



Shotgun Proteomics and Its Agricultural Insights

Shreya^{1*}, Jogender¹ and Arjoo²

¹Research Scholar, Department of Genetics and Plant Breeding, CCS HAU, Hisar, Haryana, 125001, India.

²Research Scholar, Department of Horticulture, MHU, Karnal, Haryana, India.

Corresponding Author

Shreya

Email: agpalshreya1998@gmail.com



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ABSTRACT

In the realm of biological complexity, proteins surpass genes in both abundance and intricacy. To unravel their mysteries, sophisticated techniques are imperative. Shotgun proteomics, a revolutionary approach that dissects proteins into peptides for analysis. Coined by the Yates lab, this method involves enzymatic breakdown of proteomes, followed by liquid chromatography and mass spectrometry. Its helps in enhanced peptide resolution and simplified data interpretation, making it the method of choice for large-scale protein analysis. In agriculture, its applications are manifold, from deciphering crop stress responses to optimizing yield and quality. As technology evolves and integrates with novel methodologies, shotgun proteomics holds promise as a transformative tool in advancing agricultural research and sustainable food production.

INTRODUCTION

Proteins are the bio-functional entities that outweigh genes both in terms of number and complexity. This underlines the necessity for techniques to study them to be more intricate and

multifaceted. While the inception of two-dimensional polyacrylamide gel electrophoresis (2D PAGE) in the late 1970s opened doors to resolving complex protein mixtures, further refinement was necessary to

enhance its resolution power down to a much smaller scale. Shotgun proteomics emerged as a method that could separate even low-abundance proteins, aberrant proteins and membrane proteins. This strategy showcases another facet of bottom-up proteomics. “Bottom-up” protein analysis refers to the characterization of proteins by analysis of peptides released from the protein through proteolysis.

The term ‘Shotgun proteomics’ was coined by the Yates lab because of its analogy to shotgun genomic sequencing. Shotgun proteomics is the inferential analysis of proteoforms using peptide proxies produced by enzyme-catalyzed hydrolysis of entire proteomes (Meyer, 2021). At its core, shotgun proteomics embodies an approach in which a mixture of proteins is digested using protease and the resulting mixture of peptides is then separated by single or multiple liquid chromatography and analyzed mainly by mass spectrophotometry using different fragmentation approaches. Mass spectrometry has emerged as a major tool for large-scale protein analysis. In the past decade, there has been a rapid advance in the resolution, mass accuracy, sensitivity and scan rate of mass spectrometers used to analyze proteins. Using bioinformatics and database searching algorithms, putative peptide sequences are then validated and assigned to the identified proteins. Database searching software such as SEQUEST1 and MASCOT have algorithms that assign a peptide sequence to each tandem mass spectrometry (MS/MS) spectrum by comparing experimentally acquired spectra against theoretically predicted spectra of peptides derived from a sequence database (generated from *in silico* digestion of a protein database). Scores reflecting the similarity between the measured and predicted spectra are used to discriminate between correct and incorrect peptide sequence assignments.

Starting with peptides instead of intact proteins has advantages over top-down methods, such as improved LC separation, uniform electrospray ionisation, more complete fragmentation in tandem mass spectrometry (MS/MS), and easier interpretation of simplified fragmentation patterns. Over the past two decades, bottom-up/shotgun proteomics has become the preferred framework for protein analysis due to its numerous benefits (Gao and Yates, 2019). Large scale shotgun proteomics effectively began with the introduction of multidimensional protein identification technology (MudPIT) (Gilmore and Washburn, 2010). MudPIT significantly outperformed 2D-PAGE techniques in two areas, namely the detection and identification of membrane proteins and low abundance proteins.

Technique in a nutshell:

The technique is fundamentally separated into five parts (Fig. 1), each with a straightforward strategy that leads to the separation and identification of distinct entities based on MS data.

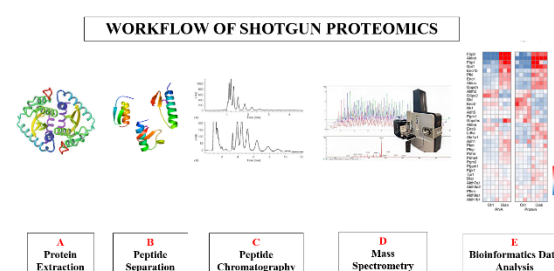


Fig. 1: Workflow of Shotgun proteomics

- 1. Protein Extraction and Isolation:** The first step involves rupturing of cells or tissues to release proteins. For this, various methods can be employed, such as mechanical disruption, chemical lysis or enzymatic digestion. After extraction, proteins need to be isolated from other cellular components. Centrifugation, filtration, or precipitation techniques can

be used to separate them out of the other cellular components. This stage requires careful optimization to maintain protein integrity and prevent degradation.

2. **Protein Separation:** Proteins extracted from complex biological samples can be highly diverse in terms of size, charge and abundance. Techniques like gel electrophoresis (SDS-PAGE) or liquid chromatography (LC) are used to separate proteins based on size or charge, respectively. Separation is often followed by protein staining or labeling to visualize and quantify proteins.
3. **Peptide Chromatography:** After protein separation, proteolytic digestion (commonly with trypsin) breaks proteins into peptides. Peptide mixture is complex and needs further separation. Liquid chromatography (LC) is used to separate peptides based on properties like hydrophobicity. High-performance liquid chromatography (HPLC) or nano-flow LC are popular choices.
4. **Mass Spectrometry:** Mass spectrometry (MS) is a key technique for analyzing peptides' mass-to-charge ratios (m/z) and deriving their sequence and identity. Peptides eluted from chromatography are ionized and then subjected to mass analysis. Tandem MS (MS/MS) involves isolating specific ions and fragmenting them to deduce amino acid sequence.
5. **Bioinformatics Data Analysis:** MS generates massive data sets of peptide masses and fragment ions. Data interpretation relies heavily on bioinformatics tools and databases to match observed mass and fragment data to known protein sequences. Search algorithms like SEQUEST, Mascot or MaxQuant are employed for peptide

identification. Statistical analysis and false discovery rate (FDR) estimation are crucial to ensure accurate results. Further analysis involves protein quantification, post-translational modification identification and pathway analysis.

