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Biosynthesis of Gliadins and Glutenin Subunits During Grain Development Under Different Environmental Conditions

SUSAN B. ALTENBACH, FRANCES M. DUPONT, WILLIAM J. HURKMAN, AND WILLIAM H. VENSEL
USDA-ARS Western Regional Research Center, 800 Buchanan Street,
Albany, California 94710 USA

ABSTRACT

The α-, γ- and ω-gliadins and LMW- and HMW-glutenin subunits, the major storage proteins in wheat endosperm, confer elasticity and extensibility properties essential for the functionality of wheat flour. These proteins are encoded by genes with highly repetitive sequences that are members of large, complex gene families. Gluten protein transcripts begin to accumulate early in endosperm development and are present throughout the grain filling period. There is a remarkable coordination in the timing of transcript accumulation for genes encoding proteins in the different classes as well as for individual genes within the different families. Changing environmental conditions shift the timing of transcript accumulation, but do not uncouple the coordinate expression of the gluten protein genes. Proteomic analyses of storage protein fractions from developing grains also reveal striking similarities in the profiles for different gluten proteins, particularly with respect to the timing of protein accumulation. Gene expression profiles and proteomic analyses of gluten proteins during grain development under different temperature, water and fertilizer regimens are discussed.

INTRODUCTION

The functional properties of flour are conferred by a complex group of monomeric gliadins and polymeric glutenins that make up the bulk of the protein in the wheat kernel. The gliadins are grouped into 3 different families of proteins, the α-, γ- and ω-gliadins, each of which is encoded by many genes with closely related and highly repetitive sequences. The glutenins consist of two different protein types, the high-molecular-weight glutenin subunits (HMW-GS), encoded by six different genes, and the low-molecular-weight glutenin subunits (LMW-GS), encoded by as many as 30-40 genes. Environmental conditions during grainfill influence the accumulation of protein in the developing wheat kernel and can alter the functional properties of the resulting flour, but the precise effects of environmental factors on the synthesis of the major gliadins and glutenins are not well understood. Quantitative studies of gene expression and protein accumulation under different environmental conditions are challenging because the complexity of the different groups of genes and proteins makes it difficult to distinguish and identify single components. Additionally, levels of gene expression and protein accumulation must be examined within the context of grain development since environmental factors such as temperature can alter the timing of grain development.

MATERIALS AND METHODS

The spring wheat *Triticum aestivum* cv. 'Butte 86' was grown in a climate-controlled greenhouse under a 24°C daytime and 17°C nighttime temperature regimen. Plants were watered by drip irrigation with a dilute solution of Plantex 20-20-20 (0.6g/l) as described in Altenbach et al (2003). Heads were tagged at anthesis. Some of the plants were transferred to a second greenhouse maintained at either 37°C days and 17°C nights or 37°C days and 28°C nights either at anthesis or at defined times after anthesis. Plants in some pots continued to be watered with a dilute fertilizer solution (+ fertilizer) while other pots received only water (- fertilizer) after anthesis. Whole grain or endosperm tissue was collected at frequent intervals during grain development for RNA and protein analysis.

RNA isolation and hybridization analysis with probes specific for α-, γ- and ω- gliadins and LMW-GS and HMW-GS were described in Altenbach et al (2002). Protein extraction and 2-DE methods are described in Hurkman and Tanaka (2004).

RESULTS AND DISCUSSION

Complexity of Gluten Protein Gene Families in 'Butte 86'

A preliminary assessment of the complexity of the α- and γ-gliadin and LMW-GS gene families in the cultivar 'Butte 86' can be obtained by examining wheat Expressed Sequence Tags (EST) databases which contain more than 580,000 sequences. Of the 3,626 ESTs from 'Butte 86' represented in The Institute for Genomic Research (TIGR) Triticum aestivum Gene Index (TaGI) Version 10.0, several hundred currently assemble into contigs for 11 different LMW-GSs, 19 γ-gliadins and 28 α-gliadins. LMW-GS contigs that contain ESTs from 'Butte 86' are shown in Table I. The number of ESTs from 'Butte 86' that make up each contig provides an approximation of the relative abundance of transcript for each gene. The Type V sequence appears to be the most prevalent in this cultivar, while the Type I sequence is represented by only a few ESTs. For the LMW-GS, variations in sequences among the different groups have made it possible to design primers that can distinguish individual genes (Altenbach 1998, Ikeda et al 2002).

