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Effect of Environmental Stress during Grain Filling on the Soluble Proteome of Wheat (*Triticum aestivum*) Dough Liquor

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The influence of genotype and environment on a soluble wheat dough liquor proteome was studied for four cultivars grown under field conditions and under hot/dry and cool/wet regimes by two-dimensional electrophoresis followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or quadrupole time-of-flight mass spectrometry. Although the four cultivars had similar patterns, differences in the relative abundances of some components were observed. Similarly, some differences were observed between the control samples and the samples grown under cool/wet and hot/dry conditions. These included differences in the abundances of storage proteins belonging to the 7S globulin (vicilin-like) and α-globulin families and of protective proteins including members of the serpin, described as allergens, and chitinase families. A number of novel annotations were made as compared to previous work on the dough liquor of cv. Hereward, including two 19 kDa α-globulins, precursors of endochitinases A and C, and several polypeptides belonging to the 7S globulin (vicilin-like) family.

KEYWORDS: Dough; environmental stress; soluble proteins; mass spectrometry; proteome; proteomics; wheat

INTRODUCTION

Wheat (*Triticum* spp.) is one of the world's most consumed crops, but it can trigger an allergic reaction (*1*). Abiotic stress such as drought, salinity, extreme temperature, chemical toxicity, and oxidative stress may adversely affect crop growth and productivity (*2*). Previous studies also have shown that the temperature and availability of water during grain filling can affect the dough and end use properties of breadmaking wheats (*3*). However, different cultivars show different levels of stability to environmental conditions and stress thresholds, suggesting that these characteristics may be under genetic control and hence amenable to selection in breeding programs (*4, 5*). However, progress in improving the environmental stability of wheat has been slow, probably because of its genetic complexity. The hexaploid wheat genome is one of the largest among crop species (16.7 billion bp) (*6*), and it is probable that genes will be regulated by both single and multiple types of abiotic stress. It is therefore important to understand the genetic control and molecular mechanisms through which plants respond to abiotic stress to facilitate the development of breeding and transgenic strategies to improve stress tolerance in crops.

Proteome analysis by two-dimensional gel electrophoresis and mass spectrometry is a powerful technique to identify changes in the types and amounts of proteins in grain that has been subjected to environmental stress during development (*6*). Using this approach, Majoul et al. (*7, 8*) observed decreases in several gliadins and in enzymes involved in starch biosynthesis when wheat plants were subjected to 34 °C soon after anthesis. A high temperature regimen (37/28 °C day/night) also influences the timing of transcript accumulation, and so several putative defense proteins such as peroxidase and serpin are detected earlier in wheat kernel development (*9*). Yan et al. (*10*) identified 31 down-regulated protein spots and 65 up-regulated spots when rice seedlings were treated at 6 °C for 6 or 24 h. However, such a severe temperature stress is not relevant to more temperate climates such as in the U.K. and Western Europe. We therefore studied the impact of stress levels that are frequently experienced in temperate climates during grain filling, specifically comparing cool/wet with hot/dry conditions to understand the molecular basis for environmental impacts on grain development and end use quality. This included studies of cell wall composition using a novel FT-IR imaging approach (*11*).

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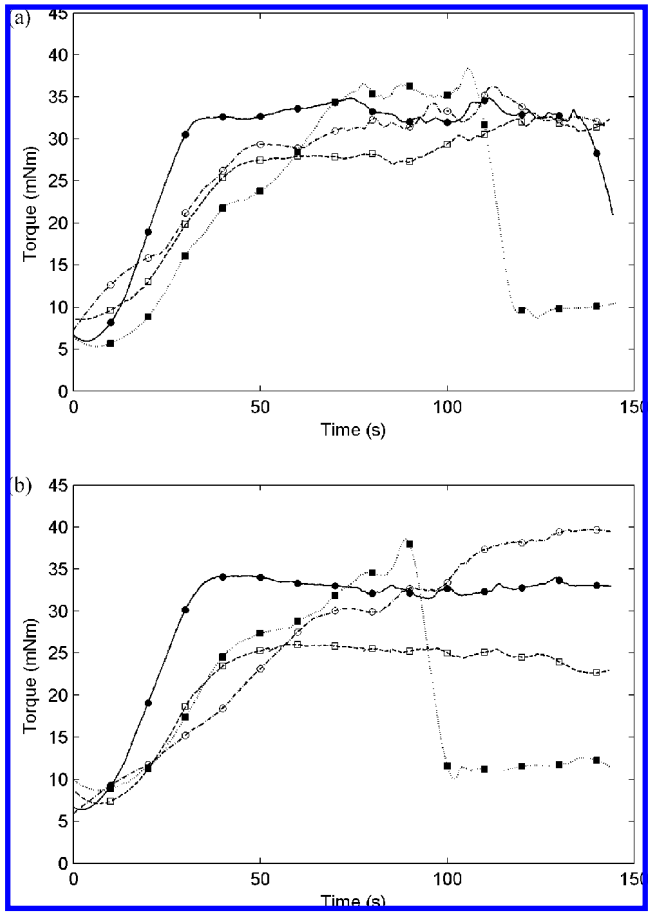

Figure 1. Mixing profile for dough prepared under set conditions (see Materials and Methods) showing torque development during dough preparation of wheat cultivars grown under dry/hot (**a**) or field-grown (**b**) conditions. Cultivars are as follows: ■, Beaver; ●, Spark; ○, Rialto; and □, Soissons.

