Comparative proteomic analysis of the metabolic seed protein fraction in the Italian durum wheat cv. Svevo after heat treatment

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INTRODUCTION

In Central and Southern Italy, where durum wheat is mostly grown and represents one of the most important crops, grain filling occurs between April and May, when sudden increases in temperature may take place. High temperature during the grain filling is reported to cause an alteration in the yield and dough quality of the bread wheat. It has been demonstrated that heat stress in hexaploid wheat modifies protein accumulation in the seed. High temperatures primarily affect the composition of the polymeric fraction (soluble/insoluble polymers) without influencing their synthesis[1]. Kernel protein content and composition are the most important factors controlling wheat quality.

Wheat proteins are divided according to their solubility proprieties into prolamins (gliadins and glutenins), albumins and globulins (water and salt soluble, respectively).

It has been established that heat stress causes a deviation in the ratio between the different prolamin components. High temperatures seem to favor the synthesis of gliadins, which remain stable or increase, whereas glutenin synthesis decreases[2]. A hypothesis to explain this effect was previously suggested by Blumenthal *et al.*[3], who observed the presence of heat stress elements (HSE) in the upstream regions of some gliadin genes. Water-soluble albumins and salt-soluble globulins constitute 10-22% of total flour protein[4,5]. These two classes of proteins were previously considered to have only metabolic activity or structural functions. However, certain globulins have been reported to behave as storage proteins in wheat seeds[6]. In contrast to prolamins, the other major protein class of wheat endosperm, the albumins and globulins have not been well characterized. In part, this is because the role of albumins and globulins in flour quality is not as well defined as that of the gliadins and glutenins.

Generally, albumins and globulins do not play a critical role in flour quality, although the ratio of albumin to globulin may correlate with breadmaking quality[4]. A relationship between α-amylase/trypsin inhibitors, misidentified as Low Molecular Weight Glutenin Subunit (LMW-GS), and pasta quality has been suggested[7,8]. Noteworthy is the observation that some albumins, such as those belonging to the family of amylase/protease inhibitors, trigger human allergies, such as baker's asthma[9].

Another effect of heat stress on metabolic protein regulation, at least in bread wheat, is a reduction in both starch accumulation and activity of soluble starch synthase[10,11,12]. More recently, a proteomic study performed on soft wheat showed the involvement of starch biosynthetic enzymes, such as glucose-1-phosphate adenyltransferase and the granule-bound starch synthase in the heat stress response[13]. In addition, over expression of large numbers of cytoplasmic heat shock proteins (HSP's) with chapherone functions was found.

Our aim is to identify the metabolic pathways that are involved in the response to heat shock treatment in durum wheat.

'Omic' technologies have great potential for identifying components of plant materials, as well as derived food products. Here we have used proteomic tools to determine those protein components of the durum wheat seed that are involved in heat stress response and that may affect the nutritional and technological quality of the derived products.

MATERIAL AND METHODS

Plant material and heat shock

The widely grown Italian durum wheat cultivar Svevo was grown in a climate chamber at 25/20°C (16 h day/8 h night). Plants were exposed to heat shock at 37/17 °C (13 h day/11 h night) during the early grain filling stage (5 days after anthesis). After 5 days of heat treatment, plants were grown for 4 h at 28°C and the growing cycle was completed at 25/20°C (16 h day/8h night), 45% humidity and 500 µmol m^{-2} s^{-1} (control condition) up to physiological maturity. The control plants were maintained at 25/20°C (16 h day/8h night), 45% humidity and 500 µmol m^{-2} s^{-1} for the same period (from seedling to seed maturity). Four biological replicates were performed for each treatment.

Comparative proteomics analysis and protein identification

Endosperm proteins were fractionated using a KCl buffer to separate the gliadins and glutenins from the albumins and globulins according to Vensel *et al*.[14]. Two-dimensional electrophoresis (IEF/SDS-PAGE) was carried out on the metabolic (non-prolamin) fraction. IPG dry strips (18 cm long) in the pH range 3-10 were loaded with the same extract volume (200 µl) rather than the same amount of protein, to visualize changes in individual protein level[15]. Three technical replicates for each biological replicate were generated (24 gels). Protein spots in 2-dimensional gel electrophoresis were revealed with Coomassie Brillant Blue (CBB) and analyzed with the software Progenesis SameSpots (Nonlinear Dynamics, UK). After gel analysis, only the spots with ANOVA and q Values lower than 0.05 were accepted as differentially expressed, and excised from the gel, washed, in gel reduced and S-Alkylated with iodoacetamide, followed by digestion with trypsin.

