Effects of High Temperature on the Wheat Endosperm Proteome

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Abstract

High temperatures during wheat grain-fill decrease the duration of grain development, storage protein accumulation, and starch deposition. To understand the molecular basis for these changes, we are using a proteomics approach to identify endosperm proteins and determine the effects of high temperature on their accumulation profiles during wheat grain development. Wheat (*Triticum aestivum* L. cv. Butte 86) plants were grown in a climate-controlled greenhouse that had an average daily maximum daytime temperature of 25ºC and nighttime temperature of 17ºC. At 10 and 20 days post anthesis, plants were transferred to a second greenhouse with identical growing conditions except that the average daily maximum daytime temperature was 37ºC and nighttime temperature was 28ºC. Developing grain from plants grown under the three regimens was harvested and endosperm collected. Salt soluble proteins were separated from the storage proteins and separated by 2-D gel electrophoresis. Qualitative and quantitative protein changes during grain development were characterized using computer-based image analysis. Many proteins were present throughout development, while some were only present early or late in development. The most noticeable difference in protein profiles was an increase in a number of proteins with low molecular weights and high pIs during the middle and late stages of development. Protein accumulation profiles reflected the shortened duration of grain development caused by high temperature. Of the 100 proteins identified in 2D gels by matrix-assisted laser desorption ionization mass spectrometry and electrospray ionization mass spectrometry, the majority function in protein synthesis, starch metabolism, and defense.

Introduction

Environmental conditions during grain-fill can affect the duration of storage protein accumulation and starch deposition, and thus play an important role in determining grain yield and flour quality of wheat. To understand the genetic basis for the effects of environment on the complex program of grain-fill, high throughput transcript and protein profiling techniques must be utilized to identify the many genes and gene products integral to this developmental program. We are currently analyzing salt soluble wheat endosperm protein fractions using two-dimensional (2-D) gel electrophoresis and mass spectrometry to identify the proteins in these fractions and establish accumulation profiles during wheat grain development. One important outcome of these studies will be a better understanding of the timing of the metabolic events that take place during grain development and the effect of the environment on these events. In this poster, we report initial results of studies on the identification of endosperm proteins and their accumulation patterns during in grain development. We also demonstrate the effects of high temperatures initiated during an early and middle stage of grain development on these accumulation patterns.

Methods

Protein Fractionation. Endosperm from developing grain of plants grown under three temperature regimens was suspended in cold (4ºC) KCl buffer (50 mM Tris-HCl, 100 mM KCl, 5 mM EDTA, pH 7.8; 200 :l of buffer per 50 mg of endosperm), incubated on ice for 5 min with intermittent mixing (Vortex), and centrifuged for 15 min at 4ºC and 14,000 rpm. The proteins in the supernatant were precipitated by the addition of 5 vol of 0.1 M ammonium acetate in methanol ammonium acetate in methanol and incubation overnight at _20ºC. The pellet rinsed with acetone and solubilized in a buffer containing 9 M urea, 4% NP-40, 1% DTT, and 2% ampholytes.

Two-dimensional gel electrophoresis. The isoelectric focusing gels were run using the Mini Protean II tube cell (BioRad Laboratories). Gels were extruded into microcentrifuge tubes and equilibration buffer (2.3% SDS, 10% glycerol, 0.05% DTT, 62.5 mM Tris-Cl pH 6.8) added. Gels were immediately frozen on dry ice and stored at –70ºC. Gels were thawed, placed on top of NuPAGE Novex Bis-Tris 4-12% acrylamide gels with 2D well (Invitrogen), and overlaid with equilibration buffer. The second dimension gels were run using NuPAGE MES SDS running buffer and stained with Coomassie G-250.

Image Analysis of 2D Gels. Digitized 2D gel images were analyzed using the 2D gel electrophoresis image analysis software Progenesis™ Workstation version 2002.01 (Nonlinear Dynamics Limited). Triplicate samples were taken at each time point, resulting in a total of 69 2D gel images. The replicates were grouped together within Progenesis™ Workstation to form averaged groups, as shown in Figure 1. Spot detection was performed using the fully automatic detection algorithm available within the software. No parameters are required thus ensuring that the detection process is as user independent and objective as possible. Post-analysis, some minor editing was carried out to rectify spot splitting of complex regions. Some editing to optimize matching was also performed. Spot volumes were automatically normalized and expressed as a percentage of the total spot volume for that gel, therefore allowing direct quantitative cross-gel comparisons to be made, regardless of loading and staining irregularities. The automatic analysis workflow is illustrated in Figure 2. All quantitative comparisons described here were based on normalized spot volumes. Averaged gel data was used to investigate and identify expression changes across the different development stages.

Mass Spectrometry and Spot Identification. A DigestPro xy robot was used to carry out alkylation and in-gel trypsin digestion of protein spots excised from 2-D gels. Matrix assisted laser desorption/ionization-time of flight (MALDI-TOF; Reflex II, Bruker Deltonics) mass spectrometry was used to obtain mass fingerprints of the peptide fragments. Mass spectra were obtained using using delayed extraction in the reflectron mode. Porcine trypsin fragments were used as internal calibrants. Electropray ionization mass spectrometry was carried out using a Finnigan LCQ Classic Ion-trap Mass Spectrometer (Finnigan MAT, San Jose, CA) using a New Objective nano-spray interface (New Objective Inc.,Woburn, MA). An LC Packings nano flow LC system (Dionex, Sunnyvale, CA) with autosampler, column switching device, loading pump, nano-flow solvent delivery system, and UV detector was used with the LCQ. The instrument was operated in the "triple play" mode with the LCQ set to acquire a full MS scan between 400 and 1400 m/z followed by a zoom scan of the most intense ion from the preceding scan followed by MS/MS fragmentation. Spectra were transferred to a PC and the batch identification was carried out using a suite of programs from ProteoMetrics.