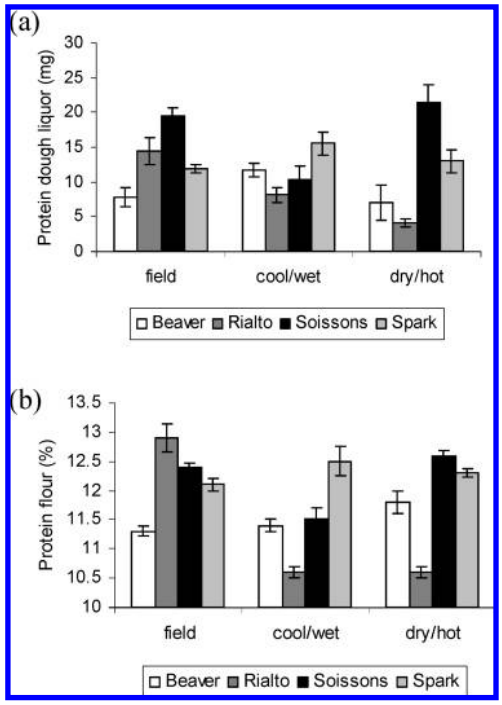

Figure 2. Total protein content of dough liquor (mg) (**a**) and wheat flour (%) (**b**).

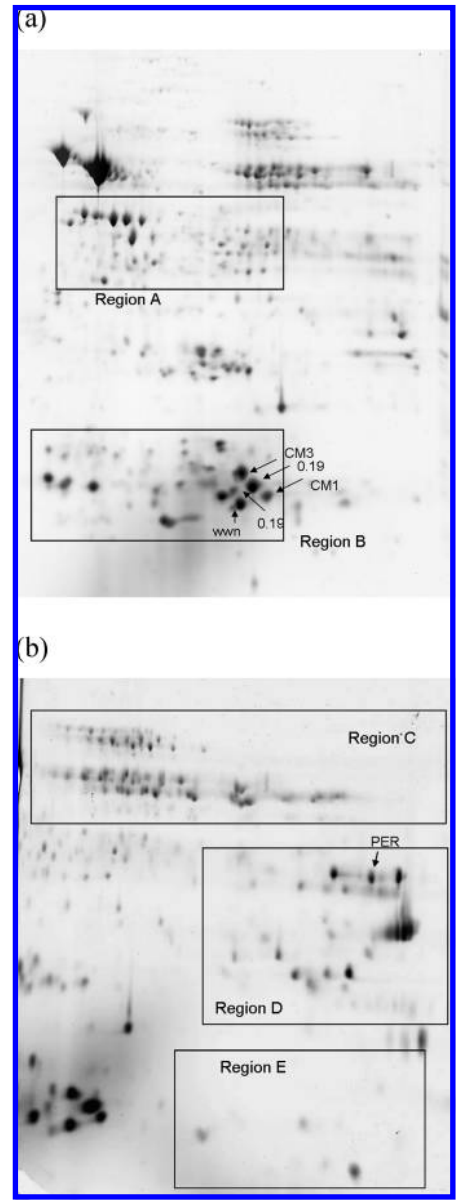

Figure 3. 2-D PAGE analysis of dough liquor proteins from wheat (cv. Rialto) grown under dry/hot conditions. Proteins were separated with an IEF gradient (**a**) pH 3−10 or (**b**) pH 6−11 for the first dimension. Boxed areas indicate regions shown in more detail in **Figures 4–8**. CM1: α-amylase/trypsin inhibitor CM1; CM3: α-amylase/trypsin inhibitor CM3; PER: peroxidase I; 0.19: 0.19 α-amylase inhibitor; and wwn: wheatwin 1.

In the present paper, we compare the effects of these growth conditions on the soluble wheat proteome, following a previous proteomic study of the aqueous phase prepared by ultracentrifugation of dough (*12*). This fraction, sometimes called dough liquor, is of particular interest in relation to effects of environmental factors on processing quality as it contains surface-active proteins that may play a key role in determining the crumb structure of baked bread (*12, 13*).

MATERIALS AND METHODS

Wheat Samples and Growing Conditions. Four bread wheat (*Triticum aestivum* L.) cultivars (Spark, Rialto, Soissons, and Beaver) were grown at the Plant Environment Laboratory (University of Reading, Reading, U.K.) in 2002 and 2003 in pots within two 272 m^2 polyethylene-covered tunnels. Pots were arranged in a latin square design with four blocks. Five hundred pots (0.12 m diameter) of each

