

Omega gliadin genes expressed in *Triticum aestivum* cv. Butte 86: Effects of post-anthesis fertilizer on transcript accumulation during grain development

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Abstract

The partial coding sequences of omega gliadin genes expressed in developing wheat kernels *Triticum aestivum* cv. Butte 86 were identified in databases of expressed sequence tags (ESTs). Three gene assemblies encode proteins with PQQFP as the predominant repetitive motif. Of these, two encode proteins with at least one cysteine and thus may be incorporated into the glutenin polymer. Another two gene assemblies encode proteins with FPQQQ and QQIPQQ repeats. Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) was used to investigate the accumulation of omega gliadin transcripts in developing wheat grains produced with or without post-anthesis fertilizer supplied as 20–20–20 nitrogen–phosphorous–potassium (NPK). Omega gliadin transcripts were detected at 8 days post-anthesis (DPA). The levels of transcript changed little in the absence of NPK during grain development, but increased when plants were supplied with post-anthesis NPK. By 32 DPA, levels of omega gliadin transcripts were considerably higher in grains that received NPK than in those that did not receive NPK. Transcripts for a gamma gliadin showed a different profile, increasing from 8 to about 22 DPA, then decreasing at later time points. In contrast to omega gliadins, both the timing and levels of gamma gliadin transcripts were similar in grains produced with or without post-anthesis NPK.

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1. Introduction

The omega gliadins comprise from ~5% to 10% of the wheat flour protein. The omega gliadins are distinguished from the other gluten proteins on the basis of their amino acid compositions, being exceptionally rich in proline and glutamine with little or no methionine and cysteine. These proteins also are unusual in that they consist almost entirely of repetitive sequences. An interesting feature of the omega gliadins is that the levels of these proteins have

been demonstrated to change in response to nitrogen (Daniel and Triboni, 2000; DuPont et al., 2006a, b; Wieser and Seilmeier, 1998; Wieser et al., 2004) and sulfur availability (Wrigley et al., 1984). Thus, it is possible that these proteins play a role in alterations in flour quality that are observed when wheat is grown at different locations. Additionally, omega gliadins have been implicated in food allergies and sensitivities. In particular, omega gliadins encoded by the 1B chromosome have been associated with wheat-dependent exercise-induced anaphylaxis (WDEIA) and urticaria in adults and immediate-type wheat allergies in children (Battais et al., 2005; Matsuo et al., 2004, 2005; Palosuo et al., 2001).

N-terminal amino acid sequences for omega gliadins from different bread wheat varieties and wheat progenitors have been determined. On the basis of N-terminal sequence, omega gliadins are sometimes referred to as ARE-, ARQ-, KEL- and SRL-type proteins (Kasarda

Abbreviations: DPA, days post-anthesis; ESTs, expressed sequence tags; NPK, nitrogen–phosphorous–potassium; nsLTP, non-specific lipid transfer protein; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; NCBI, National Center for Biotechnology Information; TaGI, The Wheat Gene Index; TIGR, The Institute for Genomic Research; WDEIA, wheat-dependent exercise-induced anaphylaxis

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et al., 1983). The ARE-, ARQ- and KEL-type proteins are encoded on the 1A and 1D chromosomes (DuPont et al., 2004) and are also called the omega-1 and omega-2 gliadins, respectively. The SRL-type protein is encoded on the 1B chromosome (DuPont et al., 2000) and is also referred to as the omega-5 gliadin. Recent data suggest that 1A- and the 1D-encoded omega gliadins beginning with KEL result from the cleavage of ARE- or ARQ-type sequences by an asparaginyl endoprotease (DuPont et al., 2004).

The omega gliadins are monomeric proteins. However, a number of proteins with similar sizes, amino acid compositions and N-terminal sequences have been found in polymeric fractions of wheat flour. These have been referred to as D-type low molecular weight glutenin subunits (Masci et al., 1993) or as glutenin-bound (omega b) gliadins (Wieser and Seilmeier, 1998). One such protein characterized by Masci et al. (1999) in cv. Chinese Spring had a M_r of 41,000–42,000 determined by mass spectrometry and an amino acid composition similar to a 1D-encoded omega gliadin, except that the protein contained small amounts of cysteine and methionine. Similar proteins also have been reported by Muccilli et al. (2005). Gianibelli et al. (2002) described a larger protein in the polymeric fraction of several wheat varieties with an apparent M_r of 71,000, an amino acid composition similar to omega gliadins encoded on chromosome 1B and an N-terminal sequence beginning with SRL. This protein was found in only 4 of the 139 Argentinian, Australian and Canadian bread wheat varieties that were screened.

Omega gliadin genes have proved difficult to clone, probably because of the repetitiveness of the sequences (Hsia and Anderson, 2001). Nonetheless, three omega gliadin genes (Accession Numbers AY667097, AB181300, AF280605), six pseudogenes (Accession Numbers AF280606, AB059812, DQ307378, DQ287981, AB181301, AY591334) and one partial cDNA (Accession Number AJ937839) have been reported in the National Center for Biotechnology Information (NCBI) non-redundant database. Genomic clones that encode proteins beginning with ARE and SRL and a cDNA that encodes a protein beginning SRL have been obtained but active genes for the ARQ-type protein have not been identified. Partial cDNAs for omega gliadin proteins also are among the more than 580,000 expressed sequence tags (ESTs) in The Wheat Gene Index (TaGI) compiled by The Institute for Genomic Research (TIGR) and maintained by the Dana Farber Cancer Institute (<http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=wheat>).

