MASS-SPECTROMETRIC ANALYSIS OF THE WHEAT GLUTEN PROTEINS

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Proteinele glutenului din bobul de grâu sunt responsabile pentru proprietăţile viscoelastice ale aluatului şi condiţionează metoda de producere a pâinii, a pastelor făinoase şi a biscuiţilor. Paternul electroforetic al acestor proteine este foarte complex şi conţine un număr mare de spoturi. Fiecare dintre acestea poate fi compus din mai multe fracţii proteice cu proprietăţi fizico-chimice asemănătoare. În prezenta lucrare se demonstrează posibilitatea caracterizării detaliate a proteinelor din gluten separate prin metoda electroforezei bidimensionale, tratate cu tripsină şi/sau chimotripsină şi urmată de MALDI masspectrometirie (MS).

The commercial value of the wheat crop is a function of the quality and amount of the storage protein and starch present in the grain, which in turn are influenced by environmental conditions during grain-fill [2]. To understand how environment modifies the size and composition of wheat grains, we set out to identify the differences of the key metabolic and regulatory proteins in the two wheat grain cultivars.

The discovery that it was possible to generate gas-phase protein ion beams of the principle storage protein of maize kernels [1] followed by subsequent developments in matrix-assisted laser desorption ionization (MALDI) [4] and electrospray ionization (ESI) [8] mass spectrometry resulted in routine peptide and protein identification by these processes. Commonly employed techniques that are outgrowths of this discovery include "peptide mass mapping" by mass spectrometry and the generation of sequence specific fragments from individual peptides by tandem mass spectrometry. We focused on the specific extraction methods of the pattern of the storage proteins [2]. Any way the protein extracts contains some enzymes and factors that are important in cellular metabolism [3]. In this paper, we report on the development of methods and instrument conditions that we found necessary to identify the proteins in this fraction. We present results of studies aimed at establishing instrument conditions that will allow us to identify cytoplasmic proteins present in wheat endosperm.

Materials and Methods

Two-dimensional gel electrophoresis (2-D): Gluten proteins were extracted from a *Triticum aestivum* L. hard spring breadwheat (Butte 86) variety and spring variety (Chinese spring) in 50% propanol containing 1% dithiothreitol (DTT). Proteins were precipitated, solubilized in urea, separated by 2-D gel electrophoresis, and stained with Coomassie blue G-250. A 3–10% ampholyte mixture was used. The second dimension gel was run in a NuPAGE 4-12% acrylamide, Bis-Tris gel (Invitrogen, Carlsbad, CA).

***Protein Spot Digestion*:** Protein spots were excised from 2-D gels with razor shortened plastic pipette tips and the gel plugs dislodged with an intact pipette tip into microcentrifuge tubes for storage or into DigestPro (INTAVIS Bioanalytical Instruments AG, Bergish Gladbach, Germany) reaction tubes for processing. The DigestPro was used to automatically carry out alkylation, in-gel tryptic and chymotryptic digestion and elution into collection tubes using programs and conditions supplied by the manufacturer. The eluent was reduced in volume to about 20 to 30 µL by use of a Speed Vac SC100 (Thermo Savant, Holbrook, NY).

***MALDI Mass Spectrometry*:** Matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry was used to obtain mass fingerprints of the peptide fragments from proteins separated by 2-D gels. Approximately 0.5 µL of the eluent from the DigestPro was spotted onto an already-prepared MALDI matrix spot, ~0.4 µL of a mixture of 20 mg/mlalpha-cyano-4-hydoxy-transcinnamic acid and 5 mg/ml of nitrocellulose dissolved in acetone-propanol (1:1) as described by Schevchenko et al. [6]. After spotting, the sample was allowed to dry for 30 min at room temperature and the matrix spot washed 3 times with 5 µL of 0.1% TFA. Mass spectra were obtained using a Reflex II MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA) that was operated using delayed extraction in the reflectron mode. Porcine trypsin fragments were used as internal calibrants.