Table 1. Protein Confidently Identified from Dough Liquor

protein class	accession number and organism	EST matches (% identity to Swissprot/Genbank)	PMF MOWSE score (95% confidence score)	coverage (%)	spot number	calcd M_r/pI	obsd M_r/pI
		Storage protein					
7S vicilin family [VIC]	Q7DMU0 (*T. aestivum*)		112 (40) Q-TOF	8	D7	72.2/6.80	38/8.55
			74 (40) Q-TOF	8	D8		37.8/8.8
			106 (40) Q-TOF	8	D9		37.6/9.33
			79 (40) Q-TOF	8	D10		37.6/9.47
	TA9588_4565 (*H. vulgare*)	gil170696 (87)	125 (75)	25	C1	n.a.	72.6−78.0/6.5−7.3
		gil170696 (83)	196 (75)	33			
		gil170696 (85)	257 (75)	48			
		gil170696 (84)	105 (75)	25			
		gil170696 (85)	247 (75)	43			
		gil170696 (85)	75 (75)	22			
		gil170696 (86)	148 (75)	36			
	TA58859_4565 (*Zea mays*)	gil170696 (38)	114 (75)	26	C2	n.a.	60.0−63.2/6.5−7.3
		gil170696 (37)	106 (75)	27			
		gil170696 (39)	86 (75)	23			
		gil170696 (39)	106 (75)	28			
		gil170696 (36)	75 (75)	22			
		gil170696 (38)	95 (75)	26			
		gil170696 (38)	105 (75)	22			
		gil170696 (39)	88 (75)	26			
		Protective proteins					
0.28 α-amylase inhibitor	P01083 (*T. aestivum*)		181 (40) Q-TOF	30	B3	13.3/6.19	11.6/5.56
0.28 α-amylase inhibitor			227 (40) Q-TOF	40	B4		11.6/5.58
seed chitinase C	Q9FRV0 (*Secale cereale*)	gil62465514(93)	67 (40) Q-TOF	15	D1	26.1/8.65	31.6/9.24
		gil62465514(93)	101 (40) Q-TOF	16	D2		26/8.4
		gil62465514(93)	67 (66)	22	D4		33.4/8.6
basic endochitinase A precursor (EC 3.2.1.14) (rye seed chitinase a) (RSC a)	Q9FRV1 (*S. cereale*)	gil62465514(94)	68 (66)	21	D3	31.8/8.30	35.7/8.63
serpin	Q41593 (*T. aestivum*)		156 (40) Q-TOF	18	A2	43.1/5.60	39/5.61
	P93692 (*T. aestivum*)		298 (75)	23	A4	43.0/5.18	40.7/5.23
	P93692 (*T. aestivum*)		76 (75)	8	A5		40.7/5.3
pathogenesis-related protein 4	Q9SQG8 (*T. aestivum*)		91 (40) Q-TOF	27	E1	13.1/7.00	11.9/7.5
CM3	P17314 (*T. aestivum*)		102 (75)	69	**Figure 1**	15.8/6.66	16.1/6.5
0.19 α-amylase inhibitor	P01085 (*T. aestivum*)		88 (75)	94	**Figure 1**	13.3/6.66	15.2/6.7
0.19 α-amylase inhibitor	Q5UHH6 (*T. aestivum*)		101 (75)	81	**Figure 1**	13.2/6.50	14.8/6.48
CM1	P16850 (*T. aestivum*)		116 (75)	45	**Figure 1**	13.1/6.72	13.2/6.8
wheatwin1 precursor	O64392 (*T. aestivum*)		76 (75)	54	**Figure 1**	13.7/6.97	12/6.48
		Unknown functions					
α-globulin	Q7XYC3 (*T. aestivum*)		66 (66)	32	D5	26.1/7.66	32.3/8.7
		Metabolic proteins					
cytosolic malate dehydrogenase	Q6XEB8 (*T. aestivum*)		298 (40) Q-TOF	40	A1	24.3/6.59	38.4/5.90
cytosolic GAPDH (fragment)	Q9M4V4 (*T. aestivum*)		140 (40) Q-TOF	25	A7	25.3/7.83	41/6.45
		Antioxidant and heat shock proteins					
DNAK-type molecular chaperone HSP70	Q40058 (*H. vulgare*)		155 (40) Q-TOF	32	A8	64.5/5.67	43.9/6.58
peroxidase I	Q8LK23 (*T. aestivum*)		118 (66)	47	**Figure 3**	38.8/8.14	39/9.30

cultivar, each containing four plants, were grown in each polytunnel. The environment within both polytunnels was kept similar to outside conditions from sowing (December 9, 2002) until 14 days after 50% anthesis. Then, two sets of environmental conditions [cool/wet (mean temperature of 19.5 °C and full irrigation) or hot/dry (mean temperature of 23.2 °C)] were imposed until full maturity, as described by Toole et al. (*11*). The temperature was controlled through manipulation of heaters and vents. Soil−water content in the hot/dry treatment was maintained to a target of 10% by weighing the pots daily and adding water by hand. Control samples consisted of plants grown outside polytunnels. Grain was Buhler milled to a 75% extraction rate by RHM Technology (High Wycombe, Bucks, U.K.), and the water absorption of the flour was determined by the Farinograph method.

