Agricultural future: Proteomics as a tool in crop breeding

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ABSTRACT

Proteins are essential for genetic products and their study contributes to obtaining regulatory information. Multiomics techniques are used to improve agronomic characteristics and increase agricultural tolerance, the use of these techniques can serve as a basis to improve the genetic heritage of crops, increasing their yields and tolerance to environmental factors. Proteomic analysis makes it possible to understand all the functions, structures, and interactions of proteins present in a biological system. Through a systematic review, a search was carried out, finding 15 articles related to the proteomic technique and its application in agriculture. In proteomic analysis, one of the main advantages is the ability to study protein expression on a large scale in diverse complex biological systems and at different times and conditions. Proteomic technology has been widely used in identifying proteome changes in signaling pathways in response to stress, detecting biotic and abiotic stress markers, which can be applied in the development of genetically modified crops. These advances in plant proteomics have the potential to revolutionize agriculture by providing sustainable and adaptable solutions to address future food production challenges.

Keywords: Omics, Proteins, Breeding.

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INTRODUCTION

Proteins are essential for genetic products and their study contributes to obtaining regulatory information (Weckwerth, 2011). Through advancements, proteomics has emerged as a popular tool for further approaches in omics and systems biology (Weckwerth et al., 2014). The term proteomics was established in 1996 by the fusion of the words "protein" and "genomics", it can be described as "the efficient and/or standardized analysis of all proteins present in tissues, cells or in the subcellular compartment" (Chaturvedi et al., 2016). Proteomics is a non-targeted technology, aiming to analyze the complete proteome of the target organism, although it may also involve the targeted identification and quantification of specific proteins/peptides. The proteomic profile reveals the level of protein abundance in different types of cells and tissues in any state, as well as between samples of various combinations (Silva-Sanchez et al., 2015; Fíla et al., 2016).

One of the current obstacles related to agricultural production is associated with the need for food and the adaptation of crops for high yield and resistance, whether to pests or atmospheric elements, in order to meet global demand (Haq et al., 2023). According to data from the United Nations, it is necessary to increase agricultural production by 75% by 2050 to ensure the food supply of the entire world population (Bates et al., 2008). In addition to large-scale production, the quality of food and its ability to meet nutritional demands are also of great importance. The study of genetic diversity to discover and create new crop variations, along with understanding the genetics of simple and complex traits and efficiently introducing these variations into new cultivars, are means to achieve these goals (Haq et al., 2023; Brozynska et al., 2016). Multiomics techniques are used to improve agronomic characteristics and increase agricultural tolerance, the use of these techniques can serve as a basis for improving the genetic heritage of crops, increasing their yields and tolerance to environmental factors (Haq et al., 2023)

Proteomic analysis makes it possible to understand all the functions, structures, and interactions of proteins present in a biological system. Since defective proteins are the main causes of diseases, they can serve as useful indicators in the development of specific diagnoses and treatments for certain diseases, whether in animal or plant cells (Anjolette, 2015). Proteomics not only provides comprehensive information about proteins, but also makes it possible to obtain quantitative profiles, evaluate post-translational modifications, investigate signaling pathways, and study interactions between proteins (Kaushik et al., 2024). In recent decades, proteomic technologies have been widely employed to assimilate plant responses to various external and internal signals, contributing significantly to an understanding of plant behavior in the face of changes in environmental conditions (Kaushik et al., 2024; Weckwerth et al., 2014).

Plants are constantly exposed to unfavorable conditions, such as biotic and abiotic stresses, which include variations in the availability of water, light, nutrients, and extreme temperatures

(Bokszczanin et al., 2013). These environmental modifications play an important role in plant performance, especially during the reproductive cycle, and can reduce crop yields (Tanou et al., 2012). To cope with these environmental constraints, most plants develop defense mechanisms that involve alterations in gene expression, resulting in changes in protein translation and metabolic reprogramming. These mechanisms are essential for metabolic adaptation and plant survival under stress conditions (Tanou et al., 2012; Chaturvedi et al., 2016). Thus, in order to promote greater crop sustainability, it is essential to understand the genetic and molecular conditions of the stress response mechanisms, as well as the physiological parameters (Chaturvedi et al., 2016).

Thus, the objective of this study was to carry out a systematic literature review to better understand the use of proteomic technology in genetic improvement, its application and benefits related to agriculture, especially in relation to biotic and abiotic factors.

METHODOLOGY

Through a systematic review, 15 articles were found in a search for studies related to proteomic biotechnology and its applications. The databases used were Medline/PubMed and Scopus. The articles that were admitted were in Portuguese and English. The keywords used in the research were: "Proteomics", "Genetic improvement", "Omics", "sustainable agriculture", "molecular biology", "genome alteration", "mass spectrometry", "genetic improvement".

The inclusion criteria used for the selection of articles were: (I) articles that report the global scenario in relation to sustainable agriculture; (II) articles with studies on genetic improvement techniques; (III) articles published in national and international journals; (IV) articles with the use of proteomic technology; (V) original articles, with a time frame from January 2004 to January 2024. The following were excluded from the study: (I) articles related only to the problem of agriculture; (II) articles related to other genetic improvement techniques unrelated to omics/proteomics technology; (III) editorial articles and case studies.

An initial search was carried out based on the title of the articles, and the selected studies were evaluated according to the criteria described. The information extracted is based on the characteristics of the study, title, publication and relevant considerations.

