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Isolation and Sequences Analysis of the α-gliadin Genes from *Aegilops sharonensis*

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Abstract: In this study, PCR primers were designed base on the known genes in wheat and its relatives to isolate α-gliadin genes from *Ae. sharonensis*, one species of the *Sitopsis* section of the genus *Aegilops*, which was traditionally considered as the B-genome donor of tetraploid and hexaploid wheat. Three novel α-gliadin genes were obtained. Analyses of the nucleotide and deduced amino acids of the obtained genes indicated that they shared the high sequence identities and similar primary structures to the known α-gliadin genes. Further more, the extensive variations were found. A phylogenic analysis based on the multigene alignment of the deduced amino acid sequences showed that the α-gliadin genes derived from *Ae. sharonensis* and *Ae. speltoides* were significantly distinguished from those of diploid A and D genome progenitors of wheat, but clustered closed to some genes from tetra- and hexaploid wheats. Moreover, genes derived from *Ae. sharonensis* were more closed to wheat than from *Ae. speltoides*. This result suggested that the origin and formation of wheat B genome might be polyphyletic.

Key words: Endosperm, storage protein, B genome, wheat

INTRODUCTION

For most of the human population, plant foods, especially cereal grains, provide the majority of nutritionally important proteins. In wheat, the most abundant storage proteins in endosperm are gliadins and glutenins, representing about 80% of the total protein in the wheat grain (Shewry *et al*., 1997). The gluten polymer is composed mainly of High-Molecular-Weight (HMW) and Low-Molecular-Weight (LMW) Glutenin Subunits (GS) linked by disulphide bonds (Shewry *et al*., 1989, 1992; MacRitchie, 1992). The genes coding for HMW-GS are located on the long arms of chromosomes 1A, 1B and 1D at the Glu-A1, Glu-B1 and Glu-D1 loci, respectively (Payne *et al*., 1987). The LMW-GS are encoded by genes on the short arm of group-1 chromosomes at the Glu-A3, Glu-B3 and Glu-D3 loci

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(Singh and Shepherd, 1988). Gliadins are normally monomeric proteins, which are classified into three groups, α, γ and ω on the basis of their electrophoretic mobility in acidic polyacrylamide gel electrophoresis (Anderson and Greene, 1997; Metakovsky *et al*., 1984). Genes coding for most of the γ- and ω-gliadins are tightly clustered at three homoeologous loci, Gli-A1, Gli-B1 and Gli-D1, on the short arms of chromosomes 1A, 1B and 1D, respectively. The α-gliadins are encoded by tightly clustered genes at three homoeologous loci, Gli-A2, Gli-B2 and Gli-D2, on the short arm of each group-6 chromosome (Metakovsky *et al*., 1984; Metakovsky, 1991).

Although gliadins account for about 50% of the gluten proteins in wheat and the allele variation in wheat and its relatives has been widely evaluated (Pan *et al*., 2007; Zhuang *et al*., 2007; Xiong *et al*., 2008), their role in determining the mixing properties of dough are not well understood (Pistóna *et al*., 2006). This is because correlations between single gliadin and functional properties are difficult to determine due to the complex patterns and overlapping fractions present in polyploidy species. The well understand of the gliadin multigene families will not only provide the possibility to express and functionally investigate a single gliadin component or a group of similar gliadins *in vitro*, but also benefit to research the origin and evolution aspects of the gene families, which might also supply some information of genome evolution in wheat and its relatives.

The *Sitopsis* section of the genus *Aegilops*, including *Ae. longissima* (2n = 2x = 14, S^lS^l), *Ae. sharonensis* (2n = 2x = 14, $S^{sh}S^{sh}$), *Ae. searsii* (2n = 2x = 14, S^sS^s), *Ae. bicornis* (2n = 2x = 14, S^bS^b) and *Ae. speltoides* (2n = 2x = 14, SS), were proposed as B genome donors of wheat (Sarkar and Stebbins 1956). In this article, the isolation and characterization of novel α-gliadin genes from *Ae. sharonensis* were reported. The comparison with known α-gliadin genes and their phylogenic relationship were also discussed.

MATERIALS AND METHODS

Plant Materials

The *Ae.sharonesis* (2n=2*x*=14, $S^{sh}S^{sh}$) accession PI 584350 was used in this study and were kindly supplied by USDA-ARS.