Dough Liquor Isolation. Dough was prepared using the following recipe: flour (5 g), 2% (w/w) NaCl, 0.02% (w/w) Na L-ascorbate, and water. The water content was as follows: cv. Soissons control 64.2%, cool/wet 64.3%, hot/dry 61.2%; cv. Beaver control 63.8%, cool/wet 60.7%, hot/dry 60.3%; cv. Spark control 64.9%, cool/wet 65.7%, hot/dry 64.0%; and cv. Rialto control 65.0%, cool/wet 61.2%, hot/dry 61.3%. Dough was prepared using a computer-controlled mini-mixer that mimics the Chorleywood dough mixing process as described by Robertson et al. (*14*). Standard mixer settings were as follows: premix 15 s, set point 1.5 W, speed set point 500 rpm, and energy input 200 J (40 J/g dough). During mixing, the speed (rpm), torque (mN m), power (W), energy (J), and run time (s) were automatically displayed and recorded for off-line processing. The dough was maintained at 30 °C for 30 min and then centrifuged at 200 000*g* at 30 °C for 30 min with a total of 90 min between dough preparation and dough liquor isolation. The supernatant (dough liquor) was dialyzed (2 kDa cutoff microdialyzers, Sigma, Dorset, U.K.) against deionized water overnight at 4 °C and stored at −20 °C until required.

The protein content of the wheat flour was determined using a near-infrared spectroscopy method (Campden and Chorleywood Food Research Association, Flour Testing Working Group (CCFRA FTWG) Method 19). Protein concentration in the dough liquor was determined in triplicate using a bicinchoninic acid assay (Sigma Diagnostics Co., St. Louis, MO) with bovine serum albumin as the standard protein (*15*).

Protein Separation by 2-D Electrophoresis. Linear IEF immobilized pH gradient (IPG) strips (18 cm, Amersham Biosciences, Bucks, U.K.), pH 3−10 or 6−11, were used as the first dimension and 10% SDS PAGE gels for the second dimension as described by Salt et al. (*12*). Dithiothreitol (DTT) was substituted by the DeStreak reagent (1.875 mg/mL, Amersham Biosciences) in a rehydration solution for IEF pH 6−11. Gels were fixed in 10% (v/v) methanol containing 10% (w/v) TCA overnight before being stained with Sypro Ruby (Bio-Rad, Hemel Hempstead, Herts, U.K.) in the dark for 4 h. After being destained (10% (v/v) methanol containing 6% (w/v) TCA) in the dark for at least 1 h, gels were scanned using a proXPRESS Proteomic

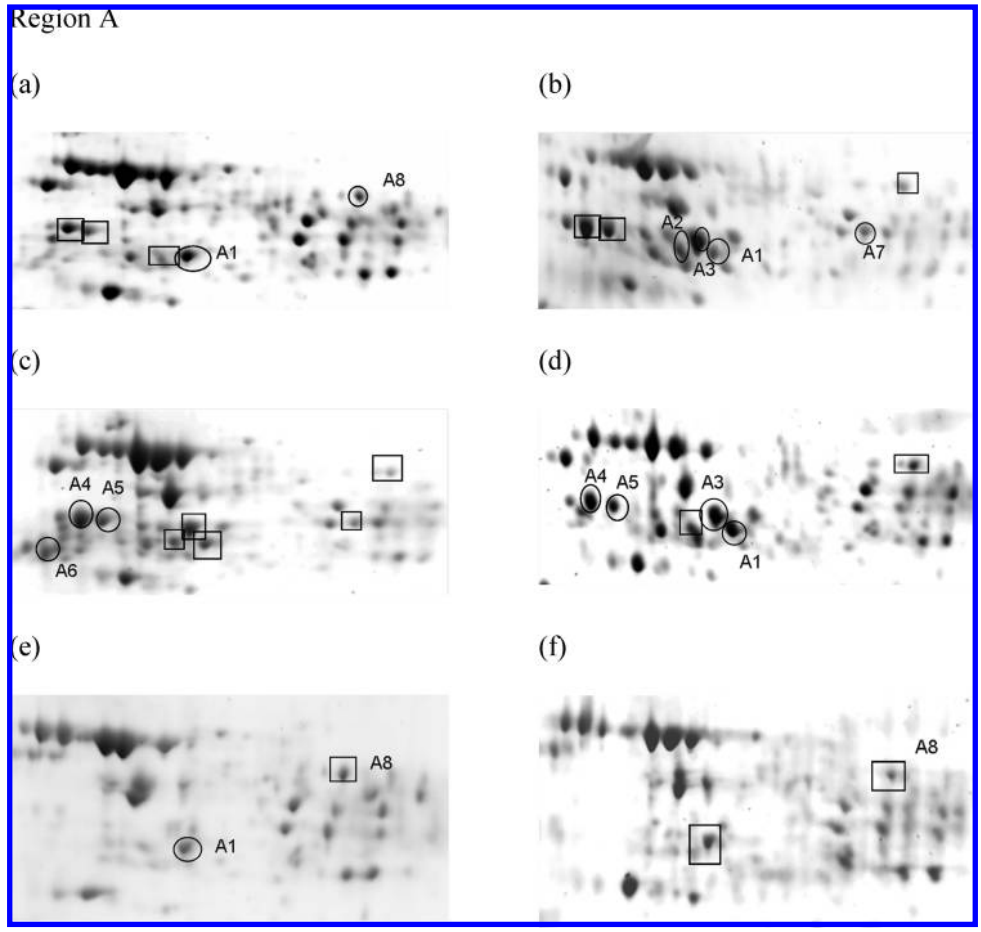

Figure 4. Enlargement of the 2-D PAGE analysis from dough liquor representing region A. Wheat cultivars were either field-grown (cv). (**a**) Soissons, (**b**) Rialto, (**c**) Beaver, and (**d**) Spark or wet/cool (**e**) or dry/hot (**f**) grown (cv. Spark). Circles indicate spots analyzed by MS. Boxes indicate identification confirmed by image analysis.

