential toxic peptides, is discussed.

2. Materials and methods

2.1. Plant materials

Ae. markgrafii (Greuter) Hammer (synonym *Aegilops caudata* L., $2n = 2 \times = 14$, genome CC) accession Y46 was kindly provided by Dr. Lihui Li, Institute of Crop Science, Chinese Academy of Agricultural Sciences Beijing.

2.2. DNA extraction and PCR amplification

Genomic DNA was extracted from seedling leaves using a Plant Genomic DNA Kit (Tiangen, Beijing). A pair of primers (P1: 5′-TATTAGTTAACGCAAATCCACC/TAT-3′ and P2: 5′-GATGAATCAGCTAAGCAACGATG-3′) were used to amplify the coding sequences of γ-gliadin genes. Twenty five μL of PCR mix containing 1.5 U LA *Taq* with GC buffer (TaKaRa, Dalian) was placed in a C1000 cycler (Bio – Rad). PCR amplification was performed as follows: an initial step of 95 °C for 5 min, 32 cycles of 94 °C for 45 s, 58 °C for 30 s, 72 °C for 2 min, and a final extension at 72 °C for 10 min.

2.3. Cloning and sequencing

The targeted DNA fragments were recovered and purified using a Universal Plant DNA Purification Kit (Tiangen), cloned into a pMD19-T vector (TaKaRa, Dalian, China), and transformed into DH5α competent cells (Tiangen). Positive clones were identified by colony PCR. DNA sequencing was performed by the Beijing Genomics Institute. A minimum of three clones was sequenced in order to minimize sequencing errors.

2.4. Sequence alignment and phylogenetic analysis

The open reading frames (ORFs) of γ-gliadin genes were translated into amino acid sequences using Expasy Network Services (http://www.expasy.org/translate). Multiple sequence alignments of the γ-gliadin sequences were constructed by DNAMAN software (version 6.0.2). Construction of a Neighbor-Joining tree was performed using MEGA 6.0 software. The bootstrap values were estimated based on 1000 replications.

2.5. CD epitope screening

Thirteen known CD epitopes in γ-gliadins, according to Wang et al. [5], were screened in the deduced amino acid sequences of γ-gliadins obtained in this work. Only exact matches were considered.

3. Results

3.1. Molecular characterization of the γ-gliadin genes from *Ae. markgrafii*

The results of PCR amplification showed that the primer pairs successfully amplified products with approximately 1000 bp (Fig. 1). We picked out 96 monoclones and 40 of them were sequenced, after which six novel full-length γ-gliadin genes were characterized (Table 1). All six sequences possessed complete ORFs; their lengths ranged from 909 to 963 bp and molecular weights of the deduced mature proteins ranged from 32.38 to 34.25 kDa (Table 1). The sequences were deposited in GenBank (JX566510 to JX566515).

3.2. Multiple alignments of the γ-gliadins

Analysis of the deduced amino acid sequences of the cloned γ-gliadin genes showed that they have primary structures similar, but not identical, to published γ-gliadins from other wheat-related species (Fig. 2). Each γ-gliadin contained six domains, a conserved signal peptide with 19 amino acid residues, a conserved N-terminal domain with 12 amino acid residues, a repetitive domain with highly variable lengths, a conserved non-repetitive domain, a glutamine-rich domain, and a conserved C-terminal domain (Fig. 2). The glutamine-rich domain in each sequence from *Ae. markgrafii* was 16 amino acid residues with six glutamine residues (Fig. 2). Along with other γ-gliadins, the non-repetitive domains contained six conserved cysteine residues, and the C-terminal domains contained two conserved