In this paper, we survey EST databases for partial sequences of omega gliadin genes from developing wheat kernels of *Triticum aestivum* cv. Butte 86, a variety in which grain development has been studied under numerous controlled environmental conditions (Altenbach et al., 2002, 2003, 2004; DuPont et al., 2006a, b). Using these sequences, we developed primers to assess accumulation profiles of transcripts for two different types of omega

gliadin genes in Butte 86 kernels by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). We compare transcript profiles for these omega gliadins in wheat grains produced under two different fertilizer regimens and highlight the response of specific gluten protein genes to the application of post-anthesis NPK.

2. Experimental

2.1. Growth of plants and collection of endosperm

The US hard red spring wheat *T. aestivum* cv. Butte 86 was grown in a climate-controlled greenhouse under a moderate temperature regimen with a daytime maximum temperature of 24 °C and a nighttime minimum temperature of 17 °C as described in Altenbach et al. (2003). Plants were watered with a dilute solution of Plantex 20–20–20 fertilizer (0.3 g L⁻¹) using an automatic drip irrigation system equipped with a fertilizer injector. At the time of anthesis, pots were divided into two groups. One group continued to receive fertilizer at the original level while the second group was supplied with only water until maturity. Developing grains were collected at various intervals after anthesis from individual heads from two separate growth experiments. In the second experiment, endosperm tissue was extracted from the developing grains. Whole grain or endosperm tissue was frozen in liquid nitrogen and stored at –80 °C until use.

2.2. RNA preparation and qRT-PCR

Total RNA was isolated from grain or endosperm as described previously (Altenbach, 1998) and treated with RQ1 RNase-free DNase (Promega, Madison, WI). RNA was reversed transcribed using the QuantiTect Reverse Transcription Kit (QIAGEN, Valencia, CA) according to the manufacturer's directions. Amplification reactions were carried out in a volume of 25 µl containing cDNA, 0.3 µM of each primer and SYBR Green Supermix (Biorad Laboratories, Hercules, CA) using a BioRad iCycler with an initial denaturation step of 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 55 °C for 45 s. At the end of the PCR cycles, a melting curve was generated and analyzed by the iCycler software.

Oligonucleotide primers for omega gliadin genes were selected using Beacon Designer 4.0 Software. Primers for a Type IV gamma gliadin gene were described by Piston et al. (2006) and primers for 18S rRNA and nsLTP were described by Altenbach et al. (in press). All primers were synthesized by Operon (Alameda, CA). Amplification efficiencies for each primer pair were calculated from standard curves generated in three independent experiments using the iCycler software. Each standard curve had a minimum of five points and *R* values greater than 0.99. Average amplification efficiencies for omega and gamma gliadin primers are reported in this manuscript. Efficiencies

for the 18S rRNA and nsLTP QF55/QR55 primers were reported in Altenbach et al. (in press).

For quantification of transcripts, PCR reactions were carried out in triplicate using cDNA from the equivalent of 10 ng RNA. The 18S rRNA served as a reference RNA and was amplified in parallel with target genes in all experiments. The Ct value was determined for each RNA sample and each primer pair. Mean normalized expression and standard errors were determined for each target gene relative to the 18S reference gene using the Q-Genie Core Module (available at <http://www.gene-quantification.de/download.html#qgene>). Amplification efficiencies of each primer pair were taken into account for all calculations using Equation #3 of Muller et al. (2002).

3. Results

3.1. Omega gliadin genes expressed in Butte 86 developing grains

More than 20% of the 580,155 ESTs represented in TaGI Version 10.0 were obtained from endosperm or grain tissue, including 3626 sequences from the bread wheat Butte 86. Of these, 15 ESTs from Butte 86 encode omega gliadin proteins and these fall into five gene assemblies (Table 1). Three of the gene assemblies encode proteins with PQQPFP as the predominant repetitive motif (Table 1, Fig. 1). These gene assemblies are most similar to an omega gliadin genomic clone isolated by Hsia and Anderson (2001) from the cultivar Cheyenne (Accession

Number AF280605). Only one of the three assemblies, TC262770, contains a full-length coding sequence. The protein encoded by TC262770 is ~43,800 and the deduced amino acid sequence shows a typical signal peptide followed by the sequence ARQ. The N-terminal sequence differs from the protein encoded by the gene reported by Hsia and Anderson (2001) that begins with ARE, but is identical to an N-terminal sequence obtained by DuPont et al. (2004) for an omega gliadin encoded on the 1A chromosome of Butte 86. The deduced amino acid sequence from TC262770 matches sequences of two CNBr peptides from the D₂ protein described in a glutenin fraction of Chinese Spring by Masci et al. (1999). The sequence also matches 29 of 30 residues from a chymotryptic peptide from the D₂ protein, differing only in the substitution of a proline for a serine. Interestingly, two of the three assemblies, TC262770 and TC263294, encode proteins that contain at least one cysteine residue, although the position of the cysteine residue is different in the two proteins (Fig. 1). In both TC262770 and TC263294, the substitution of a T with a G in the DNA sequence resulted in the replacement of a phenylalanine residue with a cysteine residue. Of the 73 ESTs that fall into TC262770, 13 cover the portion of the coding region with the cysteine residue. However, only seven of the 13 ESTs, including two from Butte 86, contain the substitution. In comparison, TC263294 is composed of 11 ESTs, and neither of the two ESTs from Butte 86 include the portion of the coding region containing the cysteine. In fact, the codon for cysteine is found in only one EST. A search of NCBI EST