Imaging System (PerkinElmer Life Sciences Beaconsfield, Bucks, U.K.). After scanning, gels were stored in the dark until required for spot picking. Three replicate gels were run for each dough liquor sample.

Protein Identification by MS. Protein spots were excised using HT Analyzer software (Genomic Solutions, Huntingdon, U.K.), and the excised gel pieces were placed into modified 96-well microtiter plates (Genomic Solutions) and stored at 2 °C overnight prior to trypsin digestion, which was performed as described by Salt et al. (*12*). In-gel trypsin digestion and tryptic peptides were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) at the joint Institute of Food Research-John Innes Centre (IFR-JIC) proteomics facility as described by Salt et al. (*12*). Peptide profiles were searched (on the basis of mass) against the NCBI nonredundant protein database using the Mascot program from Matrix Science (http://www.matrixscience.com/). The search parameters were as follows: (i) tryptic digest was assumed to have a maximum number of one missed cleavage; (ii) peptide masses were stated to be monoisotopic; (iii) methionine residues were assumed to be partially oxidized; (iv) the carbamidomethylation of cysteine residues was considered; and (v) the mass tolerance was kept at 50 ppm. The probability of identification is shown by the MOWSE score (*16*).

For quadrupole time-of-flight (Q-TOF) analysis, peptides generated from tryptic digestion were loaded at a high flow rate onto a reversed-phase trapping column (0.3 mm i.d. × 1 mm, containing 5 μm C_{18} 100 Å PepMap packing, LC Packings) and eluted through a reversed-phase capillary column (75 μm i.d. × 150 mm column, containing Symmetry C_{18} 300 Å packing, Waters Ltd.) directly into the nanoelectrospray ion source of a Q-TOF mass spectrometer (Q-ToF2, Micromass UK Ltd., Manchester, U.K.).

Image and Data analysis. The images were analyzed using nonlinear dynamics Phoretix software (Newcastle, U.K.). The protein spots on each gel were detected and matched to a reference gel. Smoothing of the mixing profile plots was performed by applying a Savitzky−Golay filter using a second-order polynomial and 250 neighboring points, giving a total filter window of 501 points (*17*).

RESULTS AND DISCUSSION

Four bread wheat cultivars were selected for comparison: the soft milling cultivar Beaver and the hard milling cultivars Spark, Rialto, and Soissons. Two of these, Beaver and Rialto, have a 1BL/1RS chromosome translocation, which is often associated with dough stickiness and hence poor breadmaking performance (*18*). These lines were grown in the field and in polytunnels with hot/dry or cold/wet regimes imposed during the grain filling period (from 14 days after 50% anthesis), milled, and used to prepare aqueous dough fractions for analysis.

Dough Mixing. Dough mixing was monitored using a computer-controlled mini-mixer for flours prepared from cvs. Spark, Rialto, Soissons, and Beaver grown in field (control), cool/wet, and hot/dry conditions. The torque associated with dough mixing is a measure of how the rheology varies with the rate of work input, measured as resistance during standardized mixing conditions. The smoothed torque (mN m) mixing profile plot comparison of all four cultivars grown under hot/dry conditions shows an initial increase after premix, reaching a plateau as mixing is complete (**Figure 1a**). The shortest torque time-to-plateau was found for cv. Spark, reaching a plateau of ~32 mN m after 37 s. Cv. Beaver had the longest torque time-to-plateau, reaching 37 mNm after 75 s and then losing structure after 100 s. This behavior is consistent with the loss of gluten