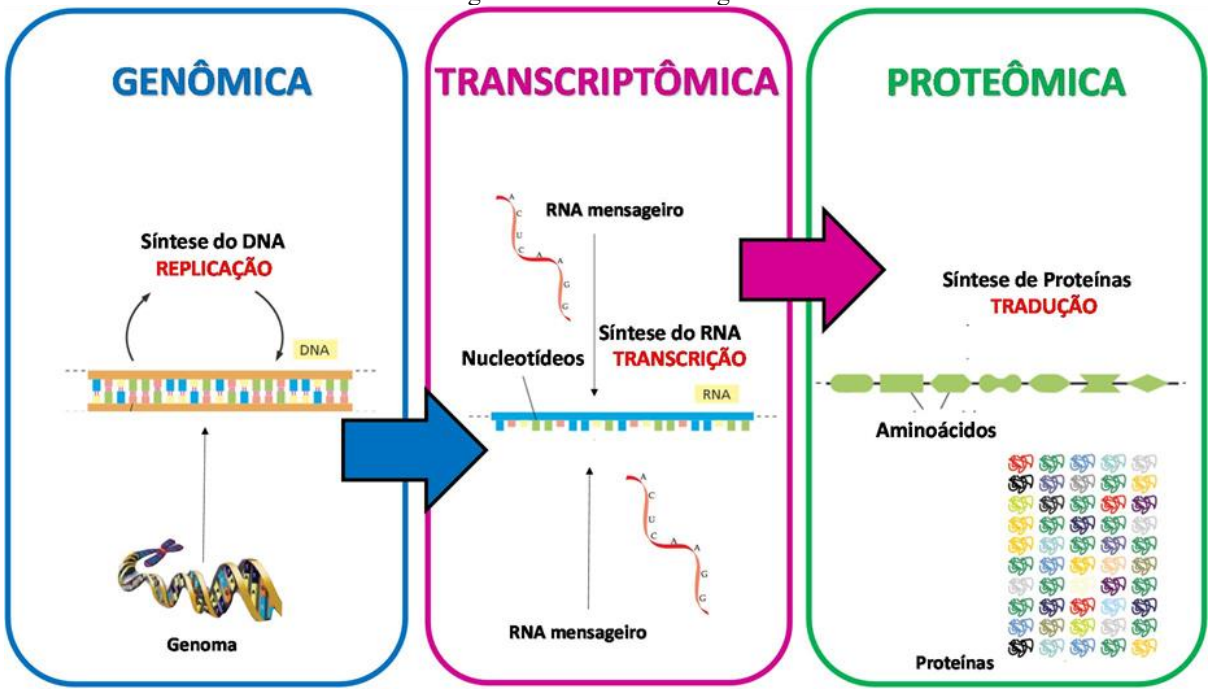
RESULT AND DISCUSSION

OMICS SCIENCES

Due to their sessile characteristic, plants develop strategies for adaptation and growth in response to changing environmental circumstances (Raza et al., 2019). At the molecular level, these tactics include changes in gene expression, from transcriptional regulation, through mRNA processing, followed by translation, protein modification, and protein renewal (Rejeb et al., 2014;

Haq et al., 2023). When exposed to controversial environmental conditions, the plant modifies its transcriptome, transcriptionally regulated genes play roles in diverse functions such as signaling, translation, transcription, metabolism, and stress response molecules (Rejeb et al., 2014). New biochemical and bioanalytical tools, such as genome sequencing, transcriptomics, proteomics, and metabolomics, are enabling more detailed analyses of these processes (Astarita & Ollero, 2015). Omics technology (figure 1) refers to the study of these processes in the genome, transcriptome, proteome, and metabolome contexts (Haq et al., 2023).

Figure 1. Omics technologies.



Overview of the fields of "omics" analyses. Genomics provides information on the complete set of genes in a complex biological system, while transcriptomics provides a complete description of the messenger RNAs in the genome. Proteomics provides information on the proteins expressed by the genome. (Anjolette, 2015)

Molecular markers are studied in genomics with the aim of discovering new patterns of variation and determining their functions in significant ecological traits (Bevan & Waugh, 2007). In crops, breeding is linked to genomic approaches to achieve advances in molecular breeding and to track elite germplasms with multi-trait assembly (Raza et al., 2019; Kole et al., 2015). Omics approaches aid in the development of crops with better yield and yield under different biotic and abiotic factors. Molecular plant breeding is an essential approach to increase crop yield and production in the presence of various factors (Raza et al., 2019).

PROTEOMICS

In recent years, studies addressing transcriptome and metabolome techniques have been conducted to identify transcriptional regulators and metabolites, providing fundamental insight into how different networks are affected and interact during the priming process (Luo et al., 2009).

However, there are limitations in estimating gene expression levels, mRNA degradation or inefficient translation, as well as in post-translational protein modifications (PTMs), protein processing and renewal, so proteomics certifies as an essential tool to bridge the gap between the transcriptome and the metabolome (Tanou et al., 2012).

The transcriptome and proteome are more complex than the genome, as distinct responses can be attributed to the same gene, and multiple proteins can be translated from the same mRNA, while the genome is a static source of information, the transcriptome and proteome are dynamic, and can vary under different conditions due to RNA processing, regulation of transcription, protein synthesis and protein modifications (Bernot, 2004). Alternative splicing, alternative promoters, or the use of polyadenylation sites can generate multiple transcripts. In addition, modifications such as glycosylation and phosphorylation can occur during or after translation, resulting in a wide variety of protein variants (Twyman, 2004; Anjolette, 2015)

Proteomic studies offer the opportunity to investigate subcellular proteomes and protein complexes, including proteins in plasma membranes, chloroplasts, mitochondria and nuclei, and, most significantly, priming-associated PTMs (Angel et al., 2012). After two-dimensional protein extract separation, the most recent advances in mass spectrometry-based proteomics (MS), such as ion mobility separations, microchip-based proteome measurements, nanoscale reversed-phase liquid chromatography, and capillary electrophoresis, have been applied as fundamental in the protein separation process (Tanou et al., 2012; Angel et al., 2012).

The variety of instruments available for proteomic analysis makes this choice complex and challenging. Currently, mass spectrometry is the main analytical method used in proteomic studies for the identification and characterization of protein compounds (Thompson; Schaeffer-Reiss; Ueffing, 2008). In proteomic analysis, one of the main advantages is the ability to study protein expression on a large scale in several complex biological systems and at different times and conditions (Barbosa et al., 2012).

