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Differential accumulation of sulfur-rich and sulfur-poor wheat flour proteins is affected by temperature and mineral nutrition during grain development

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Abstract

Hard red spring wheat (*Triticum aestivum* cv Butte86) was grown under controlled environmental conditions and grain produced under 24/17 °C, 37/17 °C or 37/28 °C day/night regimens with or without post-anthesis N supplied as NPK. Flour proteins were analyzed and quantified by differential fractionation and RP-HPLC, and endosperm proteins were assessed by two-dimensional gel electrophoresis (2-DE). High temperature or NPK during grain fill increased protein percentage and altered the proportions of S-rich and S-poor proteins. Addition of NPK increased protein accumulation per grain under the 24/17 °C but not the 37/28 °C regimen. However, flour protein composition was similar for grain produced with NPK at 24/17 °C or 37/28 °C. 2-DE of gluten proteins during grain development revealed that NPK or high temperature increased the accumulation rate for S-poor proteins more than for S-rich proteins. Flour S content did not indicate S-deficiency, however, and addition of post-anthesis S had no effect on protein composition. Although, high-protein flour from grain produced under the 37/28 °C regimen with or without NPK had loaf volumes comparable to flour produced at 24/17 °C with NPK, mixing tolerance was decreased by the high temperature regimen.

Keywords: Gliadin; Glutenin; Two-dimensional gel electrophoresis; Nitrogen

1. Introduction

Protein content and composition, key determinants of wheat flour breadmaking quality, are influenced by genetics, environment and crop management (DuPont and Altenbach, 2003; Fowler, 2003; Fowler et al., 1990; Gupta et al., 1992; Payne, 1987). Flour protein content is strongly influenced by the supply of N, as well as by environmental factors such as temperature during grainfill. High temperature affects the synthesis of protein and starch differentially, resulting in increased grain protein percentage yet lower grain weight. In contrast, N promotes the synthesis of additional protein per grain (Altenbach et al., 2003; Bhullar and Jenner, 1985; Corbellini et al., 1998; Daniel and Triboi, 2000; Fowler et al., 1990; Zahedi et al., 2004). In field studies it is difficult to determine if environmental influences on flour protein content, composition and quality should be attributed to temperature, *N*, or interactions between the two. Thus there is a need for controlled environment studies that compare the effects of high temperature and *N*.

Flour protein composition is complex, with over one hundred genes encoding the abundant proline- and glutamine-rich prolamins that influence mixing and baking. These proteins include the high molecular weight and low molecular weight glutenin subunits (HMW-GS and LMW-GS) as well as the monomeric α-, γ- and ω-gliadins. The HMW-GS and LMW-GS are linked by disulfide bonds to form the glutenin polymers responsible for the unique viscoelastic properties of wheat flour dough, while the α-, γ- and ω-gliadins contribute to dough extensibility. In addition, some albumins and globulins are relatively abundant although their role in flour quality is not known. One property that distinguishes the different groups of wheat flour storage proteins is S content, which is highest for the albumins and globulins, moderately high for γ-gliadins and

Abbreviations: 2-DE, 2-dimensional gel electrophoresis; DTT, dithiothreitol; HMW-GS, high molecular weight glutenin subunit; LMW-GS, low molecular weight glutenin subunit; MS, mass spectrometry; NIR, near infrared spectrophotometry; RP-HPLC, reverse phase high pressure liquid chromatography; TFA, trifluoroacetic acid.

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LMW-GS, intermediate for α-gliadins, low for the HMW-GS and close to zero for the ω-gliadins (Anderson and Greene, 1997; D'ovidio and Masci, 2004; Gianibelli et al., 2001; Payne, 1987; Tatham and Shewry, 1995; Zhao et al., 1999). Tatham and Shewry (1995) refer to the LMW-GS, γ- and α-gliadins as S-rich and the ω-gliadins as S-poor. Studies of mRNA profiles during grain development revealed that temporal expression of the genes for the gliadins and glutenins is coordinately regulated (Altenbach et al., 2002). In contrast, studies of protein composition reported that applications of N and S influence the proportions of the different protein types (Daniel and Triboi, 2002; Gupta et al., 1992; Timms et al., 1981; Weiser et al., 2004; Wrigley et al., 1984), perhaps through transcriptional, post-transcriptional, translational or post-translational mechanisms.

