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Effect of short heat shocks applied during grain development on wheat (*Triticum aestivum* L.) grain proteome

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ABSTRACT

In the field, developing cereal grains are often exposed to short periods of very high temperature (>35 °C) that may dramatically affect grain yield and flour quality. Here we report on the effect of 4 h of heat shock (HS) at 38 °C applied on four consecutive days during the linear phase of storage compound accumulation on grain proteome of the winter bread wheat. At maturity the average single grain dry mass and the total quantity of nitrogen per grain were 25% and 16%, respectively lower for the HS treatment than for the control, resulting in a higher (+1.6% dm) grain protein concentration in HS grains. Individual albumin–globulin, gliadin and glutenin protein fractions from grains collected just before the HS and 1, 8, and 26 (ripeness maturity) days after the HS were quantified then analysed by 2-dimensional electrophoresis followed by MALDI-TOF and MS/MS identification. The quantity per grain of 10 gliadins and 3 low molecular weight glutenin subunit proteins were significantly affected by HS. Thirty-eight HS responsive albumin and globulin proteins were identified, including several enzymes involved in carbohydrate, redox, and lipid metabolisms. One protein was transitorily induced in response to HS. Detailed discussion of the expression of these proteins is presented.

1. Introduction

Proteins are the main components of wheat (*Triticum aestivum* L.) grain that govern end-use quality. Variations in both grain protein content and composition determine whether flour is suitable for bread-making. High temperatures during the critical period of grain filling may have an adverse impact on the yield and end-use quality of wheat (Perrotta et al., 1998; Wardlaw and Wrigley, 1994). Increases in temperature throughout grain filling have a significant influence on the quantity of proteins that accumulate in the grain and on the ratios between different protein fractions.

Proteomics methods have been used to study the effects of elevated temperatures on wheat grain development. The analysis of the effect of a moderately high temperature (34 °C/10 °C, day/night) applied during most of the post-anthesis period on the protein composition of mature wheat grain identified a set of proteins affected by chronic high temperature (Majoul et al., 2003, 2004). These included three α-gliadin mature peptides up-regulated by high temperature and proteins related to HSPs possibly involved in thermotolerance. Proteins involved in plant defence mechanisms or different metabolic pathways were also identified among the high temperature responsive proteins. More recently, Hurkman et al. (2009) compared protein accumulation in the endosperm of developing wheat grains grown under normal (24 °C/17 °C, day/night) and very high temperature (37 °C/28 °C, day/night) imposed from 10 or 20 days after anthesis (daa) until grain ripeness

Abbreviations: AG, albumins–globulins; daa, days after anthesis; das, day after the end of stress; °Cd, degree-days; DHAR, dehydroascorbate reductase; FDH, formate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GST, Glutathione S-transferase; HMW-GS, high molecular weight glutenin subunits; HPLC-MS/MS, High Performance Liquid Chromatography Mass Spectrometry/ Mass Spectrometry; HSP, heat shock protein; LMW-GS, low molecular weight glutenin subunits; LTP, Lipid-transfer protein; MALDI-TOF, matrix assisted laser desorption/ ionization time-of-flight; MDH, malate dehydrogenase; NDPK, nucleoside diphosphate kinase; PDI, protein disulfide isomerase; PTMs, post-translational modifications; SDS, sodium dodecyl sulphate; sHSP, small heat shock protein; TPR, tetratricopeptide repeat; XIP, xylanase inhibitor protein.

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maturity. These authors observed a shift in the KCl-soluble/methanol-insoluble albumin–globulin (AG) protein fraction from protein active in biosynthesis and metabolism under normal temperature towards proteins with roles in storage and protection against biotic and abiotic stresses under very high temperature.

Many previous studies have dealt with the impact of a long period of above optimal temperature, e.g. from anthesis to maturity. However, short periods of HS may also affect wheat yield and quality. Skylas et al. (2002) showed that one day of very high temperature (40 °C/24 °C versus 24 °C/18 °C, day/night) applied 15 to 17 daa increased the number of small heat shock protein (HSP) isoforms present in total protein extracts of endosperm collected 1 or 2 days after the heat spell. In durum wheat (*Triticum turgidum* L. var. *Durum* Desf.) grains, 5 days of HS at 37 °C/17 °C (day/night) induced changes in sugar metabolism enzymes (e.g. nucleoside diphosphate kinase (NDPK) and glyceraldehyde 3-phosphate dehydrogenase, GAPDH) and stress-related proteins like HSPs, late embryogenesis abundant (LEA) proteins and serpins (Laino et al., 2010). It has also been noted that a gradual rise in daily maximum temperatures inflicts less damage to grain than a sudden temperature rise (Stone and Nicholas, 1994).

Here we describe how the wheat grain proteome is affected by short HS periods (4 h per day) applied during four consecutive days during the linear phase of storage compound accumulation. First, we describe the changes induced by HS on average single grain dry mass, total nitrogen content and protein concentration and the percentages of AG, amphiphilics, gliadin and glutenin protein fractions in the flour. A quantitative proteomic analysis of the AG and storage protein (gliadin and glutenin) fractions is then presented and the putative role of the identified HS responsive proteins is discussed.