The three main steps of proteomic methodologies are identification/quantification, extraction, and separation (with or without gel). Gel-free methodologies include techniques such as LC-MS, and label-based techniques such as ICAT and iTRAQ (Haq et al., 2023). Due to the complexity of the plant proteome, a single method cannot accurately assess it, so several techniques are used to improve the understanding, resolution, and comprehensiveness of the plant proteome (Altelaar et al., 2013). The choice of proteome study methodology depends on several factors, such as the availability of resources, the type of facilities and the desired applications, whether it is a global or targeted profile and the compound of interest (Anjolette, 2015). Gelless proteomics is becoming more prevalent, compared to gel-based proteomics, it has higher reproducibility and less bias (Haq et al., 2023).

GEL-FREE PROTEOMIC METHODOLOGY

Among the quantitative techniques, tag-based labeling (ICAT, iTRAQ), metabolic labeling (SILAC), and free labeling (MudPIT) stand out (Haq et al., 2023). Isotope-coded affinity labeling (ICAT) is a quantification methodology that uses a biotin affinity tag, a stable isotope ligand, and a reactive component that binds to thiol groups of proteins (cysteines) in vitro (Shiio & Aebersold, 2006). Chromatography is employed to separate the labeled triptych peptides prior to their detection by mass spectrometry (MS). ICAT is widely used to discover new proteins that influence important biological functions in specific cultivars (Barkla et al., 2013).

In the multiplex protein quantification method known as iTRAQ (isobaric labeling for relative and absolute quantification), isobaric markers are used to identify N-terminal protein and side-chain amine groups (Haq et al., 2023). This technique allows the quantification of proteins from multiple sources in a single experiment, with much higher sensitivity than ICAT. Crop researchers use iTRAQ to detect markers of biotic and abiotic stress, which can be applied in the development of genetically modified crops (Su et al., 2019).

Amino acid-stable isotope labeling in cell culture (SILAC) is the most effective metabolic labeling tool for dynamic quantitative research of the plant proteome. This technique involves in vivo labeling of cell populations cultured in medium containing N14 or N15 (Soufi & Macek, 2014). The identification of proteome changes in signaling pathways generated by PTMs in response to stress is of great value (Mastrobuoni et al., 2012).

MudPIT is a shotgun proteomic approach to analyze complex, low-abundance proteins (Zhang et al., 2010). This technology is more sensitive, it separates digested proteins using biphasic or three-phase microcapillary columns, which are then analyzed by tandem MS (Haq et al., 2023). MudPIT has been used to explore the mechanisms that regulate tiller count in rice (Lee et al., 2011).

GEL-BASED PROTEOMIC METHODOLOGIES

The gel methodology is the most popular, adaptable and recognized protein separation and quantification technique. They are more cost-effective than gel-free approaches and can be used to characterize protein isoforms and identify low-abundance proteins (Sriyam et al., 2007). Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is critical in proteomics due to its accessibility and familiarity. The isoelectric point (pI) and molecular weight (M) are applied to differentiate proteins (Marouga et al., 2005). Proteins are classified into two groups based on their molecular weight (M) and the presence or absence of 2-mercaptoethanol. They can be visualized with dyes such as Coomassie blue, silver nitrate, or SYPRO Ruby (Rabilloud, 2013).

Difference gel electrophoresis (DIGE) was developed to overcome the limitations of 2D-PAGE, such as variations between gels and low repeatability. The DIGE technique allows the

evaluation of variations in protein expression in response to biotic and abiotic stresses (Marouga et al., 2005). To reduce the co-migration interferences observed in 2D-PAGE, three-dimensional gel electrophoresis (3DGE) is used, which accurately identifies proteins and post-translational modifications (PTMs) using two distinct buffers with different ion carriers (Sriyam et al., 2007).

After digestion of the peptides, the proteins of interest are identified by mass spectrometry (MS). Computational approaches aid in the identification of proteins based on peptide mass and fragmentation (MS/MS) data (Haq et al., 2023). The process of identifying the protein by MS involves three phases: transforming molecules into gas-phase ions, separating them in an electric or magnetic field according to their mass-charge ratio (m/z), and identifying the separated ions with specific m/z values (Sriyam et al., 2007). Ionization methods include electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI), and surface-enhanced laser desorption/ionization (SELDI) (El-Aneed et al., 2009).

MASS SPECTROMETRY

This technique works by identifying signals generated by ion transitions during fragmentation, using a mass spectrometer. Additional tools include MS tandem, linear quadrupole orbitrap trap (LTQ-Orbitrap), quadrupole trap (Q-trap), and triple quadrupole trap (Haq et al., 2023). Multiple reaction monitoring (MRM) is the process of detecting multiple changes, while selected reaction monitoring (SRM) is the process of identifying transitions in a triple quadrupole (Yocum & Chinnaiyan, 2009). On the other hand, the approaches mentioned above focus on sample accuracy. To circumvent this problem, SRM/MRM methods use isotopic labeling (Yocum & Chinnaiyan, 2009).

SRM transitions are highly specific scans that aim to detect specific analytes in complex mixtures, usually using triple-quadrupole-based mass spectrometers (Anderson & Hunter, 2006). These transitions are planned so that the first mass analysis quadrupole (Q1) is configured to transmit a narrow mass window around the desired parent ion, while the third quadrupole (Q3, the second mass analysis quadrupole) is configured to transmit a narrow mass window around the desired fragment ion (Wolf-Yadlin et al., 2007). Fragmentation occurs in the second quadrupole (Q2) via collision-induced dissociation (CID). Therefore, SRM requires the presence of two ions to produce a positive result, which makes it a highly specific detection methodology with a very low noise level, thus increasing the sensitivity of detection (Yocum & Chinnaiyan, 2009). The success of SRM transitions depends not only on the ionization efficiency of the parent ion (Q1 transmission), but also on the fragmentation efficiency of this parent ion and, consequently, on the intensity of the fragment ion (Q3 transmission) (Jenkins et al., 2006). By inserting several different SRM transitions to the

same analyte or to different analytes, multiple transitions can be monitored in a single MS run. This method is known as MRM, balancing productivity and sensitivity (Yocum & Chinnaiyan, 2009).