TABLE I
Representation of 'Butte 86' ESTs for LMW-GS in TIGR TaGI Version 10.0[a]

Type	Group	N-terminal Sequence	C-terminal Sequence	Contig #	# ESTs in contig	# ESTs from 'Butte 86'
I	1	METSHIPGLEKP	VGTQVGAY	TC249993	37	2
I	2	METSHIPSLEKP	VGTRVGAY	TC250064	132	1
II	3	MENSHIPGLERP	VGTGVGGY	TC252672	25	12
III	5	METSRVPGLEKP	IGTGVGGY	TC234564	168	11
III	5	METSRVPGLEKP	VGTGVGSY	TC264038	324	3
III	5	METSRVPGLEKP	IGTGVGGY	TC264078	28	2
III	5	METSRVPGLEKP	IGTGVGGY	TC265144	111	11
IV	7	METSCISGLERP	VGTGVGAY	TC251241	63	5
IV	8	METSCIPGLERP	VGSRVGAY	TC265009	127	11
V	10	METRCIPGLERP	VGTGVGAY	TC264591	641	23
VI	11	ISQQQQAPPFS	VGIGVGVY	TC234424	58	12

[a] Classification into Types and Groups is according to Ikeda et al (2002) and based on the alignment of N- and C-terminal domains of the deduced amino acid sequences.

The α-gliadin gene family is considerably more complex with 'Butte 86' ESTs represented in 28 contigs. Six of these contigs appear to be highly expressed, containing between 9 and 23 ESTs. The deduced amino acid sequences of the most highly expressed contigs are compared in Fig. 1. The similarities among the sequences coupled with the large numbers of genes make it considerably more difficult to distinguish individual sequences within this family.

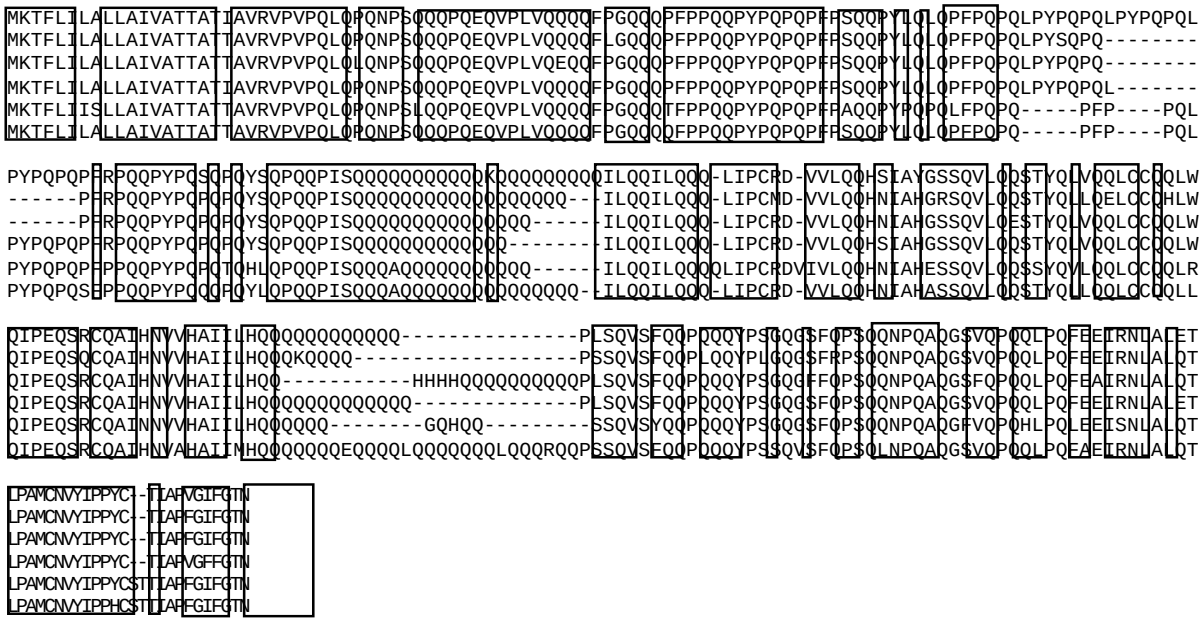

Fig. 1. Comparison of the deduced amino acid sequences of α -gliadin contigs containing large numbers of ESTs from 'Butte 86'. Sequences correspond to 1) TC234455; 2) TC249879; 3) TC264697; 4) TC234369; 5) TC249939; and 6) TC264781 from TIGR TaGI Version 10.0.