2 µl of trypsin digestions were desalted on AnchorChip™ target (Brucker Daltonics, Bremen, Germany) prior to MALDI-TOF-TOF-MS. Delayed-extraction MALDI-TOF mass spectra were recorded on a REFLEX III reflection time-of-flight mass spectrometer (Brucker Daltonics, Bremen, Germany). Identifications were considered as positive when the following criteria were fulfilled: i) significant MASCOT score, ii) at least six independent peptides matched, and iii) sequence coverage at least 13%. In case of failure, the peptide masses were re-searched using an EST database (TIGR Wheat). Additonaly, tandem MS of ambiguous identifications was performed in order to improve the sample identification rate.

RESULTS AND DISCUSSION

The 2-D gels were highly reproducible and showed well resolved spots. Gel analysis revealed 132 spots differentially expressed between heat treated and control samples (Fig. 1). Despite the lower number of spots detectable with CBB in relation to silver staining, we have chosen the former because higher quality mass spectra can be obtained due to the larger amount of sample applied.

Principal Component Analysis (P.C.A.) (Fig. 2) performed on differentially expressed polypeptides, demonstrated the presence of two different groups of spots corresponding to the two treatments, giving support to the hypothesis that the main cause of the observed differences is heat treatment. This analysis has been performed according to O'Gorman *et al*.[16] and it is included in the software SameSpots (NonLinear Dynamics, UK).

MALDI TOF analysis identified approximately 50% of the picked spots. This value was expected and was due to difficulties in manually picking up minor spots and to ambiguous identification in data bank.

As expected, proteins related to desiccation stress (LEA, and heat shock proteins, HSP70, HSP26, and glyoxalase I) were up-regulated. These proteins prevent, through the binding or interaction with other proteins, cell membrane or proteins from disruption and damage in the near-dry state[15]. Nucleoside diphosphate kinase, which is required for the synthesis of nucleotide triphosphate precursor of DNA and RNA, was also over-expressed, along with some housekeeping enzymes involved in the glycolysis and pentose phosphate pathway (glyceraldeyde 3-phosphate dehydrogenase, phosphoglycerate kinase, and glucose and ribitol dehydrogenase).

Other proteins with a defense role were up-regulated in response to heat shock and these proteins are typically identified in multiple forms on 2-D gels[15]. These are serpins, tritins, α-amylase inhibitors, globulin-like proteins and 14-3-3 protein. Most of these proteins, besides having a metabolic role, are also considered storage proteins and some of them behave as wheat grain allergens. Moreover, it is interesting to note that the 14-3-3 protein family was involved in plant-pathogen interaction and general stress response in barley[17]. Other proteins with potential role in defense responses have been identified as 14-3-3-binding proteins, including an *O* methyltransferase, which is thought to be involved in the production of antimicrobial secondary metabolites.

pH 3 **10**

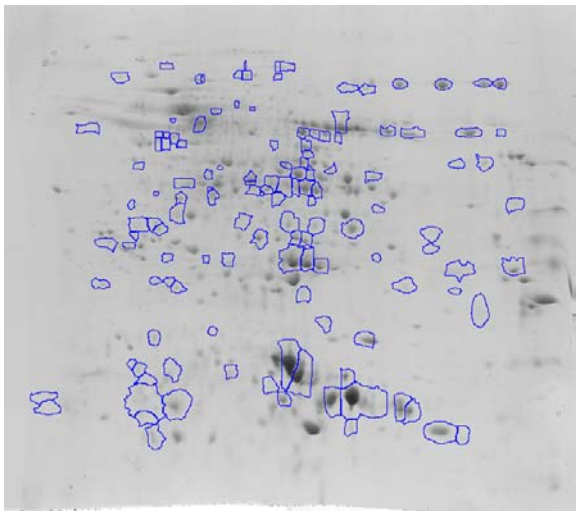

Fig. 1 IEF-SDS-PAGE of metabolic proteins in 3-10 pH range: the blue circles indicate the differential spots.