Table 1
Gene assemblies for omega gliadins from TaGI Version 10.0 that contain ESTs from Butte 86

Gene assembly Accession Number	Size of gene assembly (bp)	Total # ESTs in gene assembly	Butte 86 EST Accession Numbers	Size of EST (bp)	# Cysteines in encoded protein	Repetitive motifs in encoded protein
TC262770	1356	73	BQ804665 BQ804424 ^a BQ805896 ^a	706 633 380 ^b	1	PQQPFP
TC262980	872	120	BQ804207 BQ807007 BQ806801 BQ839049 BQ806762	773 ^b 691 ^b 684 ^b 611 ^b 586 ^b	0	PQQPFP
TC263294	1012	11	BQ838934 BQ804887 ^c	633 ^b 486	1	PQQPFP
TC250032	1078	18	BQ806240 BQ806730 ^d BQ805583	792 783 536 ^b	0	FPQQQ QQIPQQ
TC250042	1139	175	BQ805830 BQ806362	656 464 ^b	0	FPQQQ QQIPQQ

^aCoding sequence includes a cysteine residue.

^bAlso contains poly A of variable length.

^cContains likely rearrangement.

^dContains gamma gliadin sequence at 5' end.

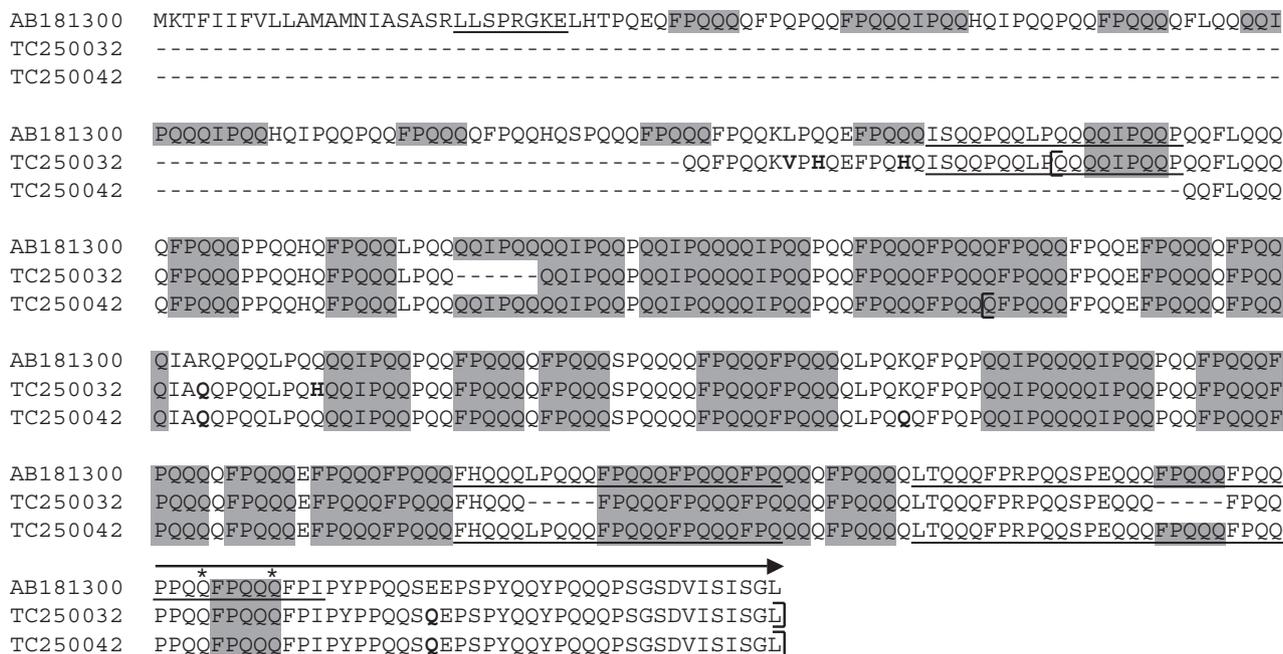


Fig. 2. Comparison of deduced amino acid sequences of omega gliadins encoded by AB181300 (Matsuo et al., 2005) and gene assemblies TC250032 and TC250042 from TaGI version 10.0. Portions of gene assemblies represented by Butte 86 ESTs are shown in brackets. Amino acids that differ from those encoded by AB181300 are shown in bold. Portions of the deduced amino acid sequences of AB181300 and TC250042 that match peptides from the 1B1-omega gliadins from Butte 86 (DuPont et al., 2000) are underlined. Amino acids within those peptides that were not identified in their study are indicated by *. FPQQQ and QQIPQQ motifs within each sequence are shaded in gray. The arrow indicates the portion of the coding region that was amplified in qRT-PCR experiments.