Biosynthesis of Gliadins and Glutenin Subunits during Grain Development under Moderate Temperature Regimens

Genes encoding the α-, γ- and ω-gliadins and the LMW-GS and HMW-GS are expressed specifically in endosperm of the developing wheat grain. One of the most striking features of their expression is the coordinate regulation of genes from the different gene families. When detected by hybridization analysis, transcripts for all major gluten protein classes were first detected at the same time, by about 8 days post-anthesis (DPA) under a 24/17^{o} C temperature regimen, and were present through much of grain development, until at least 34 DPA (Fig. 2). Reverse transcriptase-polymerase chain reaction (RT-PCR) with primer pairs specific for each of the five HMW-GS genes expressed in 'Butte 86' demonstrated that the timing of transcript accumulation for all HMW-GS genes was identical (Altenbach et al 2002). Similar experiments using primer pairs for a sampling of LMW-GS genes identified in 'Cheyenne' showed that at least 7 LMW-GS genes also shared the same temporal regulation (Altenbach et al 2002).

Protein is accumulated in developing kernels in a linear fashion from about 10 DPA until about 36 DPA under a 24/17^{o} C temperature regimen (Altenbach et al 2003). The gluten proteins, contained in the KCl-insoluble fraction, encompass the bulk of this protein. When the insoluble protein fraction from developing kernels is analyzed by two-dimensional gel electrophoresis (2-DE), a definite shift to gluten protein accumulation occurs sometime between 9 and 12 DPA under a 24/17^{o}C temperature regimen and proteins in all of the major classes accumulate coordinately (Fig. 3). While the relative amounts of some of the gluten proteins change with time, the overall pattern of proteins accumulated at 12 and 14 DPA is strikingly similar to patterns at later times in development. Interestingly, a predominant protein in the KCl-insoluble fraction, particularly at 9,

12, 14 and 21 DPA, was identified by mass spectrometry as protein disulfide isomerase (PDI). PDI catalyzes the formation of inter- and intra- molecular disulfide bonds and may play a role in the folding of the gluten proteins and the formation of glutenin polymers that are essential for quality. RT-PCR experiments demonstrated that PDI transcript is most abundant from 7-20 DPA under the same conditions (Altenbach et al 2004).

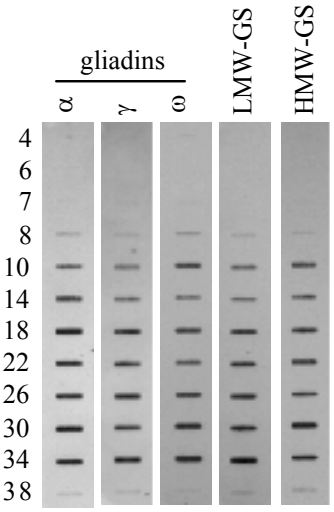

Fig. 2. Accumulation of gluten protein transcripts in developing kernels under a 24/17°C regimen with post-anthesis fertilizer. RNA was prepared from developing kernels at the times shown on the left (DPA) and hybridized to probes specific for the α-, γ- and ω-gliadins and the LMW-GS and HMW-GS.