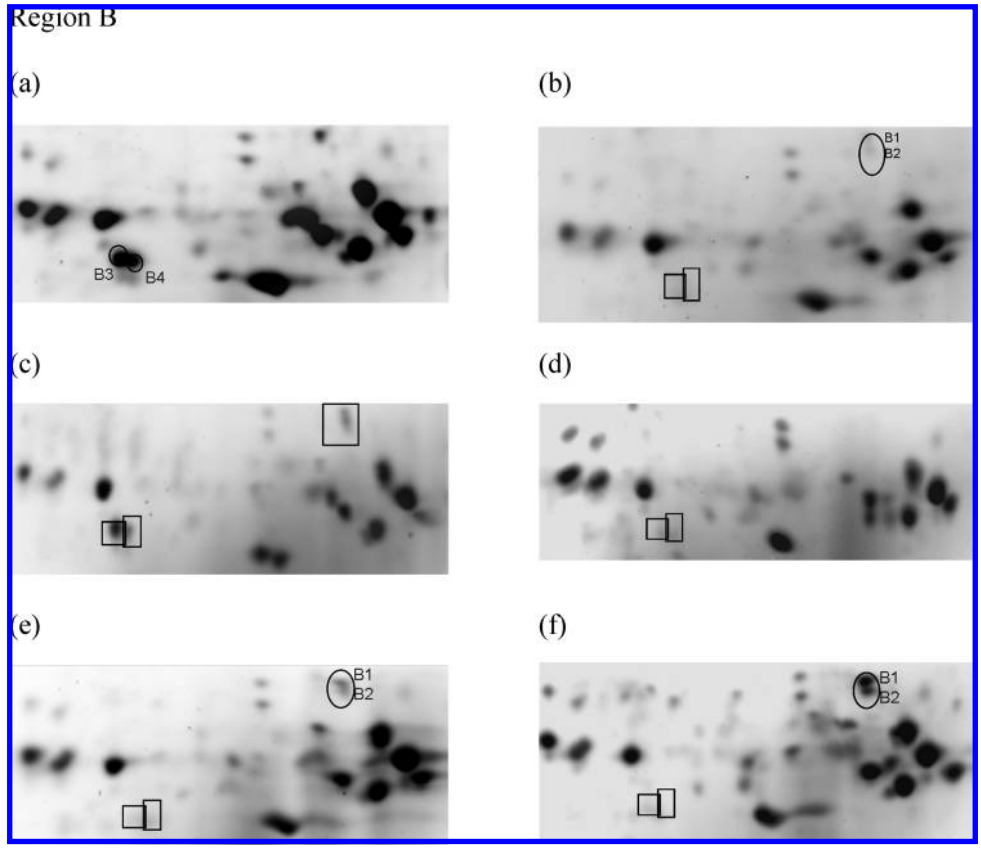

Figure 5. Enlargement of 2-D PAGE analysis from dough liquor representing region B. Wheat cultivars were either field-grown (cv). (**a**) Soissons, (**b**) Beaver, (**c**) Spark, and (**d**) Rialto or wet/cool (**e**) or dry/hot (**f**) grown (cv. Rialto). Circles indicate spots analyzed by MS. Boxes indicate identification confirmed by image analysis.

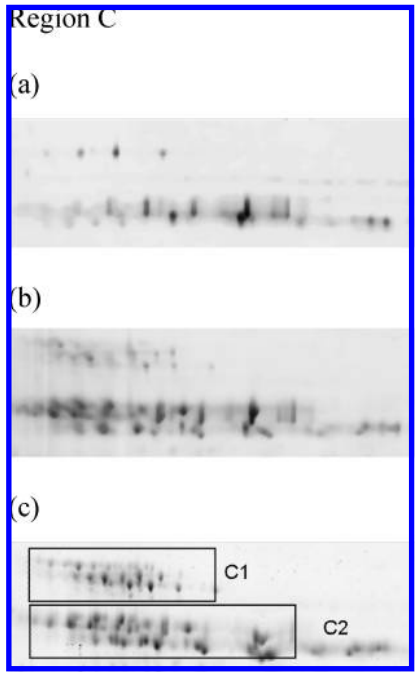

Figure 6. Enlargement of 2-D PAGE analysis from dough liquor representing region C. Cv. Rialto either grown in field (**a**) or under wet/cool (**b**) or dry/hot (**c**) conditions. Circles indicate spots analyzed by MS. Boxes indicate identification confirmed by image analysis.

structure found in soft milling wheat, resulting in dough stickiness (*19*). Mixing profiles for all cultivars grown under field conditions (**Figure 1b**) and cool/wet conditions followed a similar trend (results not shown). The total protein content of the dough liquor from the control (field-grown) seeds ranged from 21.4 to 4.1 mg (**Figure 2a**) following a similar pattern to that of the protein content in flour (%) (**Figure 2b**) for all cultivars except cv. Beaver. However, whereas the flour from the control grain of cv. Rialto contained the highest protein content, the dough liquor did not.

Changes in Soluble Wheat Dough Proteome in Response to Environmental Conditions. The effect of environmental conditions on the soluble wheat dough proteome was determined by comparison of the polypeptides patterns of dough liquor prepared from the four cultivars grown in the field and in polytunnels under hot/dry or cool/wet conditions. The relevant five areas of the 2-D gels were subjected to image analysis. The variant spots were excised from 2-D gels, digested in-gel with trypsin, and subjected to MALDI-TOF mass spectrometry for peptide mass analysis or Q-TOF mass spectrometry. The five areas selected are indicated on the 2-D PAGE separations in **Figure 3**. The proteins that varied among samples and were confidently identified by MS are listed in **Table 1**.

Whereas the overall spot patterns of 2-D gels of the dough liquor extracts were similar irrespective of the growing conditions, some spots did differ in their abundance. These spots were, in general, more intense in fractions from seeds grown under cool/wet conditions. In addition, the overall spot pattern from the four cultivars Spark, Rialto, Soissons, and Beaver was similar to those obtained for a similar fraction from cv. Hereward reported by Salt et al. (*12*). However, reproducible differences were observed between the patterns among the different cultivars. This is in agreement with the study of different barley cultivars during grain filling reported by Østergaard et al. (*20*). This showed genotypic differences that also were related to differences in functional properties. It is notable that, despite