protein encoded by BQ804665 differs from that encoded by the TC262770 consensus sequence in only 2 amino acids, one of which was the proline in the chymotryptic peptide identified by Masci et al. (1999) while the protein encoded by BQ804424 differs from the TC262770 consensus sequence by deletions of 5 and 80 amino acids, an insertion of 3 amino acids and two substitutions. Both BQ804665 and BQ804424 contain the cysteine codon. The protein encoded by BQ805896 has an 8 amino acid insertion, a 5 amino acid deletion and four substitutions when compared to that encoded by TC262770.

BQ804207 falls into a different gene assembly, TC262980, and the protein encoded by BQ804207 differs from that encoded by TC262770 by 15 substitutions and an 8 amino acid insertion (Fig. 1). Four other Butte 86 ESTs in this gene assembly are somewhat shorter, but identical in sequence to BQ804207 (Table 1).

BQ838934 falls into the third gene assembly, TC263294. The protein encoded by BQ838934 differs by 14 amino acids from that encoded by TC262770 and also contains a deletion of more than 100 amino acids as well as a 3 amino acid deletion (Fig. 1). BQ804887 also falls into TC263294, but these sequences were most likely rearranged since the 5' end of the EST encodes a portion of the protein that is found near the carboxyl end while the 3' end of the EST encodes a region in the middle of the protein.

Two other gene assemblies encode proteins with FPQQQ and QQIPQQ as their repetitive motifs. Although these sequences are not annotated in TaGI as omega gliadins, the

sequences are very similar to AB181300 reported by Matsuo et al. (2005) for the omega-5 gliadin (Fig. 2). Both gene assemblies are missing coding sequences at the 5' end. The deduced amino acid sequence from TC250042 matches three peptides of 18, 23 and 38 amino acids from the internal regions of the 1B1-omega gliadin from Butte 86 described by DuPont et al. (2000) (Fig. 2). The partial protein sequences deduced from these gene assemblies contain no cysteine. Two of the three Butte 86 ESTs that fall into TC250032 are identical to the consensus sequence, but one EST, BQ806730, has a portion of a gamma gliadin sequence at the 5' end. A search of NCBI EST databases with a sequence at the junction of the gamma and omega gliadin coding sequences failed to uncover any similar sequences, suggesting that this EST represents a cloning artifact.

TC250042, TC262980 and TC262770 are composed of 175, 120 and 73 ESTs in TaGI, respectively, and thus may represent the predominant omega gliadin sequences (Table 1). For Butte 86, the largest number of ESTs fell into TC262980.

3.2. Primer selection for qRT-PCR

Primers were designed that could be used to quantify the levels of omega gliadin transcripts in developing grains (Table 2). Proteins encoded by TC262770, TC262980 and TC263294 contain the PQQFPF motif 15, 10 and 7 times, respectively (Fig. 1) while proteins encoded by TC250032

Table 2

Primer sequences for quantitative RT-PCR, amplification efficiencies of primer pairs, and sizes and melting temperatures of amplification products

Gene name	Contig #	Primer name	Primer sequence	PCR efficiency (%)	PCR product size (bp)	Melt temperature (°C)
Omega gliadin	TC262770	QF18	AAGGCAAGCAAGCAGTAG	104.3	141	85
		QR18	GATTGTTGAGGTGATTGTAGC			
Omega gliadin	TC250032,	QF21	CAACCACCACAACAATTC	100.4	153	83.5
	TC250042	QR21	TTACATCTCTTCATTTTCATAGG			
Gamma gliadin	TC249992,	QF69	GATCCTGCGGCCACTATTTTCAGCTC	94.6	134	85
	TC250027	QR69	CAGGTGGCACATACACGTTGCACAT			