Applications of the technique:

Shotgun proteomics allows global protein identification as well as the ability to systematically profile dynamic proteome of a cell. Therefore, it has found various applications in the field of agriculture, enabling researchers to gain insights into plant biology, crop protection and agricultural practices. Here are some key applications:

1. Crop Proteomics and Stress Response:

Shotgun proteomics helps in studying the protein expression changes in crops under various stresses like drought, heat, salinity and pathogen infections. By identifying stress-responsive proteins, researchers can understand the molecular mechanisms underlying stress tolerance and develop more resilient crop varieties.

2. Plant-Pathogen Interactions:

Studying the proteomes of plants and pathogens during interactions provides insights into the molecular basis of disease resistance and susceptibility. Identifying proteins involved in defense responses and virulence factors of pathogens aids in developing strategies for disease management (Balotf *et al.*, 2022).

3. Crop Quality and Yield Improvement:

Shotgun proteomics can be used to analyze the proteomic profiles of crops with different nutritional qualities or yield potentials. Understanding the protein composition related to taste, nutritional

value and yield can guide breeding programs aimed at producing improved crop varieties.

4. Agricultural Biotechnology:

Proteomic analysis assists in characterizing genetically modified (GM) crops to ensure that the desired traits have been introduced without unintended protein changes. It can help in evaluating protein expression patterns which further aids in assessing the safety and potential allergenicity of GM crops.

5. Plant Growth and Development:

Shotgun proteomics helps in unraveling the protein networks and signaling pathways that regulate various aspects of plant growth and development, such as flowering, fruiting and senescence. Understanding these processes can lead to strategies for optimizing growth and increasing crop productivity.

6. Nutrient Management:

Analyzing proteomic responses to nutrient deficiencies or excesses provides insights into nutrient uptake, assimilation and utilization by plants. This knowledge can inform precision agriculture practices to optimize nutrient management for improved crop yield and quality.

7. Bio-fertilizers and Biostimulants:

Shotgun proteomics aids in studying the impact of bio-fertilizers and biostimulants on plant proteomes. Identifying proteins associated with enhanced nutrient uptake and stress tolerance helps in developing sustainable agricultural practices.

8. Post-Harvest Quality and Storage:

Analyzing proteomic changes in harvested crops during storage helps in

understanding the factors affecting post-harvest quality, shelf life and resistance to spoilage. This knowledge guides the development of storage conditions and treatments to extend the freshness of harvested produce.

Future prospects:

As we peer into the future, the prospects of shotgun proteomics in agriculture are indeed promising. The continuous refinement of mass spectrometry and bioinformatics tools promises higher sensitivity, accuracy and throughput, making the technique more accessible and efficient for large-scale agricultural studies. Integrating proteomic data with other 'omics' approaches, such as genomics and metabolomics, will foster a holistic understanding of complex biological systems, paving the way for the development of targeted interventions to address agricultural challenges sustainably (Santos *et al.*, 2022). Moreover, the convergence of shotgun proteomics with emerging technologies like artificial intelligence and machine learning holds tremendous potential to accelerate data interpretation, enhance predictive modeling and uncover intricate protein networks that underpin crop performance and resilience.

CONCLUSION:

In conclusion, shotgun proteomics stands as a remarkable technological advance that has revolutionized our ability to unravel the complex world of proteins within agricultural systems. Through its multifaceted applications, shotgun proteomics has significantly enriched our understanding of agricultural processes and catalyzed innovative solutions across various fronts. From gaining insights into crop stress responses, plant-pathogen interactions and nutrient management, to improving crop quality, yield and post-harvest strategies, this technique has left an indelible mark on the

agricultural landscape. In the journey ahead, shotgun proteomics will continue to shape the trajectory of agricultural research and innovation. This technology empowers the scientists to devise smarter cultivation practices, devise novel crop protection strategies and cultivate the next generation of high-yielding, nutrient-rich crops. As we harness its capabilities to address the ever-evolving demands of global agriculture, shotgun proteomics stands poised to elevate the sector to unprecedented heights, ushering in a new era of sustainability, productivity and food security.

REFERENCES:

- Meyer, J. G. (2021). Qualitative and quantitative shotgun proteomics data analysis from data-dependent acquisition mass spectrometry. *Shotgun Proteomics: Methods and Protocols*, 297-308.
- Gilmore, J. M. & Washburn, M. P. (2010). Advances in shotgun proteomics and the analysis of membrane proteomes. *Journal of proteomics*, 73(11), 2078-2091.
- Gao, Y. & Yates III, J. R. (2019). Protein analysis by shotgun proteomics. *Mass Spectrometry-Based Chemical Proteomics*, 1-38.
- Santos, M. D., Lima, D. B., Fischer, J. S., Clasen, M. A., Kurt, L. U., Camillo-Andrade, A. C. & Carvalho, P. C. (2022). Simple, efficient and thorough shotgun proteomic analysis with PatternLab V. *Nature Protocols*, 17(7), 1553-1578.
- Balotf, S., Wilson, R., Tegg, R. S., Nichols, D. S. & Wilson, C. R. (2022). Shotgun proteomics as a powerful tool for the study of the proteomes of plants, their pathogens, and plant-pathogen interactions. *Proteomes*, 10(1), 5.