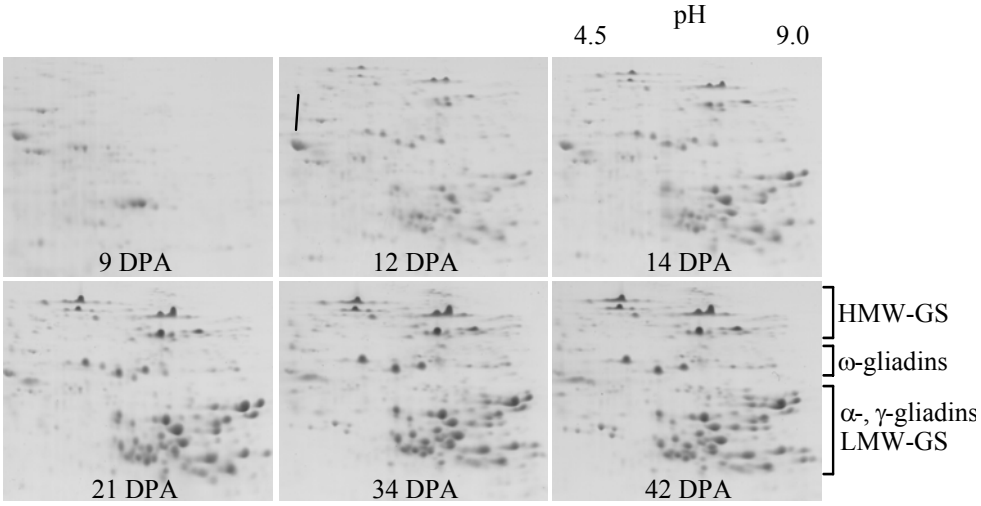

Fig. 3. 2-DE of gluten proteins isolated from kernels at different times during development under a 24/17° C temperature regimen.

Effects of High Temperatures and Fertilizer Levels on Biosynthesis of Gliadins and Glutenin Subunits during Grain Development

High temperature conditions during grain development reduced the time from anthesis to harvest maturity from 44 days, under a 24/17°C regimen, to 35 days under a 37/17°C regimen and 26 days under a 37/28°C regimen (Altenbach et al 2003). The most obvious effect of temperature on the accumulation of gliadin and glutenin subunit transcripts was on the timing of transcript accumulation. Transcripts within the entire major gene families both appeared and disappeared earlier under high temperature conditions (Table II), reflecting differences in the timing of grain development. The coordinate expression of genes within the different families was retained and

little change was observed in the levels of transcripts corresponding to any of the gluten protein gene families. However, small changes in transcript levels for individual genes may not have been detected since hybridization probes measured the accumulation of transcripts corresponding to many genes within each family (Altenbach et al 2002).

TABLE II
Effect of Temperature and Fertilizer Levels on Timing of Protein and Gluten mRNA Accumulation in Developing Kernels

	24/17°C + Fertilizer	**24/17°C – Fertilizer**	**37/17°C + Fertilizer**	**37/17°C – Fertilizer**	**37/28°C + Fertilizer**	**37/28°C – Fertilizer**
Gluten mRNA appears (DPA)	8-10	8-10	7	7	5	5
Gluten mRNA disappears (DPA)	36-38	34-38	30-34	26-30	20-22	20-22
Protein Accumulation Maximum (DPA)	36	24-30	30	24	20-22	20-22

The effects of post-anthesis fertilizer on the accumulation of gluten protein transcripts also were assessed under the three temperature regimens. Under most environmental conditions, the decline in gluten protein mRNA coincided with the end of protein accumulation. However, in the absence of fertilizer under the 24/17° C regimen, gluten protein transcripts were present well after the kernels had ceased accumulating protein, suggesting that post-translational control is important under these conditions (Table II). A comparison of transcript levels for the different classes of gluten proteins at frequent intervals during grain development revealed that levels of transcripts for ω-gliadins were somewhat higher in developing grains supplied with post-anthesis fertilizer under the 24/17°C regimen than in those that received no fertilizer, particularly at later developmental time points (DuPont et al, submitted).

2-DE patterns of gluten protein accumulation were surprisingly similar in grains developing under different environmental conditions. An example of gluten protein patterns at three time points under two temperature regimens is shown in Fig. 4. In this experiment, high temperatures were applied from 10 DPA until maturity. Striking similarities also were observed among the 2-DE patterns of gluten proteins accumulated during grain development in the presence and absence of post-anthesis fertilizer under the 24/17° C and 37/28° C regimens. However, computer analyses of the relative proportions of individual protein spots over the course of grain development have revealed quantitative changes in specific gluten proteins in response to temperature and fertilizer. Most notable were a 2-fold increase in the relative levels of ω-gliadins observed in response to post-anthesis fertilizer as well as small changes in some α-gliadins and a LMW-GS. A decrease in the relative amount of a LMW-GS also was noted in response to high temperatures and is detailed elsewhere (DuPont et al, submitted).