Results

The two-dimensional electrophoretic separation of two wheat varieties proteins is shown in Figure. Over forty six similar spots were identified and collected from Butte 86 variety and collected for Chinese spring. MALDI MS protein spectra showed the presence of one to five resolved protein components for each fraction, demonstrating the complexity of these proteins. High molecular weight subunits, which are most closely related to processing properties, were identified in the both varieties. Three of the five subunits identified showed charged *m/z* values very close to those predicted based on known DNA based sequences. This map was submitted to a database to search matched proteins using Mascot search tools.

Among matched proteins, glutenin high molecular weight chain 1Ax1 precursor-wheat with minimum sequence coverage of 27% was identified, an alpha/beta-gliadin precursor (prolamin) has been identified from chymotrypsin plus trypsin digested fractions with a minimum sequence coverage of 51% and has been confirmed by MS/MS sequencing. Increased coverage with chymotrypsin digestion was found for almost all proteins. This can be attributed to the low number of trypsin cleavage points in these proteins resulting in many peptides with MW values above the limit covered by the search engine [7]. Since many plant databases are still largely incomplete, many gluten proteins present in Butte 86 are absent in those databases. Even in this case, we expect that detailed sequence information and peptide maps will be enough to make definitive correlations with variety and/or quality. In Table are presented the unique proteins identified in the pattern of the Butte 86 and missed in the Chinese spring.

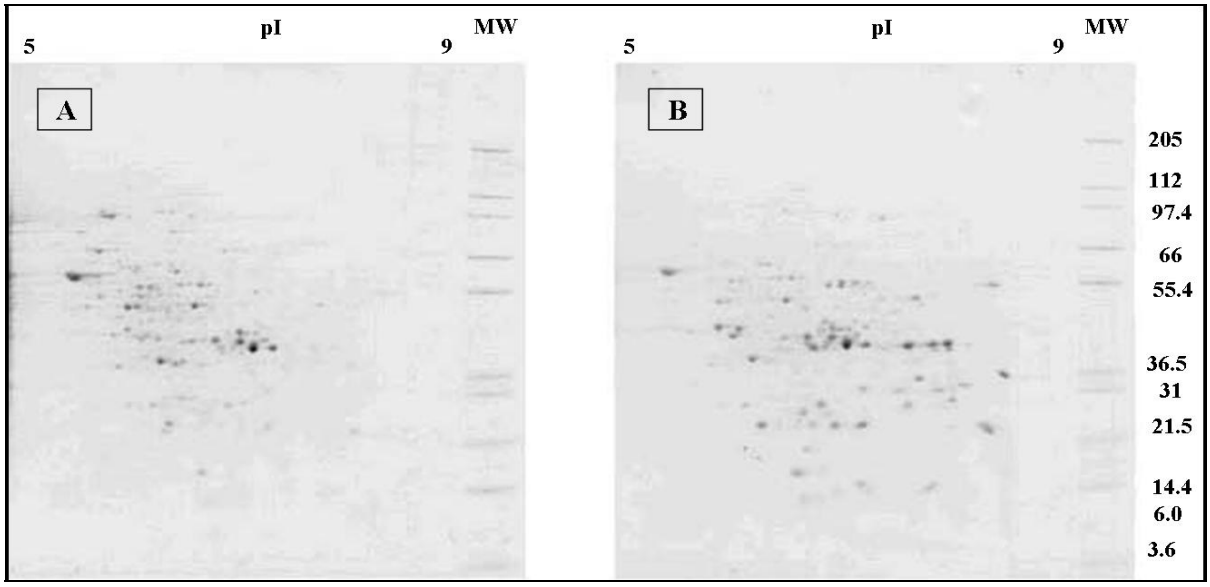

Fig. Distributions of the polypeptide patterns of the Chinese spring (A) and Butte 86 (B)