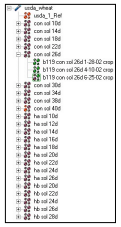


Figure 1. Experiment tree of gel set analyzed as illustrated within the Progenesis™ Workstation.

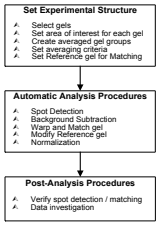


Figure 2. A flow diagram illustrating the automatic workflow through the Progenesis™ Workstation.

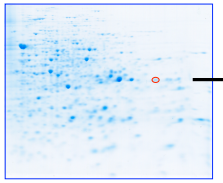


Figure 3. Coomassie-stained, 2D gel of proteins present in endosperm at 36 dpa. Spot circled in red was identified as a peroxidase by mass spectrometry.

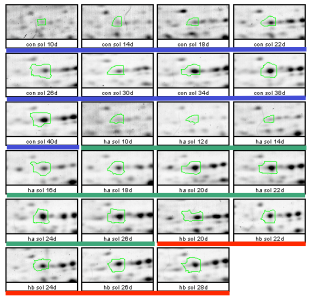


Figure 4. Peroxidase levels in developing grain grown under moderate or high temperatures. Blue: Moderate temp. from10 to 40 dpa. Green: High temp. from 10 to 26 dpa. Red: High temp. from 20 to 28 dpa.

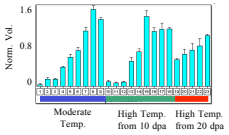


Figure 5. Accumulation profiles of peroxidase during grain development under moderate temperatures and in response to high temperatures. 1-9: moderate temperatures from 10 dpa. 10-18: high temperatures from 10 dpa. 19-23: high temperatures from 20 dpa. Error bars indicate variation between triplicate gels.

Proteins Identified in the Wheat Endosperm Proteome

Metabolism
Adenosine Kinase
ADP-Glucose Pyrophosphorylase, Large Subunit
ADP-Glucose Pyrophosphorylase, Small Subunit
Alanine Aminotransferase
Aldolase
Enolase
Formate Dehydrogenase
Malate Dehydrogenase
Methionine Synthase
Nucleoside Diphosphate Kinase
Phosphoglucomutase
Pyrophosphate Dependent 6-Phosphofructokinase
UDP-Glucose Phosphorylase
S-adenosylmethionine Synthase
Triosephosphate Isomerase

Amylase Inhibitors
Alpha-Amylase Inhibitor 0.19
Alpha- Amylase Inhibitor 0.53
Alpha- Amylase/Subtilisin Inhibitor
Alpha- Amylase/Trypsin Inhibitor

Disease and Defense
Ascorbate Peroxidase
Catalase
Chitinase
GSH-dependent Dehydroascorbate reductase
Peroxidase
Serpin
Trypsin Inhibitor (Cmx precursor)
Xylanase Inhibitor Protein I

Protein Destination
26S Proteosome Regulatory Particle Triple-A ATPase
Alpha- Subunit of 20S Proteosome
Beta-Subunit of 20S Proteosome
Chaperonin 60
ClpC Protease
Cyclophilin
HSP70
HSP80
NLS Receptor
Protein Disulfide Isomerase

Protein Synthesis
40S Ribosomal Protein S12
Elongation Factor 1alpha
Elongation Factor 2
Eukaryotic Initiation Factor

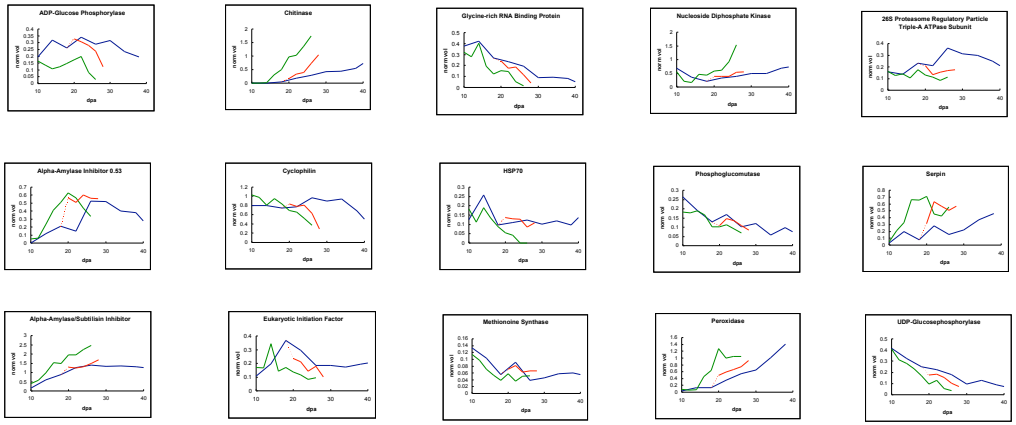
Transcription and Post-transcription
Glycine-rich RNA Binding Protein
Poly-A Binding Protein

Storage Proteins
Avenin
Low Molecular Weight Glutenin Subunit
Seed Globulin
Triticin

Cell Structure
Actin
Alpha-Amylase
Alpha-Tubulin

Transporters
Nonspecific Lipid Transfer Protein

Accumulation profiles of endosperm proteins during development of grain grown under moderate or high temperatures.



Conclusions

This proteomics approach is powerful for gaining insight into the complex processes of grain development and the effect of the environment on these processes:

Over 100 proteins were identified in the endosperm of developing wheat grain.

Accumulation profiles of these proteins are being used to understand the timing of metabolic events that take place during grain development.

Protein accumulation profiles also are being used to understand the effect of the environment on these events.

The Progenesis™ Workstation greatly simplified gel matching and protein quantification.