While small changes in the amounts of individual gliadin and LMW-GS proteins are difficult to measure convincingly, it is possible that such changes could impact flour quality, particularly if these proteins play a role in the formation of the gluten polymers. Further identification of proteins that change in response to environment is warranted so that the specific genes associated with a given protein spot can be identified. However, identification of these proteins by mass spectrometry is complicated by the poor fragmentation of many gluten proteins with trypsin and by the lack of sequences corresponding to the entire complement of gluten genes in 'Butte 86' for comparison.

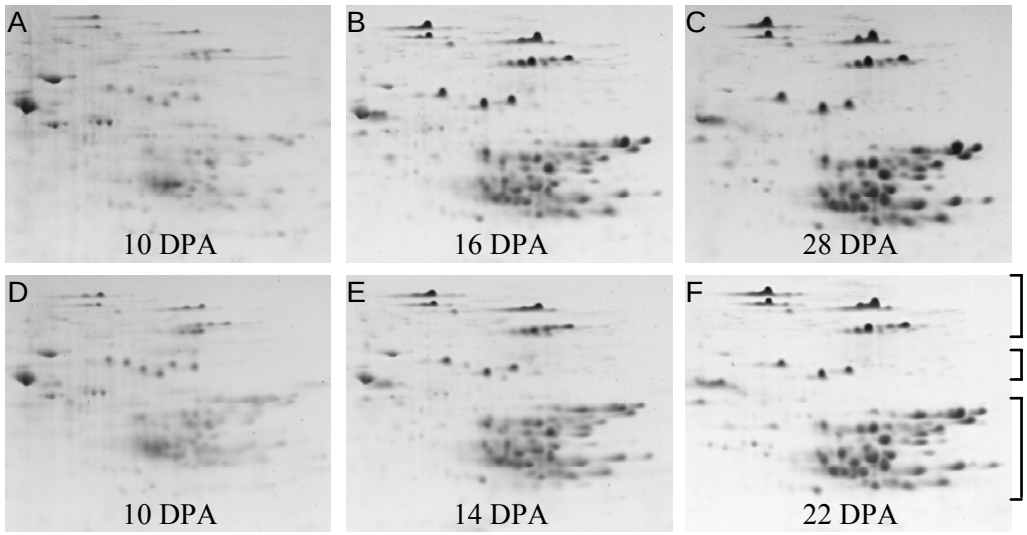

Fig. 4. 2-DE of gluten proteins isolated from endosperm at different times during development from plants grown under a 24/17°C temperature regimen (A-C) or under a 37/28°C temperature regimen from 10 DPA until maturity (D-F).

CONCLUSIONS

The complexity of the different groups of gluten proteins and their genes coupled with the remarkable coordination in the biosynthesis of the gluten proteins makes it challenging to uncover changes that result from exposure to different environmental conditions. Detailed studies of gluten protein accumulation during grain development are necessary to identify relatively small quantitative changes in specific gluten proteins that might influence quality.

LITERATURE CITED

Altenbach, S. B. 1998. Quantification of individual low-molecular-weight glutenin subunit transcripts in developing wheat grains by competitive RT-PCR. Theor. Appl. Genet. 97: 413–421.

Altenbach, S. B., DuPont, F. M., Kothari, K. M., Chan, R., Johnson E. L., and Lieu, D. 2003. Temperature, water and fertilizer influence the timing of key events during grain development in a U.S. spring wheat. J. Cereal Sci. 37: 9-20.

Altenbach, S. B., and Kothari, K. M. 2004. Transcript profiles of genes expressed in endosperm tissue are altered by high temperature during wheat grain development. J. Cereal Sci. 40: 115-126.

Altenbach, S. B., Kothari, K. M., and Lieu, D. 2002. Environmental conditions during wheat grain development alter temporal regulation of major gluten protein genes. Cereal Chem. 79: 279-285.

DuPont, F. M., Hurkman, W.J., Vensel, W.H., Tanaka, C., Kothari, K.M., Chung, O.K. and Altenbach, S.B. Effects of fertilizer and high temperature on protein accumulation in wheat grains. Eur. J. Agron. (Submitted)

Hurkman, W.J. and Tanaka, C. K. 2004. Improved methods for separation of wheat endosperm proteins and analysis by 2 dimensional gel electrophoresis. J. Cereal Sci 40: 295-299.

Ikeda, T.M. Nagamine, T., Fukuoka, H., Yano, H. 2002. Identification of new low-molecular weight glutenin subunit genes in wheat. Theor. Appl. Genet. 104: 680-687.