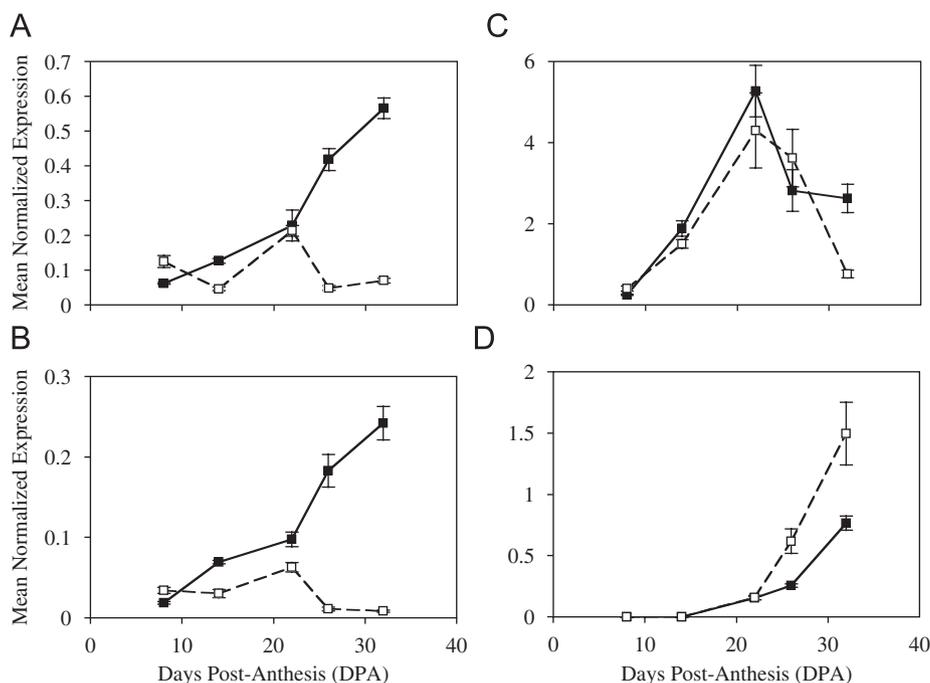


Fig. 3. Accumulation of transcripts in developing wheat grains produced in the presence (solid lines) or absence (dashed lines) of post-anthesis NPK. Transcript levels were assessed by qRT-PCR using primers specific for omega gliadins TC262770 (A), TC250032 (B), a Type IV gamma gliadin (C), or nsLTP (D).

and TC250042 contain FPQQQ 21 and 18 times and QQIPQQ 7 and 3 times, respectively (Fig. 2). The repetitiveness of the sequences precludes the use of primers in most of the coding regions. Thus, primers QF18 and QR18 were selected to amplify a 141 bp region that corresponds to the 5' untranslated region and the N-terminal coding sequences of TC262770 (Fig. 1). Primers QF21 and QR21 were selected to amplify a 153 bp region that spans the 3' end of the coding sequence and a portion of the 3' untranslated region of TC250042 that is conserved in TC250032 (Fig. 2). Primers described by Piston et al. (2006) for a group IV gamma gliadin gene also were synthesized. QF69 and QR69 amplify a 134 bp sequence of gene assemblies TC249992, which includes 19 ESTs from Butte 86, and TC250027, which does not include any Butte 86 ESTs. Amplification of cDNA derived from Butte 86 developing grains yielded single peaks with QF18/QR18, QF21/QR21 and QF69/QR69 in melting curve analyses

with melting temperatures of 85, 83.5 and 85 °C, respectively (Table 2). Analyses of amplification products by gel electrophoresis on 3% MetaPhor agarose gels verified that the single bands were of the expected sizes.

3.3. Accumulation of transcripts during grain development under different NPK regimens

Omega gliadin transcript accumulation was assessed by qRT-PCR in developing grains produced under a 24/17 °C regimen with or without post-anthesis NPK. In the experiment shown in Fig. 3, average Ct values for all samples amplified with QF18/QR18 and QF21/QR21 were less than 20.2 and 22.7, respectively. Under both fertilizer regimens, transcripts corresponding to TC262770 and TC250032 were detected by 8 DPA and the transcript profiles for the two genes were very similar (Fig. 3A, B). However, the profiles of transcript accumulation were

markedly different under the two fertilizer regimens. In the absence of post-anthesis NPK, transcripts reached maximum levels at 22 DPA and then declined at 26 and 32 DPA. When plants were supplied with post-anthesis NPK, transcript levels increased throughout grain development and maximum levels were reached at 32 DPA, the last time point examined. Maximum levels of transcripts were 2.7- and 3.8-fold higher when grains were supplied with NPK, but this occurred at 32 DPA with NPK and 22 DPA in the absence of NPK. By the 32 DPA time point, differences in the levels of transcripts under the two regimens were even more pronounced, 8.1- and 13-fold higher with NPK. Accumulation profiles of transcripts corresponding to a Type IV gamma gliadin were assessed in the same growth experiment (Fig. 3C). In these analyses, average Ct values for all samples were less than 18.1. Gamma gliadin transcripts were detected at 8 DPA, reached maximum levels at 22 DPA and declined in amount at 26 and 32 DPA. Transcript profiles and maximum levels of tran-

scripts were similar under both NPK regimens. Because transcript levels for both omega and gamma gliadins were low in the absence of NPK at the 32 DPA time point, the same samples were amplified with primers specific for a gene known to be expressed late in grain development (Altenbach and Kothari, 2004; Altenbach et al., in press). Transcript profiles for a non-specific lipid transfer protein (nsLTP) were notably different from profiles of the omega and gamma gliadins and transcript levels were about 2-fold higher at the final time point in samples produced in the absence of NPK than in those produced with post-anthesis NPK (Fig. 3D).

Similar transcript profiles were generated from endosperm tissue collected from a separate growth experiment. In the experiment shown in Fig. 4, average Ct values for all samples amplified with QF18/QR18 and QF21/QR21 were less than 18.8 and 23.3, respectively. Transcript levels reached maximum levels at 21 DPA in the absence of post-anthesis NPK and at 31 DPA when plants were supplied with NPK during grain development. Maximum levels were 6.3- and 3.2-fold higher for TC262770 and TC250032 transcripts, respectively. Differences in transcript levels were greatest at 31 DPA for both of the omega gliadins. Again, transcript levels of the nsLTP were somewhat higher late in grain development in the absence of NPK than in kernels supplied with NPK (Fig. 4), confirming that the observed differences in the omega gliadin transcript levels were not due to problems with RNA preparations at the later time points.