For the identification and quantification of proteins, MRM-MS has not been as widely used due to challenges in the development of the method (Griffiths et al., 2007). Although it is theoretically possible to introduce a standard mixture of proteins into a sample at concentrations known for quantification, similar to what is done in small molecule analyses, in practice this does not guarantee robust protein quantification (Yocum & Chinnaiyan, 2009). This is because standard proteins and analyte proteins can produce different responses in the mass spectrometer due to ion suppression and variations in fragmentation (Barnidge et al., 2004). In addition, the recovery rates of the different proteins may be affected by sample preparation steps prior to MS. For this reason, isotopically labeled peptides instead of proteins are suggested for accurate protein quantification (Yocum & Chinnaiyan, 2009).

PROTEOMICS AS A TOOL IN AGRICULTURE

The secretome encompasses all secreted proteins, accounting for up to 30% of an organism's proteome, and plays critical roles in a variety of cellular processes (Skach, 2007). These proteins are essential for functions such as signal perception and transduction, stress responses, and apoptosis. The secretion process in plants is highly sophisticated and tightly regulated, and it has been observed that secreted proteins act both locally and systemically (Gupta et al., 2011). The genes responsible for encoding these proteins are less frequently present in the central genome, but are more common in mobile regions. However, the application of proteomics to analyze the secretome is limited due to several factors, namely, the secreted proteins can be expressed in low abundance; produced by specialized cell types and expressed at specific stages of development (Ahsan et al., 2007).

Chickpeas are an important legume consumed globally as a source of vegetable protein, containing 25% protein in their composition (Gupta et al., 2011). In a study with suspended culture of chickpea calluses, Gupta (2011) the secretome was characterized using classical SDS-PAGE coupled to mass spectrometry. As a result, 773 secreted proteins were identified, providing a comprehensive view of the secretome of a dicotyledon species. Regarding the identified proteins, most were related to primary and secondary metabolism (19.1%), followed by signal transduction (14.1%), proteins with different functions (11.6%) and maintenance of the redox state (9.2%) in the extracellular space. Another category included proteins involved in cellular defense (9%) and transport (8.9%) between intercellular regions. Proteins involved in protein folding (8.9%) and modification (6.7%) of proteins were also identified. Proteins whose identity was not determined were classified as unidentified (6.6%). In addition, several proteins (5.9%) involved in cell wall modifications were detected.

Proteomic analysis is very useful for the identification of proteins extracted from wheat bread grain (*Triticum aestivum* L.), in a study by Lesage et al. (2012), a comparative analysis of the proteome of two quasi-isogenic strains (NILs) was performed with the aid of two-dimensional electrophoresis (2-DE) and mass spectrometry. As a result of the analysis, during grain development, folding and stress-related proteins were more abundant in the hard strain compared to the soft line. These results suggest that protein matrix formation occurs earlier in the hard lineage, indicating an earlier stress response, possibly the unfolded protein response, compared to the soft line, leading to earlier cell death in the endosperm. In this way, new perspectives emerge on the role of purindolins in the folding machinery of storage proteins, thus affecting the development of the wheat endosperm and the formation of the protein matrix.

PROTEOMICS IN THE INVESTIGATION OF ABIOTIC AND BIOTIC MECHANISMS OF TOLERANCE

Katam et al. (2020), in a study investigated the effects of various abiotic stresses on the regulation of leaf proteins in soybean cultivars, providing valuable data on the proteomic and enzymatic responses of soybean to these stresses under field conditions. Using 2-DE protein mapping and mass spectrometry, the results revealed that simultaneous stresses cause changes in physiology, proteome and enzymatic activity, differently from what occurs in situations of individual stress. A significant degree of genetic diversity was observed in the protein abundance between the two cultivars when subjected to different types of stresses. Multiple proteins related to metabolism, heat response, and photosynthesis exhibited cross-tolerance mechanisms. This phenomenon was especially evident in cultivar R95-1705, where proteins responsive to heat, photosynthesis, metabolism and redox proteins were abundantly present in response to heat stress, as well as to combined heat and water stress in cultivar PI-471938, suggesting a relative heat tolerance in the latter.

Heat and drought are the main abiotic stressors that limit the growth and development of soybean plants, at the cellular level, plants exhibit a variety of physiological and biochemical responses to overcome these stresses (Eldakak et al., 2013). In a study, Das et al. (2016) investigated the differential expression of proteins in soybean (Glycine max L.) in response to drought and heat stress. They identified 44 proteins responsive to abiotic stress that influenced signaling cascades and molecular processes. In addition, many proteins related to photosynthesis, which were expressed in a differential manner, impacted RuBisCO regulation, electron transport, and the Calvin cycle during periods of abiotic stress. According to the results obtained, 25 proteins related to photosynthesis were downregulated under stress conditions in both soybean varieties.

Corn (Zea mays L.) It faces severe threats due to various abiotic stresses, such as drought, salinity, cold, heat, and flooding. Among these factors, drought or water deficit is the most critical, posing a significant threat to maize production worldwide (Yousaf et al., 2023). In a study on the responses to water stress in maize at the protein level, Zenda et al. (2018), employed an iTRAQ-based quantitative strategy to perform the proteomic profiling of two contrasting inbred maize strains. A comparative proteomic analysis of the leaves of these two lines and some physiological responses under water stress were performed. They analyzed 721 differentially abundant proteins (DAPs) in two maize strains, identifying both common and single proteins accumulated in response to water limitation in maize. Using an iTRAQ-based method, which resulted in a total of 721 differentially abundant proteins (DAPs), five essential sets of drought-responsive DAPs were identified, including 13 specific DAPs.