2. Materials and methods

2.1. Plant material and growth conditions

Crops of the winter wheat (*T. aestivum* L.) cultivar Récital were grown outside at INRA Clermont-Ferrand, France (45°46′ N, 03°09′ E, 329 m a.s.l.) in 2 m^2 containers 0.5-m deep, filled with a 2:1 (v/v) mixture of black soil:peat. Ammonium phosphate (N:P, 18:46; 20 g m^{-2}) and potassium sulphate (K_2SO_4; 20 g m^{-2}) were hand-dressed on 2 November 2000. Seeds were sown on 6 November 2000 at a density of 578 seeds m^{-2}. During the growing season, the crops received 10 g N m^{-2} as ammonium–phosphate (20 g m^{-2}) and ammonium nitrate (20 g m^{-2}) on 6 February 2001 and 15 g N m^{-2} as ammonium nitrate (45.5 g m^{-2}) on 2 April 2001. Rate and frequency of watering was adjusted to maintain soil water potential above −0.3 MPa. All other crop inputs were applied at levels to prevent pests, diseases or weeds from limiting crop growth.

At anthesis (15 May 2001), two containers were transferred under transparent enclosures under natural light in the Crop Climate Control and Gas Exchange Measurement (C3-GEM) experimental platform. The C3-GEM platform allows monitoring and controlling air temperature, air CO_2 concentration, water supply and gas exchange (Triboï et al., 1996). Air CO_2 concentration was maintained at 378 ± 5 ppm. Except during heat-shock exposure, day/night air temperature was controlled at 18 °C/10 °C ± 0.2 °C (average daily temperature).

One of the two containers placed under the transparent enclosures received HS treatments consisting of 4 h a day at 38 °C (air temperature), between 12:00 and 16:00 and 21 °C (air temperature) the rest of the day (average daily air temperature was 23.8 °C). The rate of heating or cooling was 8.5 °Cd h^{-1}. HS treatments were applied for four consecutive days starting at 18 daa (300 °Cd after anthesis). At anthesis, all the containers were irrigated to field capacity by applying 90 mm of water, they then received 25–50 mm of water every 4–7 days until harvest to replace measured crop evapotranspiration. Spikes were tagged at anthesis to allow accurate determination of the developmental stage when harvesting.

2.2. Plant sampling and grain dry mass determination

Three replicates of 20 plants (ca. 0.25 m^{-2}) were collected from the HS treated containers prior to the HS period at stage named S1 (300 °C/17 daa) and one day after the end of HS (1 das) at stage S2 (400 °C/22 daa), 8 days after the end of HS (8 das), at stage S3 (500 °Cd/29 daa), and 26 days after the end of HS (26 das), at ripeness maturity (940 °Cd/47 daa). The corresponding thermal times after anthesis were chosen for the control containers (i.e. at 17, 25, 31, and 47 daa). Grains were rapidly hand-threshed from ears, then frozen in liquid nitrogen and stored at −80 °C before being freeze-dried. After freeze-drying grains were stored at 4 °C before analysis. Grain dry mass was determined on subsamples after oven drying at 80 °C to constant mass.

2.3. Total N content determination and protein fractions extraction and quantification

Freeze-dried grains (1.5 g) were ground to wholemeal flour using a Quadrumat Jr mill (Brabender, Duisburg, Germany). Total flour (250 mg) N content was determined by the Kjeldhal method using a Kjeltec 2300 analyser (Foss Tecator AB, Hoeganaes, Sweden). Protein content was calculated from the percentage of total N by multiplying by a conversion factor of 5.7.

The protein fractions AG, amphiphilic, gliadin, and glutenin protein fractions were sequentially extracted from 833 mg of flour as described by Triboï et al. (2003). Briefly, soluble and insoluble fractions were separated by centrifugation at 8000 g for 30 min at different temperatures. Albumins–globulins were extracted at 4 °C with 25 ml 0.05 M NaCl, 0.05 M sodium phosphate buffer pH 7.8. Amphiphilic proteins were extracted at 4 °C from the previous pellet with 25 ml 2% (v/v) Triton X-114, 0.1 M NaCl, 0.05 M sodium phosphate buffer pH 7.8. Gliadins were extracted at 20 °C from the previous pellet with 25 ml 70% (v/v) ethanol. Glutenins were extracted at 20 °C from the previous pellet with 25 ml 20 g l^{-1} SDS (sodium dodecyl sulphate), 2% (v/v) 2-mercaptoethanol (2-SH), 0.05 M tetraborate buffer pH 8.5. After centrifugation, the glutenins were recovered in the supernatant. After the supernatant solutions were evaporated to approximately 5 mL in a forced-air oven at 50 °C, total N content of the different protein fractions and the residue of the extraction was determined using the Kjeldhal method. As a control, total N content of the whole meal flour was systematically determined on a different subsample, and analyses were redone if the difference between whole meal flour N content and the sum of N content for the different protein fractions was greater than 5%.

2.4. Albumin–globulin and total protein extraction and two-dimensional gel electrophoresis

Freeze-dried grains (5 g) were ground to wholemeal flour using a Cyclotec 14920 mill with a 0.75-mm sieve (Hilleröd, Denmark). For two-dimensional electrophoresis (2-DE) analysis, AG were extracted from 100 mg of flour as above (Triboi et al., 2003) with the optimization for 2-DE separation described in Branlard and Bancel (2006), and total proteins with gliadins and glutenins were extracted from 40 mg flour using a urea/thiourea/CHAPS protein extraction protocol as described in Majoul et al. (2003).