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PROTEOMIC ANALYSIS: AN EFFICIENT APPROACH FOR INVESTIGATING THE GENETIC AND ENVIRONMENTAL BASES OF WHEAT QUALITY.

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ABSTRACT

The proteomic approach encompasses the analysis of the whole set of proteins in a given tissue, or in cells, or in sub-cellular components, at a given time in the life of an organism. The identification of all expressed proteins, the study of their genetic determinism (gene mapping and gene sequencing), the study of their possible polymorphism, the identification of their function in the life of a cell and in physiological metabolism are the main tasks for which the proteomic approach is appropriate. Wheat genetic research can greatly benefit from this approach and some examples on kernel components are reported .

INTRODUCTION

Wheat storage proteins have received a great deal of attention in the two last decades. These early findings on genetic determinism, allelic diversity, protein sequences, and functional properties (for review see Lafiandra 1999) were in fact the main steps of proteomic analysis long before - thanks to progress in mass spectrometry - this approach became a powerful tool. Although plant proteomics is still in its infancy many different approaches have already been used for genetic and physiological studies (for review see Thiellement et al., 1999, Van Wijk 2001). Wheat endosperm proteins first separated using two-dimensional electrophoresis (2DGE) were partly identified after Edman amino-acid sequencing (Skylas et al., 2000, Nakamura 2001). Here we briefly report on the usefulness of this type of approach for analysing the characteristics of the kernel and the components of the endosperm. The identification of proteins in the total endosperm extract, or from subcellular organites or fractions, in response to both genotype and /or environmental effects, may provide a valuable tool for identifying potentially useful genes.

MATERIALS and METHODS

Plant Materials

Hexploid wheat cultivars were grown at the INRA Research Centre in Clermont Ferrand, France. Chinese Spring Synthetic and Opata cultivars were obtained from a field nursery in 2000, where they had been grown in normal conditions with full fungicide protection. The Thésée cultivar was grown in field conditions at the Clermont Ferrand agronomy research unit, then subjected to heat treatment soon after flowering as previously described (Majoul et al., 2003a)

Methods

The following methods are the main steps in proteomic analysis:

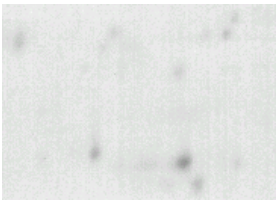
1. **2DGE** This is currently the most efficient method for protein separation [Rabilloud, 2002]. In our case, Immobiline pH gradient gel electrophoresis (IPG) was used as the first dimension and SDS poly-acrylamid gel electrophoresis as the second dimension. 2DGE was performed of the total protein extract from mature grains (Majoul et al., 2003a). In order to identify the common spots that characterise each proteic sample, 4 to 6 replicates were performed. Coomassie or silver staining procedures were used before gel scanning in transmission mode.
2. **Image analysis**. Melanie-3 software (GeneBio, Geneva, Switzerland), was used to compare images and spots. The SAS GLM procedure was used to test spot volume and percentage of spot volume in response to heat treatment versus normal growing conditions.
3. **Mass spectrometry** (MS). Spots of interest were excised and submitted to trypsic digestion. Two spectrometers were used (1) matrix-assisted laser desorption – the time of flight (MALDI-TOF) spectrometer giving peptide mass fingerprints and /or (2) Electrospray ion trap MS/MS spectrometer giving partial sequences of peptides.
4. **Protein identification**. The peptide masses resulting from the trypsic digest were compared with peptide masses in databases such as SwissProt, TrEMBL and NCBI, using for example Profound software (http://129.85.19.192/profound_bin/WebProFound.exe). The peptide sequences obtained from MS/MS can be used to identify proteins using dedicated software or manually against non-redundant SWISS-PROT and TrEMBL using Fasta software.

RESULTS and DISCUSSION

1-Puroindolines and grain hardness: Puroindolines are endosperm lipid binding proteins that have been associated to grain softness and the *Pin-a* gene has been found linked to the *Ha* gene (Sourdille et al., 1996). We developed a 2DGE method that has enabled many of the proteins focusing between pI 10.5 and 11 to be clearly identified (Branlard et al., 2003). For Synthetic, among its 10 proteins identified using mass spectrometry, 7 were of the puroindoline type, 2 of the grain softness type and 1 was a dehydrin. All these proteins belong to the amphiphilic class of proteins, i.e. are both hydrophilic and hydrophobic. The wheat amphiphilic proteins that are soluble in the non-ionic detergent Triton X114 were studied in the mapped ITMI progeny using the above 2DGE. Many of the segregating spots were mapped and identified using MS (Amiour et al., 2002). Further analysis of these proteins revealed that, in addition to some puroindolines, the dehydrin spot was involved in grain hardness.(Amiour et al., 2003)