Wang et al. (2019), in proteomic analysis of the filling core proteomes of two drought-tolerant maize strains, used an iTRAQ-based strategy to identify protein expression profiles during grain development and to compare the water stress responses of the same strains (YE8112 and MO17) after a 14-day exposure to moisture deficit. As a result, a variety of molecular elements have been identified that are involved in mediating drought tolerance, There was a change in the proteome of the stressed plants compared to the control conditions. A total of 5,175 DAPs were identified in the four experimental comparisons, DAPs expressed exclusively in YE8112 were mainly involved in pathways related to "protein processing in the endoplasmic reticulum" and "tryptophan metabolism", while DAPs in MO17 were associated with "starch and sucrose metabolism" and "oxidative phosphorylation" pathways. Thus, the results revealed that YE8112 grains were comparatively more drought tolerant than MO17 grains.

Barley (Hordeum vulgare L.) stands out as one of the most salinity-tolerant crops, being an excellent model for studies on the mechanisms and inheritance of salinity tolerance, in addition to being fundamental for the development of tools that improve salt tolerance in cereals (Wang et al., 2018). In order to perform a biochemical and proteomic analysis of barley, Zhu et al. (2020), verified two pairs of quasi-isogenic strains (NILs), which are genetically almost identical, except for the target region containing QSl.TxNn.2H. According to the study, 53 and 51 differentially expressed proteins were identified in leaves and roots, respectively. QTL QSl.TxNn.2H can improve salinity tolerance by controlling the load of Na+ in the xylem, reducing the toxicity of Na+ in leaves. In addition, this QTL induces the expression of proteins related to photosynthesis, elimination of reactive oxygen species (ROS) and ATP synthase genes.

Huang et al. (2016) applied proteomics to analyze the interaction between tomato cultivars resistant to and susceptible to TYLCV infection. Proteins extracted from leaves of the resistant tomato cultivar 'Zheza-301' and the vulnerable cultivar 'Jinpeng-1', after infection by TYLCV, were

confirmed by two-dimensional gel electrophoresis. A total of 86 differentially expressed proteins were identified, defined into seven groups based on their functions, which are responsible for photosynthesis, proteometabolism, carbohydrate metabolism, signal transduction, accompanying proteins, detoxification, antioxidant and amino acid metabolism. The results help identify key proteins involved in the interaction between tomatoes and TYLCV, potentially increasing resistance to the virus and providing protection against infection.

In proteomic analyses with maize infected with MCMV (Virus chlorotic stain), Dang et al. (2019), applied a comparative approach with isobaric tags for relative and absolute quantification (iTRAQ) to analyze maize infected with MCMV. A total of 972 differentially abundant proteins (DAPs) were identified, of which 661 showed increased abundance and 311 reduced abundance. Functional annotations and orientation of photosynthetic activity revealed decreased photosynthesis and significant changes in ribosomal proteins, stress responses, oxidation-reduction, and redox homeostasis. Thus, the results suggest that the combination of comparative proteomic analyses and virus-induced gene silencing can help in the identification of host proteins that modulate MCMV infection, thus contributing to guide the development of strategies.

Shah et al. (2012) used proteomic analysis of proteins released in the microenvironment of the infection sites of green and red tomato fruits with B. cinerea to identify the proteins produced by ripe green and ripe red fruits in response to infection, as well as the proteins released by B. cinerea. Thus, the analysis characterized 186 proteins in tomatoes infected with *B. cinerea*, being a viable approach both to obtain sufficient information to identify pathogen and host proteins from sites of infection, and to describe the various classes of proteins. The results provided simultaneous information on host and pathogen proteomes, identifying a significant number of several proteins involved in pathogenicity and proteins related to protection against the host oxidative stress response.

CONCLUSION

Addressing the growing challenges of agricultural production requires innovative and integrative approaches. Understanding the genetic diversity of crops and their effective introduction into new cultivars are crucial to meeting the world's growing demand for food. In this context, omics techniques, including proteomics, play a key role. Not only does proteomics provide comprehensive understanding of proteins, but it also offers the opportunity to investigate post-translational modifications, signaling pathways, and interactions between proteins, thereby contributing to a deeper understanding of plant behavior in the face of environmental changes.

Methods such as LC-MS, ICAT, and iTRAQ have been widely used to analyze the plant proteome, each bringing its own advantages and specific applications. In addition, approaches such as MudPIT have been valuable for exploring regulatory mechanisms in important cultures. By

integrating these innovative methodologies, we can not only identify markers of biotic and abiotic stress, but also contribute to the development of more resilient and productive genetically modified crops. These advances in plant proteomics have the potential to revolutionize agriculture by providing sustainable and adaptable solutions to address future food production challenges.

REFERENCES

1. Ahsan, Nagib, Sang-Hoon Lee, Dong-Gi Lee, Hyoshin Lee, Shin Woo Lee, Jeong Dong Bahk, et al. "Physiological and Protein Profiles Alternation of Germinating Rice Seedlings Exposed to Acute Cadmium Toxicity." *Comptes Rendus. Biologies* 330, no. 10 (2007): 735–46. doi:10.1016/j.crvi.2007.08.001.

2. Altelaar, A. F. Maarten, Javier Munoz, and Albert J. R. Heck. "Next-Generation Proteomics: Towards an Integrative View of Proteome Dynamics." *Nature Reviews. Genetics* 14, no. 1 (2013): 35–48. doi:10.1038/nrg3356.

3. Anderson, Leigh, and Christie L. Hunter. "Quantitative Mass Spectrometric Multiple Reaction Monitoring Assays for Major Plasma Proteins." *Molecular & Cellular Proteomics: MCP* 5, no. 4 (2006): 573–88. doi:10.1074/mcp.M500331-MCP200.