4. Discussion

New insight into the family of genes encoding omega gliadin proteins in the bread wheat Butte 86 was gained by examining EST databases. This work complements previous studies in which the complexity of the omega gliadin protein fractions from Butte 86 was characterized by MALDI-TOF and N-terminal sequencing. DuPont et al. (2000, 2004) demonstrated that Butte 86 kernels accumulate six chromosome 1A-encoded omega gliadins ranging in mass from 34,523 to 39,447 and beginning with ARQ, RQL or KEL and two chromosome 1D-encoded omega gliadins with masses of 42,700 and 41,923 and N-terminal sequences of ARE and KEL. Ten of the 15 ESTs from Butte 86 represent either 1A- or 1D-encoded omega gliadins. Although these fell into three gene assemblies, deletions and insertions observed in the repetitive regions of many of these ESTs suggest that the sequences are derived from different genes or that some were altered during cloning. The 1B-encoded omega gliadin fractions from Butte 86 were even more complex and consisted of at least 10 proteins that range in size from 48,900 to 51,500 and begin with minor variations of SRL (DuPont et al. 2004). Only five ESTs representing two of the 1B-encoded omega gliadin proteins from Butte 86 were found in the database and these were similar to a genomic sequence amplified from the bread wheat Norin 61 (Matsuo et al.,

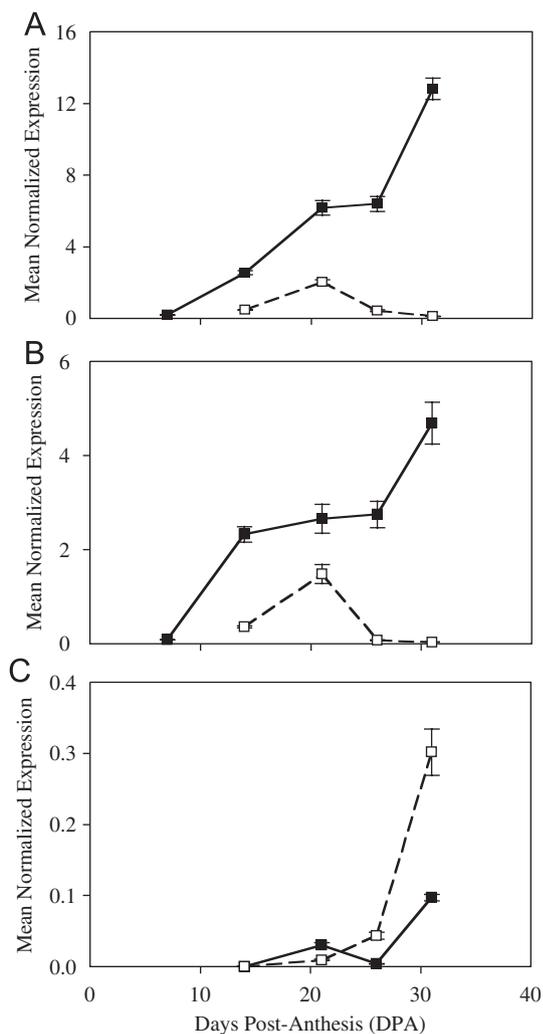


Fig. 4. Accumulation of transcripts in developing endosperm from wheat grains produced in the presence (solid lines) or absence (dashed lines) of post-anthesis NPK. Transcript levels were assessed by qRT-PCR using primers specific for omega gliadins TC262770 (A), TC250032 (B), or nsLTP (C).

2005). Both chromosome 1B-encoded omega gliadin sequences from Butte 86 contain epitopes found to bind IgE from patients with symptoms of the life-threatening food allergy WDEIA (Matsuo et al., 2004, 2005). Within TC250032 and TC250042, QQFPQQQ was found 18 and 19 times, QQLPQQQ was found 2 and 4 times and QQIPQQQ was found 2 and 3 times, respectively. The epitopes QQSPQQQ, QQYPQQQ, PYPP, QSPEQQQ, YPPYPQQ, QQPPQQ and QQFHQQQ also were identified in both sequences. These epitopes were specific to the 1B-encoded omega gliadins and were not found in omega gliadins encoded by ESTs within TC262770, TC262980 and TC263294.