Isolation of α-Gliadin Genes

Genomic DNA was isolated using cetytrimethylammonium bomide (CTAB) procedure as reported by Murray and Thompson (1980). Based on the conserved sequences of the known α-gliadin genes, a pair of degenerate primers, α-F: 5'-G (G/C) TCAATACAAATCCA(C/T)CATG-3' and α-R: 5'- TTCTCTTCTCAGTT (A/G) GTACC (A/G) -3' was designed for the complete open reading frame (ORF) of α-gliadin genes. Polymerase Chain Reactions (PCR) were performed in 100 μL reaction volume, consisting of 4U E*xTaq*™ DNA polymerase (TaKaRa) with high fidelity, 10 μL PCR buffer (supplied with Taq DNA polymerase), 200 ng genomic DNA, 1.5 mM $MgCl_2$ and 100 mM of each dNTP. PCR amplifications were conducted according to the following program: 95°C for 5 min denaturation followed by 35 cycles of 45s at 95°C, 45s at 57°C and 45s at 72°C. PCR products were separated in 1.5% agarose gels. The desired DNA fragments were cloned into the pMD18-T plasmid vector (TaKaRa) and several predominated clones were randomly selected for DNA sequencing. All the experiments were conducted in the laboratory of Triticeae Research Institute, Sichuan Agricultural University, during March, 2008 to May, 2009.

Sequence Analyses

The obtained sequences by PCR amplification and DNA sequencing were confirmed to be α-gliadin genes by using ORF finder and Blastp programs deposited in NCBI network

(http://www.ncbi.nlm.nih/gov/). Sequence analyses were conducted by using the programs DNAMAN, BioEdit and MEGA 3.1 (Kumar *et al*., 2004).

RESULTS AND DISCUSSION

Isolation of α-Gliadin Genes from *Ae. sharonesis*

With genomic PCR using primers α-F and α-R, a DNA fragment of about 900 bp was amplified from the genomic DNA of *Ae. sharonesis* (Fig. 1). Ten positive clones with expected insert were randomly selected for DNA sequencing and three unique sequences, s47a-2, s47a-4 and s47a-11, were finally obtained. Analysis using ORFfinder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) indicated that all three sequences contained a unique continuous ORF which were 930, 900 and 873 bp long, respectively.

Sequence Analysis

Sequence analysis indicated that three sequences could be translated into the proteins consisting of 309, 299 and 290 amino acid residues, respectively. Alignment of the deduced amino acid sequences showed that the obtained genes contained six domains (Fig. 2) as suggested by Anderson and Greene (1997). The leader sequences of the deduced α-gliadin of *Ae. sharonesis* encode a signal peptide consisting of 20 amino acid residues (Fig. 2). It was conserved among the three obtained genes.

The repetitive domain was composed of repeat motifs which were rich in proline and glutamine. S42-2 and S42-11 shared same repetitive domain. Comparing to S42-2 and S42-11, a deletion (six amino acids) and an insertion (7 amino acids) and several amino acid substitutions were present in S42-4 (Fig. 2).

Polyglutamine domains I and II were mainly composed of glutamines (Fig. 2). The Polyglutamine domain I in S42-2, S42-4 and S42-11 consisted of 31-32 amino acids and contained 23-26 glutamines (About 74-81%). The Polyglutamine domain II had a higher glutamine content and more variable than I. S42-2 had a longest Polyglutamine domain II with 29 amino acids, among which 28 were glutamines. The Polyglutamine domain II in S42-4 contained 19 amino acids and 17 were glutamines. However, S42-11 had only nine amino acids in this domain and among eight were glutamines.