4. Angel, Thomas E., Uma K. Aryal, Shawna M. Hengel, Erin S. Baker, Ryan T. Kelly, Errol W. Robinson, et al. "Mass Spectrometry-Based Proteomics: Existing Capabilities and Future Directions." *Chemical Society Reviews* 41, no. 10 (2012): 3912–28. doi:10.1039/C2CS15331A.

5. Anjolette, Fernando Antonio Pino. "Análise proteômica comparativa das secreções das glândulas parotoides e mucosas do sapo Rhinella schneideri e avaliação, in vitro, da atividade antimicrobiana." Doutorado em Toxicologia, Universidade de São Paulo, 2015. doi:10.11606/T.60.2015.tde-29102015-135110.

6. Astarita, Giuseppe, and Mario Ollero. "Lipidomics: An Evolving Discipline in Molecular Sciences." *International Journal of Molecular Sciences* 16, no. 4 (2015): 7748–52. doi:10.3390/ijms16047748.

7. Barkla, Bronwyn J., Rosario Vera-Estrella, and Omar Pantoja. "Progress and Challenges for Abiotic Stress Proteomics of Crop Plants." *Proteomics* 13, no. 12–13 (2013): 1801–15. doi:10.1002/pmic.201200401.

8. Barnidge, David R., Marcia K. Goodmanson, George G. Klee, and David C. Muddiman. "Absolute Quantification of the Model Biomarker Prostate-Specific Antigen in Serum by LC-Ms/MS Using Protein Cleavage and Isotope Dilution Mass Spectrometry." *Journal of Proteome Research* 3, no. 3 (2004): 644–52. doi:10.1021/pr049963d.

9. Bates, Bryson, Zbigniew Kundzewicz, and Shaohong Wu. *Climate Change and Water*. Intergovernmental Panel on Climate Change Secretariat, 2008. Accessed May 7, 2024. [Link](https://www.taccire.sua.ac.tz/handle/123456789/552).

10. Bevan, Michael, and Robbie Waugh. "Applying Plant Genomics to Crop Improvement." *Genome Biology* 8, no. 2 (2007): 302. doi:10.1186/gb-2007-8-2-302.

11. Bokszczanin, Kamila L., Sotirios Fragkostefanakis, Hamed Bostan, Arnaud Bovy, Palak Chaturvedi, Maria L. Chiusano, et al. "Perspectives on Deciphering Mechanisms Underlying Plant Heat Stress Response and Thermotolerance." *Frontiers in Plant Science* 4 (2013). doi:10.3389/fpls.2013.00315.

12. Brozynska, Marta, Agnelo Furtado, and Robert J. Henry. "Genomics of Crop Wild Relatives: Expanding the Gene Pool for Crop Improvement." *Plant Biotechnology Journal* 14, no. 4 (2016): 1070–85. doi:10.1111/pbi.12454.

13. Chaturvedi, Palak, Arindam Ghatak, and Wolfram Weckwerth. "Pollen Proteomics: From Stress Physiology to Developmental Priming." *Plant Reproduction* 29, no. 1 (2016): 119–32. doi:10.1007/s00497-016-0283-9.

14. Dang, Mingqing, Qi Cheng, Ya Hu, Jianxiang Wu, Xueping Zhou, and Yajuan Qian. "Proteomic Changes during MCMV Infection Revealed by iTRAQ Quantitative Proteomic Analysis in Maize." *International Journal of Molecular Sciences* 21, no. 1 (2019): 35. doi:10.3390/ijms21010035.

15. Das, Aayudh, Moustafa Eldakak, Bimal Paudel, Dea-Wook Kim, Homa Hemmati, Chhandak Basu, et al. "Leaf Proteome Analysis Reveals Prospective Drought and Heat Stress Response Mechanisms in Soybean." *BioMed Research International* 2016 (2016): e6021047. doi:10.1155/2016/6021047.

16. El-Aneed, Anas, Aljandro Cohen, and Joseph Banoub. "Mass Spectrometry, Review of the Basics: Electrospray, MALDI, and Commonly Used Mass Analyzers." *Applied Spectroscopy Reviews* 44, no. 3 (2009): 210–30. doi:10.1080/05704920902717872.

17. Eldakak, Moustafa, Sanaa I. Milad, Ali I. Nawar, and Jai S. Rohila. "Proteomics: A Biotechnology Tool for Crop Improvement." *Frontiers in Plant Science* 4 (2013). doi:10.3389/fpls.2013.00035.

18. Griffiths, John R., Richard D. Unwin, Caroline A. Evans, Siân H. Leech, Bernard M. Corfe, and Anthony D. Whetton. "The Application of a Hypothesis-Driven Strategy to the Sensitive Detection and Location of Acetylated Lysine Residues." *Journal of the American Society for Mass Spectrometry* 18, no. 8 (2007): 1423–28. doi:10.1016/j.jasms.2007.04.021.

19. Gupta, Sonika, Vijay Wardhan, Shikha Verma, Saurabh Gayali, Uma Rajamani, Asis Datta, et al. "Characterization of the Secretome of Chickpea Suspension Culture Reveals Pathway Abundance and the Expected and Unexpected Secreted Proteins." *Journal of Proteome Research* 10, no. 11 (2011): 5006–15. doi:10.1021/pr200493d.

20. Haq, Syed Anam Ul, Tanzeel Bashir, Thomas H. Roberts, and Amjad M. Husaini. "Ameliorating the Effects of Multiple Stresses on Agronomic Traits in Crops: Modern Biotechnological and Omics Approaches." *Molecular Biology Reports* 51, no. 1 (2023): 41. doi:10.1007/s11033-023-09042-8.