2- Wheat storage proteins (WSP), namely gliadins and glutenins were mostly separated by 1D electrophoresis. Most of the allelic variants of the high and low molecular weight subunits of glutenins were described using SDS-PAGE. 2DGE of the WSP offers a powerful tool to reveal genetic diversity resulting from both ionic charge and mass modifications. WSP sequences were known from partial N terminal Edman analysis or deduced from DNA sequences of coding genes. The proteomic approach is a useful tool to answer questions such as: What are the different peptides encoded at the WSP locus? What are the covalently linked WSP that are simultaneously present in a given genotype? What are the mechanisms of WSP biosynthesis and of their accumulation in protein bodies. The use of the nullisomic lines in proteomic analysis has already shown that intergenomic interactions were involved in WSP synthesis (Islam et al., 2002, Dumur et al., 2003).

3- Environmental influence: Considerable effort is needed to identify the key genes involved in the stability of wheat quality. Gliadin accumulation has been shown to increase and glutenin synthesis to remain stable or to decrease at high temperatures (Blumenthal et al., 1993, Daniel et al., 2000). The proteomic approach used for the analysis of endosperm proteins from wheat kernel subjected or not to heat treatment revealed that a total of 37 spots were significantly changed at grain maturity (Majoul et al., 2003a). Further proteomic analyses of non storage proteins evidenced that some enzymes associated with starch biosynthesis were quantitatively modified after heat treatment (Majoul et al., 2003b).



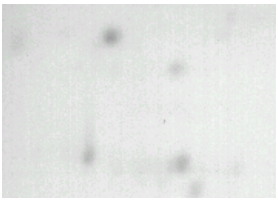


Figure 1:

The proteomic approach, which is complementary to transcriptome analysis, can also be advantageously used to track the expressed sequence tags (ESTs) encoding proteins of interest. Future progress in functional genomics and particularly in understanding the genetic causes of the stability of wheat quality characteristics will be facilitated by the use of proteomic analysis.

BIBLIOGRAPHY

Amiour, N., Merlino M., Leroy, P., Branlard, G. (2002). Proteomics 2, 632-641.

Blumenthal C.S., Barlow E.W.R., Wrigley C.W. (1993). J. Cereal Sci. 18, 3-21

Branlard, G., Amiour, N., Igrejas, G., Gaborit, T., Herbette, S., Dardevet, M., Marion, D. (2003). Proteomics 3, 168-174.

Dumur J., Jahier J., Branlard G. (2003). In: Proceeding of Eucarpia Cereal Section Meeting (Stanca M. ed), Salsomaggiore, Italy, 21-25 November 2002

Daniel C. Triboï E. (2000). J. Cereal Sci. 32, 45-56

Islam N., Woo S.H., Tsujimoto H., Kawasaki H., Hirano H. (2002). Proteomics 2, 319-326

Lafiandra D., Masci S., Blumenthal C., Wrigley C.W. (1999). Cereal Food World 44, 572-578

Majoul T., Bancel E., Triboï E., Ben Hamida J., Branlard G. (2003a). Proteomics 3, 175-183

Majoul T., Bancel E., Triboï E., Ben Hamida J., Branlard G. (2003b). Proteomics submitted.

Nakamura H.(2001). Cereal Chem. 78, 79-83.

Payne P. I., Corfield k. G., Blackman J. A. (1979). Theor. Appl. Genet. *55*, 153-159.

Rabilloud T. (2002*).* Proteomics 2, 3-10

Skylas D. J., Mackintosh J. A, Cordwell S. J., Basseal D. J. *et al*., (2000). J. Cereal Sci. 32,169-188.

Stone P. J., Nicolas M. E., (1994). Aust. J. Plant Physiol. 21, 887-900.

Sourdille P., Perretant M.R., Charmet G., Leroy P., Gautier M.F., Joudrier P., Nelson J.C., Sorrells M.E., Bernard M. (1996). Theor. Appl. Genet 92, 1542-1546

Thiellement H., Bahrman N., Damerval C., Plomion C., Rossignol M., Santoni V., de Vienne D., Zivy M. (1999). Electrophoresis 20, 2013-2026

Van Wijk K. J. (2001). Plant Physiology 126, 501-508