In previous papers, we reported the effects of post-anthesis temperature and mineral nutrients on grain fill (Altenbach et al., 2002, 2003; DuPont et al., 2006). Grains produced under a 24/17 °C cool days/cool nights temperature regimen were large, with high starch content. Protein per grain doubled when post-anthesis NPK was supplied from anthesis to maturity, but NPK had little effect on the pattern of grain development, rate and duration of grainfill, or rate and duration of starch accumulation. When grains were produced under a 37/28 °C hot days/warm nights temperature regimen, the duration of grain fill was greatly reduced, and post-anthesis NPK had little effect on protein accumulation. Effects of a 37/17 °C hot days/cool nights temperature regimen were intermediate, with moderate reductions of starch with or without NPK, and moderate increases in rate and amount of protein accumulation with added NPK.

In this paper, proteins were separated by differential fractionation, RP-HPLC and 2-DE to quantify the effects of NPK and temperature on albumin/globulin, gliadin and glutenin fractions and on individual flour and endosperm proteins. Relationships to protein S, Cys and Met content and to flour baking quality were analyzed. Effects of the 24/17 °C regimen, where NPK had large effects on protein accumulation, and the 37/28 °C regimen, where NPK had little effect on protein accumulation, were examined in detail to determine effects specific to temperature or NPK. Intermediate treatments at 37/17 °C and half-strength NPK were also analyzed.

2. Experimental

2.1. Plant material and growth conditions

Plants of the US hard red spring wheat *Triticum aestivum* 'Butte86' were grown at 24 °C days, 17 °C nights with drip irrigation (24/17 °C regimen) as described in Altenbach et al. (2003). Grain was produced in the 13 experiments outlined in Table 1. Except for experiment 12, there was one set of pots per treatment. For the different NPK regimens, plants were watered with 0, 1 or 2 emitters (0×, 0.5× and 1× NPK) from anthesis until maturity and hand watered to maintain similar pot weights. For the high temperature regimens, pots were transferred at anthesis to a climate-controlled greenhouse maintained at 37 °C days and 17 or 28 °C nights (37/17 °C and

Table 1
Post-anthesis growing conditions and protein percentages for flour produced under various environmental regimens

Expt		Flour protein percentage[a,b]										
	Temp[c]	24/17	24/17	24/17	24/17	37/17	37/17	37/17	37/28	37/28	37/28	37/28
	NPK[d]	0× (%)	0.5× (%)	1× (%)	1×+S (%)	0× (%)	0.5× (%)	1× (%)	0× (%)	0.5× (%)	1× (%)	1×+S (%)
1[a]			13.9+0.1				14.4+0.7					
2[a]		9.4+0.2	14.5+0.1	15.4+0.2								
3[a]		9.4+0.2				10.6+0.1						
4[a]		6.9+0.5		16.4+0.1		9.4+0.5		16.5+0.2				
5[a]				14.3+0.3				14.6+0.1				
6[b]				17.3							18.2	
7[b]				17.4							16.7	
8[b]		8.8							14.9			
9[b,e]			14.0							18.8		
10[b,e]			12.5							18.7		
11[a]				14.3+0.1	14.6+0.3						18.7+0.0	18.6+0.0
12[a,f]		7.3+0.4		14.3+1.0								
13[a]		7.8+0.01		14.2+0.0					16.3+0.0		18.2+0.1	
Avg[g]		8.3+1.0	13.7+0.7	15.5+1.4	14.6	10.0+0.6	14.4	15.6+1.3	15.6+0.7	18.8+0.1	18.0+0.8	18.6

All plants were grown under the 24/17 °C regimen with 1× fertilizer until anthesis, except as indicated.
[a] Flour protein percentage + STD was determined by N analysis in triplicate of the single batch of flour.
[b] Flour protein percentage was determined by NIR with a standard error of prediction of 0.13% of the single batch of flour.
[c] Temperature regimen from anthesis to maturity.
[d] NPK fertilizer regimen from anthesis to maturity.
[e] Pots were supplied with 0.5× NPK from sowing until maturity.
[f] Experiment 12 was divided into three separate replicates at 0× and three at 1× NPK, providing a total of six separate flour samples.
[g] Average plus STD.