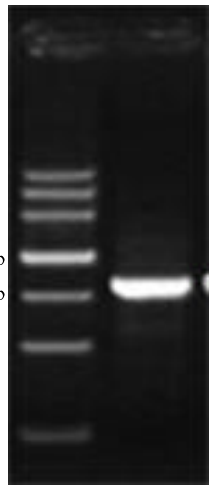

Fig. 1: Amplification product obtained from genomic DNA of *Ae. sharonesis* using primers α-F and α-R

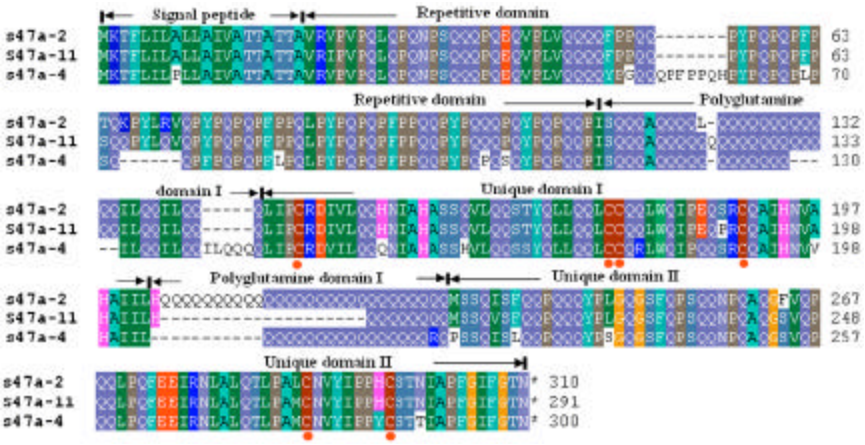

Fig. 2: Alignment and primary structure of the amino acid sequences of the α-gliadin genes obtained in this study

Table 1: Sequence similarity to the known α-gliadin genes

Clone	Most homologous sequences	Sequence identity (%)	Resource	Reference
S47a-2	DQ296195	94.6	*T. durum*	Wang *et al.* (2007)
	EF569978	94.7	*T. sphaerococcum*	[b]
	EF561274	94.9	*T. aestivum*	Wang *et al.* (2007)
	K03075	95.0	*T. aestivum*	Sumner-Smith *et al.* (1985)
	X02540	95.0	*T. aestivum*	Sumner-Smith *et al.* (1985)
S47a-4	EU018250[a]	94.2	*Lophopyrum elongatum*	[b]
	EU401791	95.2	*T. paleocolchicum*	[b]
	EU018334	95.6	*L. elongatum*	[b]
	U51309[a]	95.7	*T. aestivum*	Anderson and Greene (1997)
	FJ159430	96.0	*T. armeniacum*	[b]
S47a-11	EF569971	94.8	*T. sphaerococcum*	[b]
	M16496	94.6	*T. urartu*	Reeves and Okita (1987)
	X02538	94.8	*T. aestivum*	Sumner-Smith *et al.* (1985)
	K03074	94.8	*T. aestivum*	Sumner-Smith *et al.* (1985)
	EF569975	94.7	*T. sphaerococcum*	[b]

[a]Pseudogene; [b]Directly submitted to GenBank

The unique domains I and II were extremely conserved the three obtained α-gliadin genes (Fig. 2). Only nine amino acid substitutions were found. Six cysteines were in these two domains and four and two cysteines were in unique domains I and II, respectively.

Sequences Similarity and Phylogenic Analysis

Sequence comparison indicated that the obtained three α-gliadin genes show high degree similarities with the known α-gliadin genes from hexaploid, tetraploid and diploid wheat (Table 1). Phylogenic analysis using several known α-gliadin genes from different species was conducted. It indicated that the S47a-4 was close to the genes from *Ae. speltoides*, whereas S47a-2 and S47a-11 were clustered with genes from hexa- (AABBDD) and tetraploid (AABB) wheats (Fig. 3).

Previous study found that approximately 50% of the α-gliadin genes are pseudogenes (Anderson and Greene, 1997). Van Herpen *et al.* (2006) reported that the fraction of pseudogenes is much higher in diploid wheat species, which were 72% in A genome species (*T. monococcum*) and 95% in B genome species (*Ae. speltoides* and *Ae. longissima*). In this study, three α-gliadin genes were isolated from *Ae.sharonesis*, another species of *Sitopsis*