The presence of a single cysteine residue in the proteins encoded by TC262770 and TC263294 suggests that these proteins may be incorporated into the glutenin polymer whereas proteins encoded by TC262980, TC250032 and TC250042 are likely to be present as monomers. As a result, these proteins may play different roles in flour functionality. The expression of omega gliadin proteins containing a single cysteine residue in developing kernels is of particular significance since these proteins could have negative effects on glutenin polymer size by serving as chain terminators (Masci et al., 1993). It is interesting that portions of TC262770 matched peptides identified by Masci et al. (1999) in a protein purified from a polymer fraction of flour from the variety Chinese Spring. Furthermore, an omega gliadin protein referred to as 1dS has been found in the glutenin fraction of Butte 86 flour (DuPont et al., 2005). The fact that only 7 of 13 ESTs within TC262770 contained the cysteine codon suggests that a similar protein also resides in the monomeric fractions of flour from some wheat varieties and supports the hypothesis that the single cysteine is the result of a single base mutation. Previously, efforts by Masci et al. (1999) to determine the location of the cysteine were unsuccessful, largely because it is difficult to obtain internal sequence information since these proteins are not readily cleaved with proteases. The data presented here suggests that the single cysteine residue is located in the carboxyl-half of the protein, more than 60 amino acids beyond the single methionine residue that was cleaved by CNBr in the study by Masci et al. (1999). The single cysteine in the protein encoded by TC263294 is located more than 120 amino acids upstream from the cysteine in TC262770. However, since the cysteine codon was found in only one EST from a variety other than Butte 86, further evidence is needed to support this finding. Gianibelli et al. (2002) reported the presence of cysteine in proteins similar to chromosome 1B-encoded omega gliadins from several wheat varieties. However, none of the ESTs for 1B-encoded omega gliadins identified from Butte 86 contained codons for cysteine.

Although the different types of omega gliadins may play different roles in flour functionality and allergenicity, transcript profiles of TC262770 and TC250032 were remarkably similar. Responses of the omega gliadin genes

to NPK also were similar to each other but notably different from that of a major gamma gliadin. In controlled growth experiments, the application of NPK during grain development resulted in nearly a doubling of protein content in Butte 86 kernels. Protein accumulated between 10 and 36 DPA at a maximum rate of 0.28 mg per day in the presence of NPK. In the absence of NPK, the rate was significantly less, 0.17 mg per day and protein accumulation leveled off after about 24 DPA (Altenbach et al., 2003). The increase in protein content was primarily due to increases in the amount of the major gluten proteins in the grain. While both gliadins and glutenins increased in amount with added NPK, the omega gliadins and the HMW-GS increased more than the other gluten protein types, with the end result that the protein composition of the flour was altered (DuPont et al., 2006a, b). The data demonstrate that the 3.5- to 5-fold increase observed in the rate of accumulation for the omega gliadins in Butte 86 kernels in the presence of NPK results from an increase in the steady state levels of transcripts and that regulation of omega gliadins by NPK occurs at the level of transcript accumulation. These data are consistent with and expand upon previous observations using hybridization analysis with a probe based on the omega gliadin sequence of Hsia and Anderson (2001) (DuPont et al., 2006b).

It is likely that there are differences in the regulatory regions of the omega and gamma gliadin genes that account for the observed differences in response to NPK. However, the gamma gliadins also represent a complex family of closely related genes and proteomic analyses in Butte 86 have shown that individual gamma gliadins respond differentially to NPK (DuPont et al., 2006b). Thus, it will be important to survey the response of different gamma gliadin genes to NPK before selecting gene sequences for promoter comparisons. Piston et al. (2006) recently grouped the sequences of nine new gamma gliadin cDNAs with other genes reported in NCBI into four different groups. The timing of gamma gliadin transcript accumulation in Butte 86 was similar to that of three of the four groups in the bread wheat cultivars 'Perico' and 'Anza'. However, the effects of NPK were not assessed nor was the fertilizer regimen reported in their experiments.

The application of post-anthesis NPK can make a substantial contribution to grain protein content under moderate temperature regimens and thereby improve one important component of flour quality. However, NPK also may have negative consequences for flour quality since it may increase the expression of genes that encode omega gliadins that are either allergenic or likely to decrease the size of the glutenin polymer. Thus far, the omega gliadins have received less attention than some of the other gluten proteins, in part because the importance of the HMW-GS and LMW-GS in flour quality has been well documented, but also because the omega gliadins have been difficult to study at the gene level. Clearly, further work is warranted on the characterization of omega gliadin genes and on the

roles of these proteins in both flour quality and allergenicity. Characterization of the regulatory regions of these genes also is important since these may prove useful in transgenic strategies to alter the levels of transcripts for other endosperm proteins in response to NPK.