21. Huang, Ying, Hong-Yu Ma, Wei Huang, Feng Wang, Zhi-Sheng Xu, and Ai-Sheng Xiong. "Comparative Proteomic Analysis Provides Novel Insight into the Interaction between Resistant vs Susceptible Tomato Cultivars and TYLCV Infection." *BMC Plant Biology* 16, no. 1 (2016): 162. doi:10.1186/s12870-016-0819-z.

22. Jenkins, Rosalind E., Neil R. Kitteringham, Christie L. Hunter, Sally Webb, Tony J. Hunt, Robert Elsby, et al. "Relative and Absolute Quantitative Expression Profiling of Cytochromes P450 Using Isotope-Coded Affinity Tags." *Proteomics* 6, no. 6 (2006): 1934–47. doi:10.1002/pmic.200500432.

23. Katam, Ramesh, Sedigheh Shokri, Nitya Murthy, Shardendu K. Singh, Prashanth Suravajhala, Mudassar Nawaz Khan, et al. "Proteomics, physiological, and biochemical analysis of cross tolerance mechanisms in response to heat and water stresses in soybean." *PLOS ONE* 15, no. 6 (2020): e0233905. doi:10.1371/journal.pone.0233905.

24. Kaushik, Neha, Ravi Gupta, Manorma Negi, Ajeet Kaushik, June Hyun Kim, Eun Ha Choi, et al. "Integrating Cutting-Edge Plasma Technology for Environmentally Friendly Smart Horticulture: A Proteomics Approach." *Applied Materials Today* 37 (2024): 102142. doi:10.1016/j.apmt.2024.102142.

25. Kole, Chittaranjan, Mehanathan Muthamilarasan, Robert Henry, David Edwards, Rishu Sharma, Michael Abberton, et al. "Application of Genomics-Assisted Breeding for Generation of Climate Resilient Crops: Progress and Prospects." *Frontiers in Plant Science* 6 (2015): 563. doi:10.3389/fpls.2015.00563.

26. Lee, Joohyun, Wenzhu Jiang, Yongli Qiao, Young-Il Cho, Mi-Ok Woo, Joong-Hyun Chin, et al. "Shotgun Proteomic Analysis for Detecting Differentially Expressed Proteins in the Reduced Culm Number Rice." *Proteomics* 11, no. 3 (2011): 455–68. doi:10.1002/pmic.201000077.

27. Lesage, Véronique S., Marielle Merlino, Christophe Chambon, Brigitte Bouchet, Didier Marion, and Gérard Branlard. "Proteomes of Hard and Soft Near-Isogenic Wheat Lines Reveal That Kernel Hardness Is Related to the Amplification of a Stress Response during Endosperm Development." *Journal of Experimental Botany* 63, no. 2 (2012): 1001–11. doi:10.1093/jxb/err330.

28. Luo, Zhi-Bin, Dennis Janz, Xiangning Jiang, Cornelia Göbel, Henning Wildhagen, Yupeng Tan, et al. "Upgrading Root Physiology for Stress Tolerance by Ectomycorrhizas: Insights from Metabolite and Transcriptional Profiling into Reprogramming for Stress Anticipation." *Plant Physiology* 151, no. 4 (2009): 1902–17. doi:10.1104/pp.109.143735.

29. Marouga, Rita, Stephen David, and Edward Hawkins. "The Development of the DIGE System: 2D Fluorescence Difference Gel Analysis Technology." *Analytical and Bioanalytical Chemistry* 382, no. 3 (2005): 669–78. doi:10.1007/s00216-005-3126-3.

30. Mastrobuoni, Guido, Susann Irgang, Matthias Pietzke, Heike E. Aßmus, Markus Wenzel, Waltraud X. Schulze, et al. "Proteome Dynamics and Early Salt Stress Response of the Photosynthetic Organism Chlamydomonas Reinhardtii." *BMC Genomics* 13, no. 1 (2012): 215. doi:10.1186/1471-2164-13-215.

31. Rabilloud, Thierry. "When 2D Is Not Enough, Go for an Extra Dimension." *PROTEOMICS* 13, no. 14 (2013): 2065–68. doi:10.1002/pmic.201300215.

32. Raza, Ali, Ali Razzaq, Sundas Saher Mehmood, Xiling Zou, Xuekun Zhang, Yan Lv, et al. "Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review." *Plants* 8, no. 2 (2019): 34. doi:10.3390/plants8020034.

33. Rejeb, Ines Ben, Victoria Pastor, and Brigitte Mauch-Mani. "Plant Responses to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms." *Plants* 3, no. 4 (2014): 458–75. doi:10.3390/plants3040458.

34. Schaeffer-Reiss, Christine. "A Brief Summary of the Different Types of Mass Spectrometers Used in Proteomics." *Methods in Molecular Biology (Clifton, N.J.)* 484 (2008): 3–16. doi:10.1007/978-1-59745-398-1_1.

35. Shah, Punit, Ann L. T. Powell, Ron Orlando, Carl Bergmann, and Gerardo Gutierrez-Sanchez. "Proteomic Analysis of Ripening Tomato Fruit Infected by Botrytis Cinerea." *Journal of Proteome Research* 11, no. 4 (2012): 2178–92. doi:10.1021/pr200965c.

36. Shiio, Yuzuru, and Ruedi Aebersold. "Quantitative Proteome Analysis Using Isotope-Coded Affinity Tags and Mass Spectrometry." *Nature Protocols* 1, no. 1 (2006): 139–45. doi:10.1038/nprot.2006.22.

37. Silva-Sanchez, Cecilia, Haiying Li, and Sixue Chen. "Recent Advances and Challenges in Plant Phosphoproteomics." *PROTEOMICS* 15, no. 5–6 (2015): 1127–41. doi:10.1002/pmic.201400410.

38. Skach, William R. "The Expanding Role of the ER Translocon in Membrane Protein Folding." *Journal of Cell Biology* 179, no. 7 (2007): 1333–35. doi:10.1083/jcb.200711107.