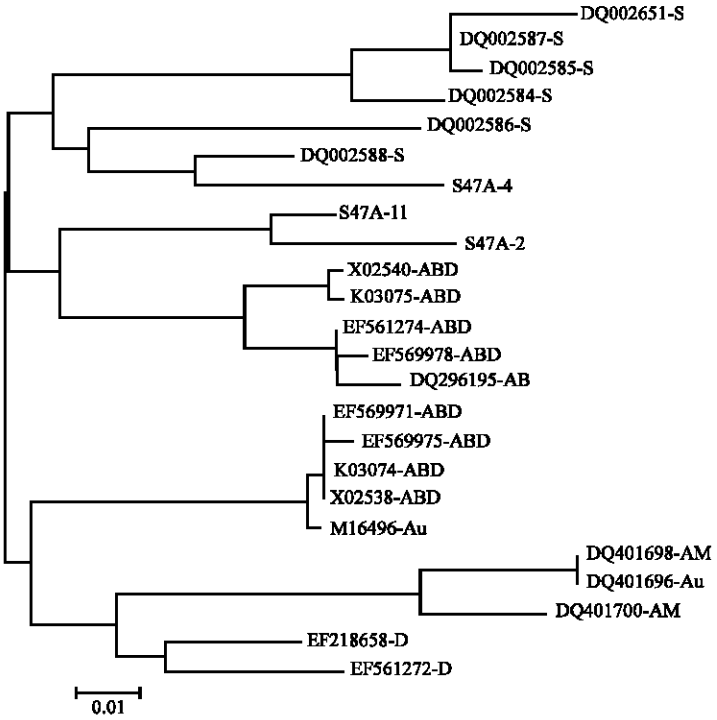

Fig. 3: Phylogenic analyses of the α-gliadin genes of tetraploid and hexaploid wheats and their diploid progenitors. The genes with accession No. as DQ 002588, DQ002586, DQ002584, DQ002585, DQ002587, DQ002651 were derived from *Aegilops speltoides*; EF218658 and EF561272 were from *Ae. tauchii*; DQ401698 and DQ401700 were from *T. monococcum*; DQ401696 and M16496 were from *T. urartu*, DQ296195 was from *T. durum*; EF561274, K03075, X02540, EF569975, K03074 and X02538 were from *T. aestivum*, EF569978 and EF569971 were from *T. sphaerococcum*

section of the genus *Aegilops*. However, no pseudogene was obtained from the ten randomly sequenced clones. Though the exact copy number of the α-gliadin genes in *Ae. sharonesis* has not been revealed and their sequences have not also been fully obtained, the results from the present study indicated that the percentage of the pseudogenes in *Ae. sharonesis* is lower than those in wheat and its relatives. This may result from different evolution process of these species.

The common wheat, *T. aestivum*, possesses three sets of homologous genomes, designated as AABBDD, of which the wild diploid *Ae. tauschii* and *T. urartu* or *T. monococcum* were the D and A genome donors (Huang *et al*., 2002). One or more species in the section *Sitopsis* of the genus *Aegilops*, were traditionally considered as the wild diploid B genome donor of wheat. The α-gliadin genes have been isolated from *Ae. speltoides* and *Ae. longissima*. But their relationship with those in wheat remains unknown. In this study, three novel genes were obtained from *Ae. sharonensis*. Phylogenic analysis indicated that the α-gliadin genes from A, D genomes or *Sitopsis* species were significant divergent from each other. This means the sequences of α-gliadin genes were genome-specific, similar to the previously found in another type of seed storage protein genes (Long *et al*., 2005, 2006). Further more, genes from *Ae. sharonensis* and *Ae. speltoides*

were close to some from wheat with the ABD and AB genomes and the former seems closer than the latter. Though the exact chromosome location of these wheat α-gliadin genes is unknown, they apparently divergent from A or D genome. These results suggested that they might be derived from the B genome. Although *Ae. speltoides* was suggested as the exact B genome donor of polyploidy wheat more recently (Petersen *et al*., 2006). The results obtained in this study were not consistent with it, for that the α-gliadin genes in *sharonensis* seemed to be more closed to wheat than those from *Ae. speltoides*. This result suggested the polyphyly for the B genome which would come from intergenome recombination between different species in section *Sitopsis* of genus *Aegilops*. Further research need to isolate the α-gliadin genes from other species of section *Sitopsis* of the genus *Aegilops* and obtain more information of α-gliadin genes derived from A, B and D genomes of wheat. This may help to better understand the structure and relationship of α-gliadin multigene families.

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