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References

- Altenbach, S.B., 1998. Quantification of individual low-molecular-weight glutenin subunit transcripts in developing wheat grains by competitive RT-PCR. *Theoretical and Applied Genetics* 97, 413–421.
- Altenbach, S.B., Kothari, K.M., 2004. Transcript profiles of genes expressed in endosperm tissue are altered by high temperature during wheat grain development. *Journal of Cereal Science* 40, 115–126.
- Altenbach, S.B., Kothari, K.M., Lieu, D., 2002. Environmental conditions during wheat grain development alter temporal regulation of major gluten protein genes. *Cereal Chemistry* 79, 279–285.
- Altenbach, S.B., DuPont, F.M., Kothari, K.M., Chan, R., Johnson, E.L., Lieu, D., 2003. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. *Journal of Cereal Science* 37, 9–20.
- Battais, F., Courcoux, P., Popineau, Y., Kanny, G., Moneret-Vautrin, D.A., Denery-Papini, S., 2005. Food allergy to wheat: differences in immunoglobulin E-binding proteins as a function of age or symptoms. *Journal of Cereal Science* 42, 109–117.
- Daniel, C., Triboui, E., 2000. Effects of temperature and nitrogen nutrition on the grain compositions of winter wheat: effects on gliadin content and composition. *Journal of Cereal Science* 32, 45–56.
- DuPont, F.M., Vensel, W.H., Chan, R., Kasarda, D.D., 2000. Characterization of the 1B-type ω -gliadins from *Triticum aestivum* cultivar Butte. *Cereal Chemistry* 77, 607–614.
- DuPont, F.M., Vensel, W., Encarnacao, T., Chan, R., Kasarda, D.D., 2004. Similarities of omega gliadins from *Triticum urartu* to those encoded on chromosome 1A of hexaploid wheat and evidence for their post-translational processing. *Theoretical and Applied Genetics* 108, 1299–1308.
- DuPont, F.M., Chan, R., Lopez, R., Vensel, W.H., 2005. Sequential extraction and quantitative recovery of gliadins, glutenins, and other proteins from small samples of wheat flour. *Journal of Agricultural and Food Chemistry* 53, 1575–1584.
- DuPont, F.M., Hurkman, W.J., Vensel, W.H., Chan, R., Lopez, R., Tanaka, C.K., Altenbach, S.B., 2006a. Differential accumulation of sulfur-rich and sulfur-poor wheat flour proteins is affected by temperature and mineral nutrition during grain development. *Journal of Cereal Science* 44, 101–112.
- DuPont, F.M., Hurkman, W.J., Vensel, W.H., Tanaka, C., Kothari, K.M., Chung, O.K., Altenbach, S.B., 2006b. Protein accumulation and composition in wheat grains: effects of mineral nutrients and high temperature. *European Journal of Agronomy* 25, 96–107.
- Gianibelli, M.C., Masci, S., Larroque, O.R., Lafiandra, D., MacRitchie, F., 2002. Biochemical characterization of a novel polymeric protein subunit from bread wheat (*Triticum aestivum* L.). *Journal of Cereal Science* 35, 265–276.
- Hsia, C.C., Anderson, O.D., 2001. Isolation and characterization of wheat ω -gliadin genes. *Theoretical and Applied Genetics* 103, 37–44.
- Kasarda, D.D., Autran, J.-C., Lew, E.J.-L., Nimmo, C.C., Shewry, P.R., 1983. N-terminal amino acid sequences of ω -gliadins and ω -secalins. Implications for the evolution of prolamin genes. *Biochimica et Biophysica Acta* 747, 138–150.
- Masci, S., Lafiandra, D., Porceddu, E., Lew, E.J.L., Tao, H.P., Kasarda, D.D., 1993. D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. *Cereal Chemistry* 70, 581–585.
- Masci, S., Egorov, T.A., Ronchi, C., Kuzmicky, D.D., Kasarda, D.D., Lafiandra, D., 1999. Evidence for the presence of only one cysteine residue in the D-type low molecular weight subunits of wheat glutenin. *Journal of Cereal Science* 29, 17–25.
- Matsuo, H., Morita, E., Tatham, A.S., Morimoto, K., Horikawa, T., Osuna, H., Ikezawa, Z., Kaneko, S., Kohno, K., Dekio, S., 2004. Identification of the IgE-binding epitope in ω -5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *Journal of Biological Chemistry* 279, 12135–12140.
- Matsuo, H., Kohno, K., Morita, E., 2005. Molecular cloning, recombinant expression and IgE-binding epitope of ω -5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *FEBS Journal* 272, 4431–4438.
- Muccilli, V., Cunsolo, V., Saletti, R., Foti, S., Masci, S., Lafiandra, D., 2005. Characterization of B- and C-type low molecular weight glutenin subunits by electrospray ionization mass spectrometry and matrix-assisted laser desorption/ionization mass spectrometry. *Proteomics* 5, 719–728.
- Muller, P.Y., Janovjak, H., Miserez, A.R., Dobbie, Z., 2002. Processing of gene expression data generated by quantitative real-time RT-PCR. *BioTechniques* 32, 1372–1379.
- Palosuo, K., Varjonen, E., Kekki, O.-M., Klemola, T., Kalkkinen, N., Alenius, H., Reunala, T., 2001. Wheat ω -5 gliadin is a major allergen in children with immediate allergy to ingested wheat. *Journal of Allergy and Clinical Immunology* 108, 634–638.
- Piston, F., Dorado, G., Martin, A., Barro, F., 2006. Cloning of nine γ -gliadin mRNAs (cDNAs) from wheat and the molecular characterization of comparative transcript levels of γ -gliadin subclasses. *Journal of Cereal Science* 43, 120–128.
- Wieser, H., Seilmeier, W., 1998. The influence of nitrogen fertilisation on quantities and proportions of different protein types in wheat flour. *Journal of the Science of Food and Agriculture* 76, 49–55.
- Wieser, H., Gutser, R., Von Tucher, S., 2004. Influence of sulphur fertilisation on quantities and proportions of gluten protein types in wheat flour. *Journal of Cereal Science* 40, 239–244.
- Wrigley, C.W., Du Cros, D.L., Fullington, J.G., Kasarda, D.D., 1984. Changes in polypeptide composition and grain quality due to sulfur deficiency in wheat. *Journal of Cereal Science* 2, 15–24.