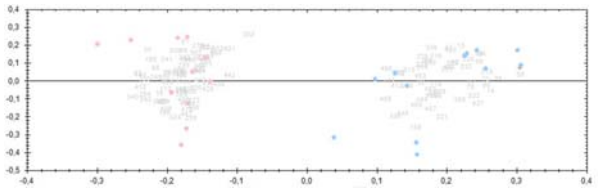

Fig.2 P.C.A representation in which differential spots relative to each gel analysed are reported. Red spots: heat stress samples; blue spots: control samples. Number indicate the differentially expressed polypeptides

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REFERENCES

1. Don C., Lookhart G., Naeem H., MacRitchie F., Hamer R.J., 2005. Heat stress and genotype affect the glutenin particles of the glutenin macropolymer–gel fraction. *J. Cereal Sci.* 42: 69–80.
2. Majoul T., Bancel, E., Triboi E., Hamida J.B., Branlard G., 2003. Proteomic analysis of the effect of heat stress on hexaploid wheat grain: Characterization of heat-responsive proteins from total endosperm**.** *Proteomics*. 3: 175-183.
3. Blumenthal, C. S., Batey, I. L., Bekes, F.,Wrigley, C.W. Barlow, E. W. R., 1990. Gliadin genes contain heat-shock elements: possible relevance to heat-induced changes in grain quality. *J. Cereal Sci.* 11: 185–187.
4. Pence J.W., Weinstein N.E., Mecham D., 1954. The albumin and globulin contents of wheat flour and their relationship to protein quality. *Cereal Chem.* 31: 303–311.
5. Singh J., Skerritt J., 2001. Chromosomal control of albumins and globulins in wheat grain assessed using different fractionation procedures. *J. Cereal Sci.* 33: 163–181.
6. Singh N. K., Shepherd K. W., 1985, The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm *Theor. Appl. Genet.* 71: 79–92.
7. Kobrehel K., Alary R., 1989a. The role of a low molecular weight glutenin fraction in the cooking quality of durum wheat pasta. *J. Sci. Food Agric.* 47: 487–500.
8. Kobrehel K., Alary R., 1989b. Isolation and partial characterization of two low molecular weight durum wheat (*Triticum durum*) glutenins. *J. Sci. Food Agric.* 48: 441–452.
9. Fränken J., Stephan U., Meyer H.E., König W., 1994. Identification of alpha amylase inhibitor as a major allergen of wheat flour. *Int. Arch. Allergy Immunol.* 104: 171–174.
10. Shy Y.C., Seib P.A., Bernardin J.E., 1994. Effects of temperature during grain-filling on starches from six wheat cultivar. *Cereal Chem.* 71: 369-383.
11. Hawker J.S., Jenner C.F., 1993. High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. *Austr. J. Plant Physiol.* 20: 197-209.
12. Keeling P.L., Bacon P.J., Holt D.C., 1993. Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch syntase. *Planta*. 191: 342-348.
13. Majoul T., Bancel E., Triboï E., Hamida J.B., Branlard G., 2004. Proteomic analysis of the effect of heat stress on hexaploid wheat grain: Characterization of heat-responsive proteins from non-prolamins fraction. *Proteomics*. 4: 505-513.
14. Vensel W. H., Tanaka C. K., Cai N., Wong J. H., Buchanan B.B., Hurkman W. J., 2005. Developmental changes in the metabolic proteins profiles of wheat endosperm. *Proteomics*. 5: 1594-1611.
15. Østergaard O., Finnie C., Laugesen S., Roepstorff P., Svensson B., 2004. Proteome analysis of barley seeds: Identification of major proteins from two-dimensional gels (p*I* 4-7). *Proteomics*. 4: 2437-2447.
16. O'Gorman M., Beauvallet C., Lepercq P. David O., Seksik Ph., Beaugerie L., Doré J.,. Martin P., . Bogard P,. Juste C., 2007. An Investigation into Crohn's Disease using the Progenesis SameSpots Analysis Platform. *24th Journée Françaises de Spectrométrie de Masse, PAU 2007.*
17. Finnie C., Borch J., Collinge D.B., 1999. 14-3-3 proteins: eukaryotic regulatory proteins with many functions. *Plant Molec. Biol.* 40: 545–554.