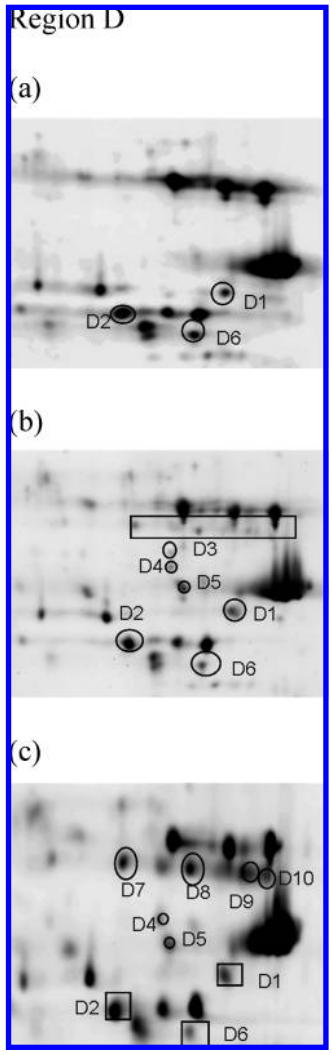

Figure 7. Enlargement of 2-D PAGE analysis from dough liquor representing region D. Cv. Rialto either grown in field (**a**) or under wet/cool (**b**) or dry/hot (**c**) conditions. Circles indicate spots analyzed by MS. Boxes indicate identification confirmed by image analysis.

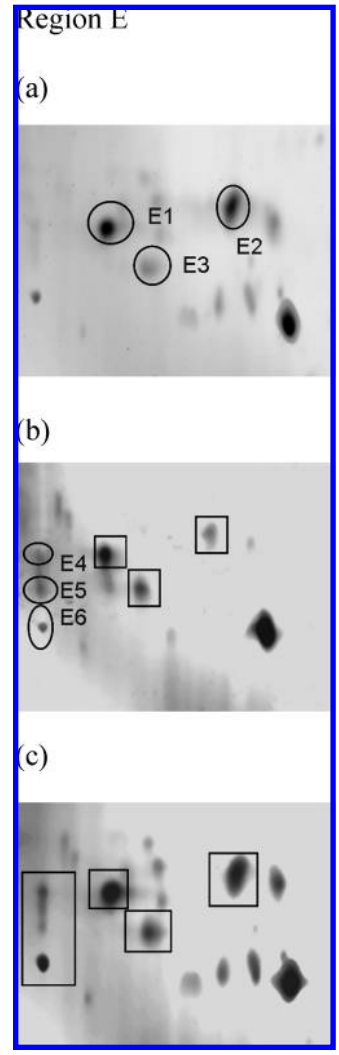

Figure 8. Enlargement of 2-D PAGE analysis from dough liquor representing region E. Cv. Spark either grown in field (**a**) or under wet/cool (**b**) or dry/hot (**c**) conditions. Circles indicate spots analyzed by MS. Boxes indicate identification confirmed by image analysis.

their slightly better solubility properties, no rye prolamins (secalins) expressed in the rye translocation lines (Rialto and Beaver) were found in the dough liquor. These observations support the data of Salt et al. (*12*) who failed to observe any wheat prolamins in dough liquor, indicating that they all become entrained in the gluten network of the dough.

Several reproducible differences in spot intensity were observed in region A (**Figure 4**). Cytosolic malate dehydrogenase (spot A1 identified by Q-TOF; **Table 1**) was less intense in the samples that had been grown in the polytunnels than in those grown in the field, irrespective of the cultivar or of the polytunnel growth conditions (shown only for cv. Spark in **Figure 4e**,**f**). A cluster of polypeptides identified as members of the serpin family (spots A2, A4, and A5) varied in intensity between the different cultivars (**Figure 4a**−**d**). This group of serine proteinase inhibitors was studied in some detail in wheat and may prevent premature proteolysis of the seed storage proteins (*21*). Serpins also were identified as wheat allergens (*22*). One variant (spot A2) was absent from the material of cv. Spark, which was grown in the polytunnels but was present in the other lines. Two other serpin variants (spots A4 and A5) were very weak or absent from the material grown under cool/wet and hot/dry conditions, irrespective of the cultivar. Spot A3 could not be identified by Q-TOF MS, and it was present in the field-grown grain of all cultivars except cv. Soissons and in the polytunnel samples of all but cv. Spark. Similarly, a second protein that could not be identified by Q-TOF MS (spot A6) was only present in cv. Beaver. This protein was not observed either by Salt et al. (*12*) in dough liquor from cv. Hereward nor by Vensel et al. (*23*) in the albumin and globulin fractions of cv. Butte 86. A fragment of GAPDH (spot A7) was identified by Q-TOF, which was present only in cvs. Rialto and Beaver. This suggests that it might be associated with the 1BL/1RS translocation, and its expression was not affected by the growth conditions.

Plants, including wheat, respond to high temperatures by the induction of heat-shock proteins (HSPs) (*24*), and some members of the HSP70 family also were reported to be constitutively expressed (*7*). Spot A8 was identified as a member of the HSP70 family corresponding to the DNAK-type molecular chaperone HSP70. This protein was present in all of the material and showed no apparent differences in intensity under the different growth conditions. It is therefore probable that A8 represents a constitutively expressed form of HSP and that the moderate temperature regime used in the study (below 30 °C) did not induce a classic heat-shock response.