39. Soufi, Boumediene, and Boris Macek. "Stable Isotope Labeling by Amino Acids Applied to Bacterial Cell Culture." In *Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC): Methods and Protocols*, edited by Bettina Warscheid, 9–22. Springer, 2014. doi:10.1007/978-1-4939-1142-4_2.

40. Sriyam, Supawadee, Supachok Sinchaikul, Payungsak Tantipaiboonwong, Ching Tzao, Suree Phutrakul, and Shui-Tein Chen. "Enhanced Detectability in Proteome Studies." *Journal of Chromatography B, Analytical Tools for Proteomics* 849, no. 1 (2007): 91–104. doi:10.1016/j.jchromb.2006.10.065.

41. Su, Jiangshuo, Jiafu Jiang, Fei Zhang, Ye Liu, Lian Ding, Sumei Chen, et al. "Current Achievements and Future Prospects in the Genetic Breeding of Chrysanthemum: A Review." *Horticulture Research* 6 (2019): 109. doi:10.1038/s41438-019-0193-8.

42. Tanou, Georgia, Vasileios Fotopoulos, and Athanassios Molassiotis. "Priming against Environmental Challenges and Proteomics in Plants: Update and Agricultural Perspectives." *Frontiers in Plant Science* 3 (2012). doi:10.3389/fpls.2012.00216.

43. Twyman, Richard M. *Principles of Proteomics*. New York: BIOS Scientific Publishers, 2004. [Link](http://archive.org/details/principlesofprot0000twym).

44. Van Der Kelen, Katrien, Rudi Beyaert, Dirk Inzé, and Lieven De Veylder. "Translational Control of Eukaryotic Gene Expression." *Critical Reviews in Biochemistry and Molecular Biology* 44, no. 4 (2009): 143–68. doi:10.1080/10409230902882090.

45. Wang, Haiyang, Lana Shabala, Meixue Zhou, and Sergey Shabala. "Hydrogen Peroxide-Induced Root Ca2+ and K+ Fluxes Correlate with Salt Tolerance in Cereals: Towards the Cell-Based Phenotyping." *International Journal of Molecular Sciences* 19, no. 3 (2018): 702. doi:10.3390/ijms19030702.

46. Wang, Xuan, Tinashe Zenda, Songtao Liu, Guo Liu, Hongyu Jin, Liang Dai, et al. "Comparative Proteomics and Physiological Analyses Reveal Important Maize Filling-Kernel Drought-Responsive Genes and Metabolic Pathways." *International Journal of Molecular Sciences* 20, no. 15 (2019): 3743. doi:10.3390/ijms20153743.

47. Weckwerth, Wolfram. "Green Systems Biology — From Single Genomes, Proteomes and Metabolomes to Ecosystems Research and Biotechnology." *Journal of Proteomics* 75, no. 1 (2011): 284–305. doi:10.1016/j.jprot.2011.07.010.

48. Weckwerth, Wolfram, Stefanie Wienkoop, Wolfgang Hoehenwarter, Volker Egelhofer, and Xiaoliang Sun. "From Proteomics to Systems Biology: MAPA, MASS WESTERN, PROMEX, and COVAIN as a User-Oriented Platform." In *Plant Proteomics: Methods and Protocols*,

edited by Jesus V. Jorrin-Novo, Setsuko Komatsu, Wolfram Weckwerth, and Stefanie Wienkoop, 15–27. Humana Press, 2014. doi:10.1007/978-1-62703-631-3_2.

49. Wolf-Yadlin, Alejandro, Sampsa Hautaniemi, Douglas A. Lauffenburger, and Forest M. White. "Multiple Reaction Monitoring for Robust Quantitative Proteomic Analysis of Cellular Signaling Networks." *Proceedings of the National Academy of Sciences of the United States of America* 104, no. 14 (2007): 5860–65. doi:10.1073/pnas.0608638104.

50. Yocum, Anastasia K., and Arul M. Chinnaiyan. "Current Affairs in Quantitative Targeted Proteomics: Multiple Reaction Monitoring–Mass Spectrometry." *Briefings in Functional Genomics* 8, no. 2 (2009): 145–57. doi:10.1093/bfgp/eln056.

51. Yousaf, Muhammad Irfan, Muhammad Waheed Riaz, Aamar Shehzad, Shakra Jamil, Rahil Shahzad, Shamsa Kanwal, et al. "Responses of Maize Hybrids to Water Stress Conditions at Different Developmental Stages: Accumulation of Reactive Oxygen Species, Activity of Enzymatic Antioxidants and Degradation in Kernel Quality Traits." *PeerJ* 11 (2023): e14983. doi:10.7717/peerj.14983.

52. Zenda, Tinashe, Songtao Liu, Xuan Wang, Hongyu Jin, Guo Liu, and Huijun Duan. "Comparative Proteomic and Physiological Analyses of Two Divergent Maize Inbred Lines Provide More Insights into Drought-Stress Tolerance Mechanisms." *International Journal of Molecular Sciences* 19, no. 10 (2018): 3225. doi:10.3390/ijms19103225.

53. Zhang, Xiang, Aiqin Fang, Catherine P. Riley, Mu Wang, Fred E. Regnier, and Charles Buck. "Multi-Dimensional Liquid Chromatography in Proteomics--a Review." *Analytica Chimica Acta* 664, no. 2 (2010): 101–13. doi:10.1016/j.aca.2010.02.001.

54. Zhu, Juan, Yun Fan, Sergey Shabala, Chengdao Li, Chao Lv, Baojian Guo, et al. "Understanding Mechanisms of Salinity Tolerance in Barley by Proteomic and Biochemical Analysis of Near-Isogenic Lines." *International Journal of Molecular Sciences* 21, no. 4 (2020): 1516. doi:10.3390/ijms21041516.