Table

The specific proteins identified in the patterns of the Butte 86 varieties

Nr.	Estimated MW	Name	Range	Code Data Bases	Amino Acid Sequence
1.	25.5	putative RNA binding	-1.5	rs-1|CT3295	**AVAEGAELLEDIVAEPLMHAHLQHFD**
2.	62.8	high affinity nitrate transptasa	-2.3	fr2|gnl|UG|Ta_S12923278	**AAGTIAAXXGMANXVARPMGGYLSDLGAR**
3.	35.5	gamma gliadin with 9 cys	-1.5	rs1|CT14965|-30_3pri_N_7e-26_AAK84776.1_	**RPLFQLVQGQGIIQPQQPAQLEVIR**
4.	41.7	LMW-GS Group I	-2.6	gi|17425164|	**VFLQQQCSPVAMPQSLAR**

5.	42.0	LMW-GS Group 3 type II	-2.2	gi|17425170|	**VFLQQQCSPVAMPQSLAR**
6.	41.7	LMW-GS Group I	-2.6	gi|17425164|	**VFLQQQCSPVAMPQSLAR**
7.	42.0	LMW-GS Group 3 type II	-2	gi|17425170|	**VFLQQQCSPVAMPQSLAR**
8.	35.5	gamma gliadin with 9 cys	-1.9	rs1|CT14965|-30_3pri_N_7e-26_AAK84776.1	**RPLFQLVQGQGIIQPQQPAQLEVIR**

This approach has the potential to better assess the complexity and structure of these proteins and to identify unique protein or peptide patterns that can be used as markers for quality. Previously used electrophoretic techniques for Quality Score identifications [5] for Moldovan wheat varieties have shown some problems, especially for fine differentiation of the near parental lines. We expect that detailed sequence information and peptide maps will be enough to make definitive correlations with variety and/or quality. Polypeptide score once identified, much more rapid by MS based techniques, can be potentially developed for quality marker selection in breeding programs.

Conclusions

Two-dimensional gel electrophoresis was used to compare the accumulation patterns of reserves proteins for two wheat varieties at the final stage of grain development. As would be expected, the 2-D gel patterns were significantly different. Mass spectrometry was used to identify the proteins in the 2-D gels by means of "peptide mass maps" of the in-gel enzymatically digested protein spots. We found that we could identify approximately 80% by ESI-MS/MS. Based on these results we plan to continue using MALDI-TOF in the first stage of sample screening. Identification of many more proteins and careful analysis of changes in protein accumulation profiles will allow us to determine the effect of environment on these events and more precise identification of wheat varieties.

References

1. Clegg G.A., Dole M. Molecular beams of macroions. III. Zein and polyvinylpyrrolidone // Biopolymers. - 1971. - Vol.10(5). - P.821-826.
2. DuPont F.M., Samoil V., Chan R. Extraction of up to 95% of Wheat (*Triticum aestivum* L.) Flour Protein Using Warm Sodium Dodecyl Sulfate (SDS) without Reduction or Sonication // J. Agric. Food Chem. - 2008. - Vol.56 (16). - P.7431-7438.
3. Hurkman W., Tanaka Ch., et. al. Germin-Like Polypeptides Increase in Barley Roots during Salt Stress // Plant Physiol. - 1991. - Vol.97(1). - P.366-374.
4. James P., Quadroni M., Carafoli E., Gonnet G. Protein identification by mass profile fingerprinting // Biochem. Biophys. Res. Commun. - 1993. - Vol.195(1). - P.58-64.
5. Samoil V., Serghei T., Postolatii A. Protein polymorphism and quality characteristics data for some wheat varieties cultivated in Moldova // Stiinţa agricolă. - 2008. - *in press*.
6. Shevchenko A., Wilm M., Vorm O., Mann M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels // Anal Chem. - 1996. - Vol.68(5). - P.850-858.
7. Sorensen D., Aagaard H., et.al. Variety identification of wheat using mass spectrometry with neural networks and the influence of mass spectra processing prior to neural network analysis // Rapid Communications in Mass Spectrometry. - 2002. - Vol.16. - P.1232-1237.
8. Wilm M., Mann M. Analytical properties of the nanoelectrospray ion source // Anal Chem. - 1996. - Vol.68(1). - P.1-8.

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