Two protein spots in region B (B1 and B2) were less intense in cvs. Rialto and Beaver but increased in material grown under

cool/wet and hot/dry conditions (**Figure 5**). However, these proteins could not be identified by Q-TOF MS as reported by Salt et al. (*12*). Spots B3 and B4 also showed some variation in intensity between cultivars. It was less abundant or absent in cvs. Rialto and Beaver as compared to the other cultivars irrespective of growth conditions. Although these were not identified by Salt et al. (*12*), we obtained a positive identification as the 0.28 α-amylase inhibitor (**Table 1**) (also called wheat monomeric α-amylase inhibitor: WMAI-1). No differences were observed between material grown under different conditions.

One of the most striking differences in region C (**Figure 6a−c**) was the marked increase in the intensities of several spots in the material of cv. Rialto grown in the polytunnels. While the intensity of the C2 group of spots increased under both hot/dry and cool/wet growth regimes, the C1 group increased only in the material grown under hot/dry conditions. Some spots of this group were identified as belonging to the 7S globulin (vicilin-like) family of seed storage proteins (spots C1 and C2). These proteins are located in the embryos and aleurone cells of cereal grains rather than in the starchy endosperm, which is milled to give white flour (*25–27*). Hence, their presence in the dough liquor fractions indicates the presence of these tissues in the flour fractions. Although the 7S globulins initially are synthesized as subunits with a mass of ∼55 000−70 000, they may undergo post-translational proteolysis, leading to the presence of components of M_r ranging from ∼70 000 to 20 000 in barley aleurone cells (*25*). This is consistent with the present study in which the spots identified as 7S globulins had masses of 60 000−78 000. Salt et al. (*12*) also described a group of 7S globulin proteins, as indicated by MOWSE scores, with similar molecular masses and pI values. However, the present study gives a more complete description.

Region D contains spots identified as precursors of endochitinase A (D3) (based on homology with a rye gene) and endochitinase C (D1, D2, and D4). While the intensity of spot D4 was greater in the material grown in the polytunnels, the intensity of spot D2 remained unchanged (**Figure 7a−c**). Chitinases are enzymes that catalyze the hydrolysis of the *β*-1,4-*N*-acetyl-D-glucosamine linkages in chitin polymers. Therefore, they are thought to be involved in defense against fungal pathogens and insect pests that contain chitin. None of these proteins were identified in dough liquor from cv. Hereward by Salt et al. (*12*). Region D also contained a protein (spot D5) identified as a 19 kDa α-globulin that increased in intensity in the material of cv. Rialto grown in the polytunnels. This spot may correspond to an α-globulin described by Vensel et al. (*23*) in wheat endosperm. However, Salt et al. (*12*) failed to identify a protein of similar molecular mass in the dough liquor of cv. Hereward. α-Globulins are members of the prolamin superfamily and have been characterized as storage proteins in rice (*28*) and maize (*29*). They have not been characterized at the protein level in wheat, but the sequence determined showed 59% identity to α-globulin from *Z. mays* (accession number Q946V3).

One of the clearest differences between the cultivars was in spot D6. This was absent from cv. Spark and less intense in the samples of cv. Rialto under the different growth conditions. This spot was not identified by Q-TOF MS in the present study or by Salt et al. (*12*). Furthermore, no additional spots of similar molecular mass were observed in cv. Spark, which indicates that the differences were not due to modifications affecting pI during preparation or electrophoresis. Spots D7−D10, which were newly identified as 7S storage globulins by Q-TOF, only increased in intensity in the sample of cv. Rialto grown under hot/dry conditions.

Within region E an increase was observed in the intensity of spot E2 (**Figure 8a−c**) that was not identified by Q-TOF (as reported by Salt et al. (*12*)). Spot E1 was identified as a pathogenesis-related protein 4 (PR4) by Q-TOF as described previously by Vensel et al. (*23*). Members of the PR4 family such as WWN are thought to have antifungal activity (*30*) and might play a role in the response of the developing grain to environmental stress since their gene expression is enhanced by high temperature conditions (*31*).

Increases in the intensities of a series of spots (E3, E4, E5, and E6) were observed in cv. Spark when grown under cool/wet and hot/dry conditions; one spot (E3) being absent from cv. Rialto. However, none of these components could be identified by Q-TOF MS.

In conclusion, we report changes in individual protein spots in response to environmental conditions using 2-D gel electrophoresis followed by MALDI-TOF MS to identify proteins based on information available in nonredundant protein and EST databases. Reproducible differences were observed between the spot patterns in aqueous dough fractions from the four cultivars Spark, Rialto, Soissons, and Beaver. Minor differences in the stability of the soluble wheat dough proteome to environmental conditions were observed, and it was clear that cv. Rialto showed the greatest variability in intensity and Spark the least when comparing the three samples grown under different environmental regimes. The differences in composition could not in general be related to the presence of the 1BL/1RS translocation or to the grain texture. The variable spots included storage proteins of the 7S globulin (vicilin-like) and α-globulin families and protective proteins belonging to the serpin, previously described as allergens, and chitinase families. These data suggest that gross changes in protein expression do not underlay environmentally induced variations in dough liquor. It may be that more subtle changes, such as effects of grain drying on the collective behavior of flour proteins and other components, such as nonstarch polysaccharides, may play a more important role than changes in expression per se.

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